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REISSUE PATENT APPLICATION TRANSMITTAL

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Attorney Docket No.	30610/44556C1-R2
First Named Inventor	Daniel J. Wendt
Original Patent Number	8,598,121
Original Patent Issue Date (Month/Day/Year)	12/03/2013
Express Mail Label No.	

APPLICATION FOR REISSUE OF: *(Check applicable box)* [X] Utility Patent [ ] Design Patent [ ] Plant Patent

APPLICATION ELEMENTS (37 CFR 1.173)

1. [ ] **Fee Transmittal Form** (PTO/SB/56)
2. [ ] **Applicant asserts small entity status.** See 37 CFR 1.27
3. [ ] **Applicant certifies micro entity status.** See 37 CFR 1.29. Applicant must attach form PTO/SB/15A or B or equivalent.
4. [X] **Specification and Claims** in double column copy of patent format (*amended, if appropriate*)
5. [X] **Drawing(s)** (*proposed amendments, if appropriate*)
6. [ ] **Reissue Oath/Declaration or Substitute Statement** (37 CFR 1.175) (PTO/AIA/05, 06, or 07)
7. [X] **Application Data Sheet** **NOTE:** Benefit claims under 37 CFR 1.78 and foreign priority claims under 37 CFR 1.55 MUST be set forth in an Application Data Sheet (ADS).
8. [X] **Original U.S. Patent currently assigned?** [X] Yes [ ] No (*If Yes, check applicable box(es)*)
   - [X] Written Consent of all Assignees (PTO/AIA/53)
   - [X] 37 CFR 3.73(c) Statement (PTO/AIA/96)
9. [ ] **CD-ROM or CD-R** in duplicate, Computer Program (*Appendix*) or large table
   - [ ] Landscape Table on CD
10. **Nucleotide and/or Amino Acid Sequence Submission** (*if applicable, items a. – c. are required*)
    - a. [X] Computer Readable Form (CRF)
    - b. [x] Specification Sequence Listing on:
      - i. [ ] CD-ROM (2 copies) or CD-R (2 copies); **or**
      - ii. [X] Paper
    - c. [X] Statements verifying identity of above copies

ACCOMPANYING APPLICATION PARTS

11. [ ] **Statement of status and support for all changes to the claims.** See 37 CFR 1.173(c).
12. [X] **Power of Attorney**
13. [ ] **Information Disclosure Statement (IDS)** PTOSB/08 or PTO-1449
    - [ ] Copies of citations attached
14. [ ] **English translation of Reissue Oath/Declaration** (*if applicable*)
15. [ ] **Return Receipt Postcard** (MPEP § 503) (Should be specifically itemized)
16. [X] **Preliminary Amendment** (37 CFR 1.173; MPEP § 1453)
17. [ ] **Other:**

[ ] This is a continuation reissue or divisional reissue application (*i.e.*, a second or subsequent reissue application for the same issued patent). (*Check box if applicable.*)

18. CORRESPONDENCE ADDRESS

[X] The address associated with Customer Number: 90849 **OR** [ ] Correspondence address below

Name					
Address					
City		State		Zip Code	
Country		Telephone			
Email					

Signature	/Katherine L. Neville/	Date	July 11, 2017
Name (Print/Type)	Katherine L. Neville	Registration No.	53,379

Ascendis EX1064 (Part 1 of 2)
Ascendis v. BioMarin
PGR2026-00013

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STATEMENT UNDER 37 CFR 3.73(c)

Applicant/Patent Owner: BioMarin Pharmaceutical Inc.

Application No./Patent No.: 8,598,121 Filed/Issue Date: December 3, 2013

Titled: Variants of C-Type Natriuretic Peptide

BioMarin Pharmaceutical Inc.. , a Corporation
(Name of Assignee) (Type of Assignee, e.g., corporation, partnership, university, government agency, etc.)

states that, for the patent application/patent identified above, it is (choose **one** of options 1, 2, 3 or 4 below):

1. [x] The assignee of the entire right, title, and interest.

2. [ ] An assignee of less than the entire right, title, and interest (check applicable box):

   [ ] The extent (by percentage) of its ownership interest is _____ %). Additional Statement(s) by the owners holding the balance of the interest must be submitted to account for 100% of the ownership interest.

   [ ] There are unspecified percentages of ownership. The other parties, including inventors, who together own the entire right, title and interest are:

   Additional Statement(s) by the owner(s) holding the balance of the interest must be submitted to account for the entire right, title, and interest.

3. [ ] The assignee of an undivided interest in the entirety (a complete assignment from one of the joint inventors was made). The other parties, including inventors, who together own the entire right, title, and interest are:

   Additional Statement(s) by the owner(s) holding the balance of the interest must be submitted to account for the entire right, title, and interest.

4. [ ] The recipient, via a court proceeding or the like (*e.g.*, bankruptcy, probate), of an undivided interest in the entirety (a complete transfer of ownership interest was made). The certified document(s) showing the transfer is attached.

The interest identified in option 1, 2 or 3 above (not option 4) is evidenced by either (choose **one** of options A or B below):

A. [x] An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel 028206 , Frame 0517 , or for which a copy thereof is attached.

B. [ ] A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as follows:

1. From: _____ To: _____
   The document was recorded in the United States Patent and Trademark Office at
   Reel _____ , Frame _____ , or for which a copy thereof is attached.

2. From: _____ To: _____
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STATEMENT UNDER 37 CFR 3.73(c)

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☐ Additional documents in the chain of title are listed on a supplemental sheet(s).

☒ As required by 37 CFR 3.73(c)(1)(i), the documentary evidence of the chain of title from the original owner to the assignee was, or concurrently is being, submitted for recordation pursuant to 37 CFR 3.11.

[NOTE: A separate copy (i.e., a true copy of the original assignment document(s)) must be submitted to Assignment Division in accordance with 37 CFR Part 3, to record the assignment in the records of the USPTO. See MPEP 302.08]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.

/Katherine L. Neville/	July 11, 2017
Signature	Date
Katherine L. Neville	53,379
Printed or Typed Name	Title or Registration Number

PTO/AIA/53 (09-12)
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REISSUE APPLICATION: CONSENT OF ASSIGNEE; STATEMENT OF NON-ASSIGNMENT	Docket Number (Optional) 30610/44556C1-R2

This is part of the application for a reissue patent based on the original patent identified below.

Name of Patentee(s) BioMarin Pharmaceutical Inc.

Patent Number	Date Patent Issued
8,598,121	December 3, 2013

Title of Invention
Variants of C-Type Natriuretic Peptide

1. [x] Filed herein is a statement under 37 CFR 3.73(c). (Form PTO/AIA/96)

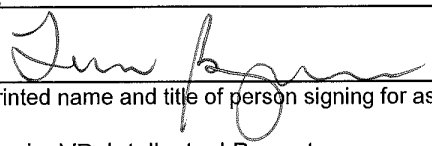
2. [ ] Ownership of the patent is in the inventor(s), and no assignment of the patent is in effect.

One of boxes 1 or 2 above must be checked. If multiple assignees, complete this form for each assignee. If box 2 is checked, skip the next entry and go directly to "Name of Assignee."

The written consent of all assignees and inventors owning an undivided interest in the original patent is included in this application for reissue.

The assignee(s) owning an undivided interest in said original patent is/are BioMarin Pharmaceutical Inc., and the assignee(s) consents to the accompanying application for reissue.

Name of assignee/inventor (if not assigned)
BioMarin Pharmaceutical Inc.

Signature	Date
	07-05-2017

Typed or printed name and title of person signing for assignee (if assigned)

Luisa Bigornia, VP, Intellectual Property

Doc Code: PA..
Document Description: Power of Attorney

PTO/AIA/82B (07-13)
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POWER OF ATTORNEY BY APPLICANT

I hereby revoke all previous powers of attorney given in the application identified in either the attached transmittal letter or the boxes below.

Application Number	Filing Date

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[X] I hereby appoint the Patent Practitioner(s) associated with the following Customer Number as my/our attorney(s) or agent(s), and to transact all business in the United States Patent and Trademark Office connected therewith for the application referenced in the attached transmittal letter (form PTO/AIA/82A) or identified above: 90849

OR

[ ] I hereby appoint Practitioner(s) named in the attached list (form PTO/AIA/82C) as my/our attorney(s) or agent(s), and to transact all business in the United States Patent and Trademark Office connected therewith for the patent application referenced in the attached transmittal letter (form PTO/AIA/82A) or identified above. (Note: Complete form PTO/AIA/82C.)

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OR

Firm or Individual Name					
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Country					
Telephone		Email			

I am the Applicant (if the Applicant is a juristic entity, list the Applicant name in the box):

BIOMARIN PHARMACEUTICAL INC.

[ ] Inventor or Joint Inventor (title not required below)

[ ] Legal Representative of a Deceased or Legally Incapacitated Inventor (title not required below)

[X] Assignee or Person to Whom the Inventor is Under an Obligation to Assign (provide signer's title if applicant is a juristic entity)

[ ] Person Who Otherwise Shows Sufficient Proprietary Interest (e.g., a petition under 37 CFR 1.46(b)(2) was granted in the application or is concurrently being filed with this document) (provide signer's title if applicant is a juristic entity)

SIGNATURE of Applicant for Patent

The undersigned (whose title is supplied below) is authorized to act on behalf of the applicant (e.g., where the applicant is a juristic entity).

Signature	Luisa Bigornia	Date (Optional)	
Name	Luisa Bigornia		
Title	Vice President, Intellectual Property, BioMarin Pharmaceutical Inc.		

NOTE: Signature - This form must be signed by the applicant in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. If more than one applicant, use multiple forms.

[X] Total of 1 forms are submitted.

US008598121B2

(12) **United States Patent**
Wendt et al.

(10) **Patent No.: US 8,598,121 B2**
(45) **Date of Patent: Dec. 3, 2013**

(54) **VARIANTS OF C-TYPE NATRIURETIC PEPTIDE**

(75) Inventors: **Daniel J. Wendt**, Walnut Creek, CA (US); **Shinong Long**, Milpitas, CA (US); **Sianna Castillo**, San Francisco, CA (US); **Christopher P. Price**, Kentfield, CA (US); **Mika Aoyagi-Scharber**, Mill Valley, CA (US); **Michel Claude Vellard**, San Rafael, CA (US); **Augustus O. Okhamafe**, Concord, CA (US)

(73) Assignee: **Biomarin Pharmaceutical Inc.**, Novato, CA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **13/466,672**

(22) Filed: **May 8, 2012**

(65) **Prior Publication Data**

US 2012/0316114 A1 Dec. 13, 2012

Related U.S. Application Data

(63) Continuation of application No. 12/784,117, filed on May 20, 2010, now Pat. No. 8,198,242.

(60) Provisional application No. 61/180,112, filed on May 20, 2009, provisional application No. 61/254,563, filed on Oct. 23, 2009.

(51) **Int. Cl.**

A61K 38/22	(2006.01)
A61K 38/00	(2006.01)
A61K 38/16	(2006.01)
C12P 21/04	(2006.01)

(52) **U.S. Cl.**
USPC **514/12.4**; 514/16.7; 514/21.3; 435/69.4; 435/69.7; 435/70.1; 530/324

(58) **Field of Classification Search**
None
See application file for complete search history.

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(Continued)

Primary Examiner — Shulamith H Shafer
(74) *Attorney, Agent, or Firm* — Marshall, Gerstein & Borun LLP

(57) **ABSTRACT**

The present disclosure provides variants of C-type natriuretic peptide (CNP), pharmaceutical compositions comprising CNP variants, and methods of making CNP variants. The CNP variants are useful as therapeutic agents for the treatment of diseases responsive to CNP, including but not limited to bone-related disorders, such as skeletal dysplasias (e.g., achondroplasia), and vascular smooth muscle disorders (e.g., restenosis and arteriosclerosis).

14 Claims, 61 Drawing Sheets

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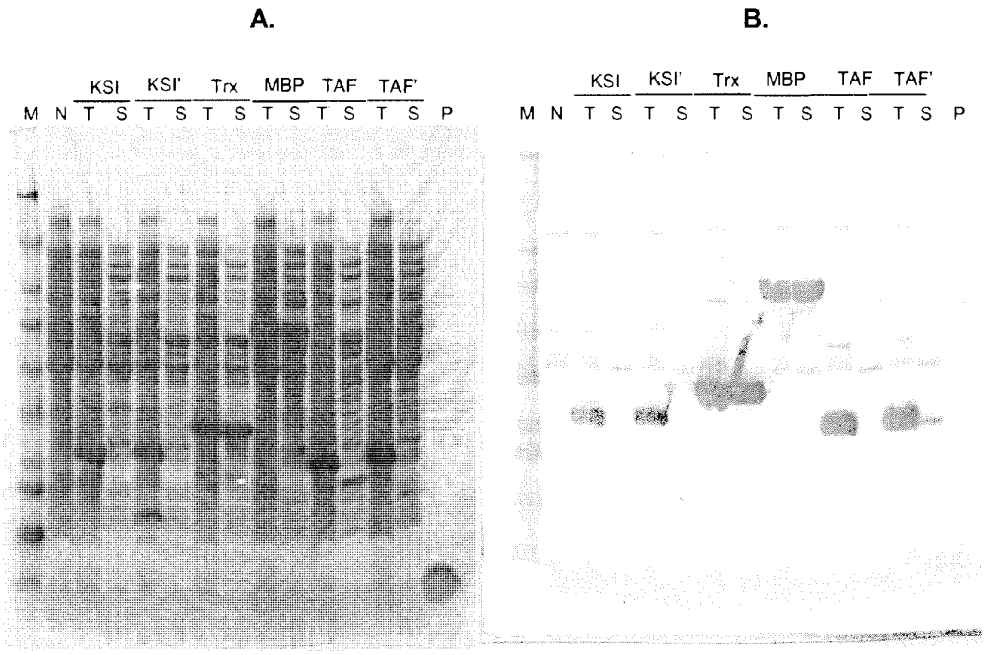
FIGURE 1



Coomassie blue stain

Western blot with anti-CNP antibody

FIGURE 2

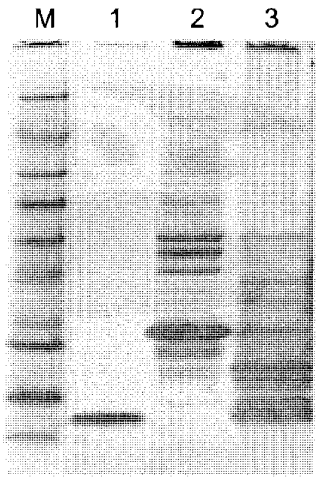


FIGURE 3

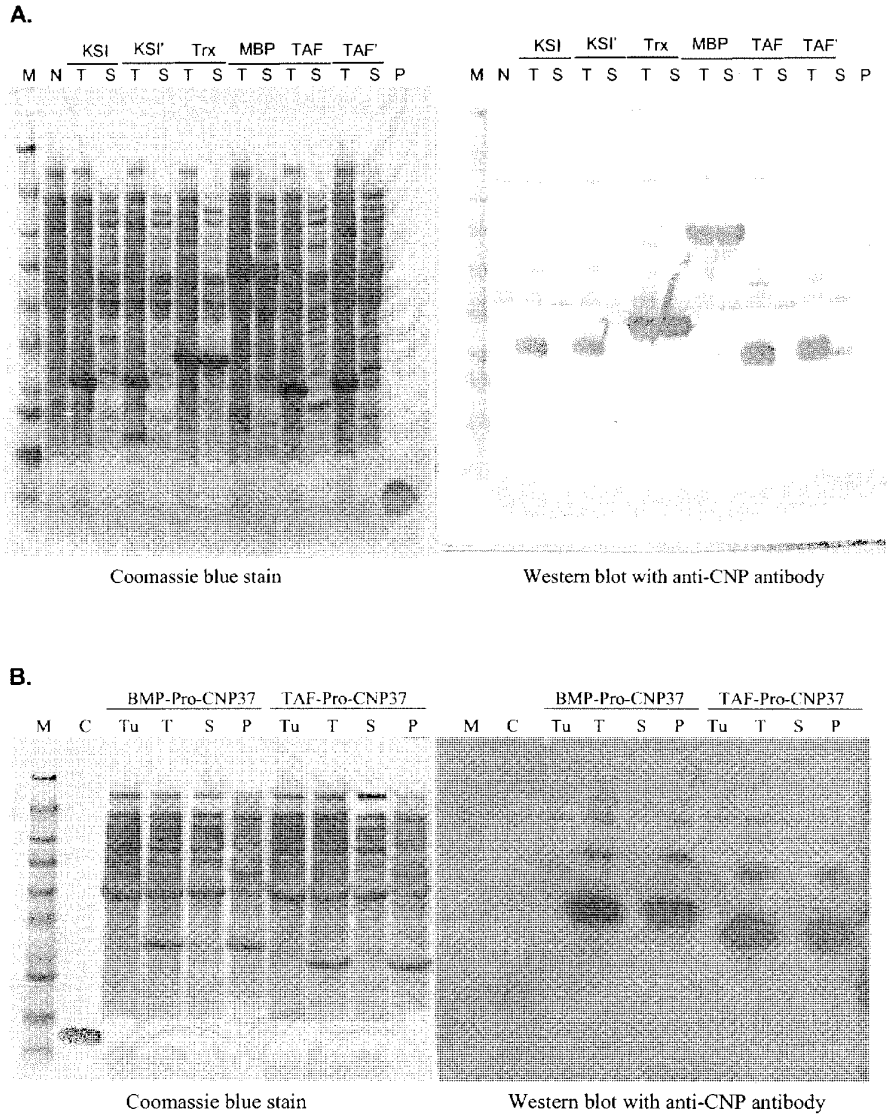

A.
KSI KSI' Trx MBP TAF TAF'
M N T S T S T S T S T S T S P
KSI KSI' Trx MBP TAF TAF'
M N T S T S T S T S T S T S P
Coomassie blue stain
Western blot with anti-CNP antibody
B.
BMP-Pro-CNP37 TAF-Pro-CNP37
M C Tu T S P Tu T S P
BMP-Pro-CNP37 TAF-Pro-CNP37
M C Tu T S P Tu T S P
Coomassie blue stain
Western blot with anti-CNP antibody

FIGURE 3 continued

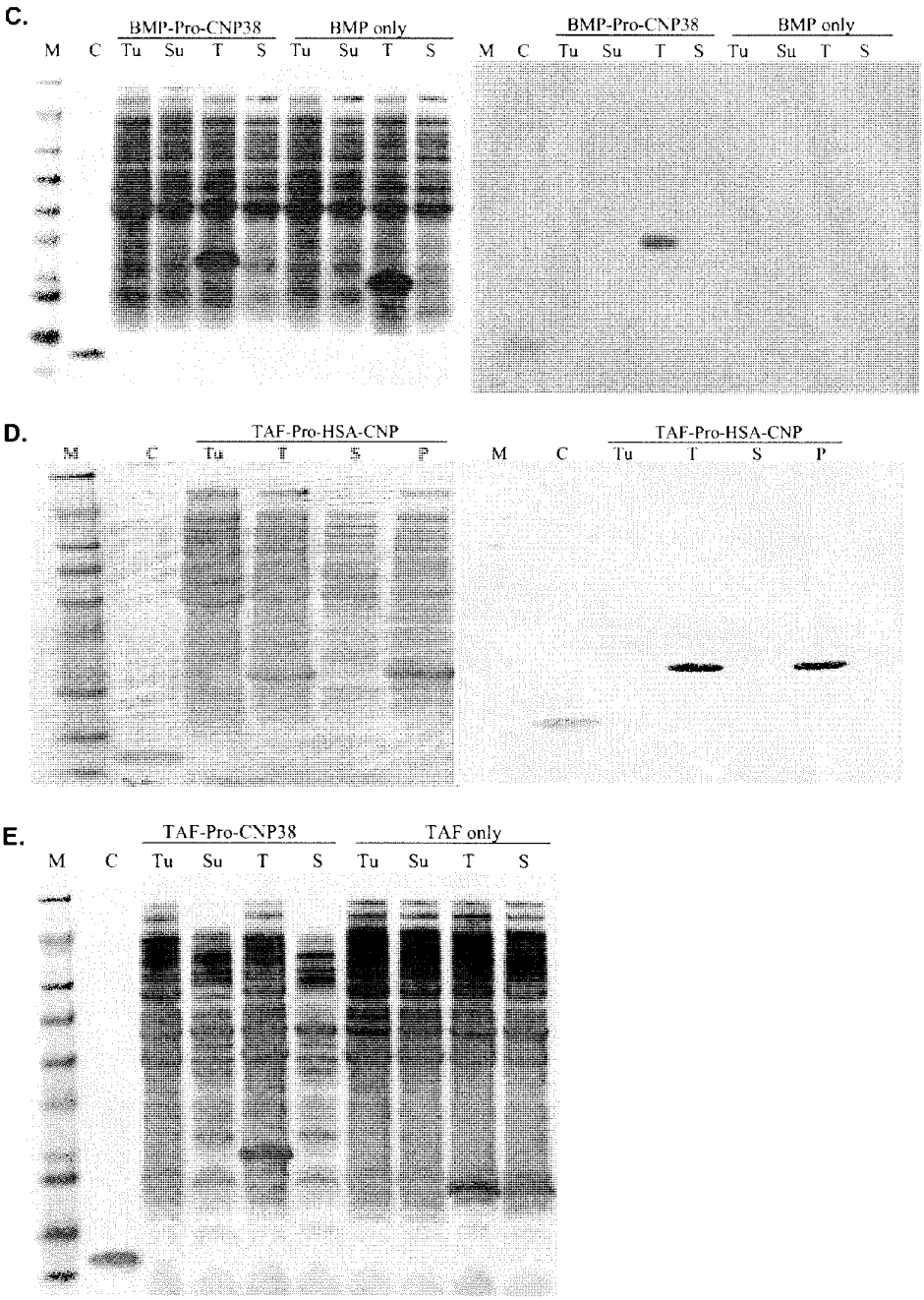


FIGURE 4

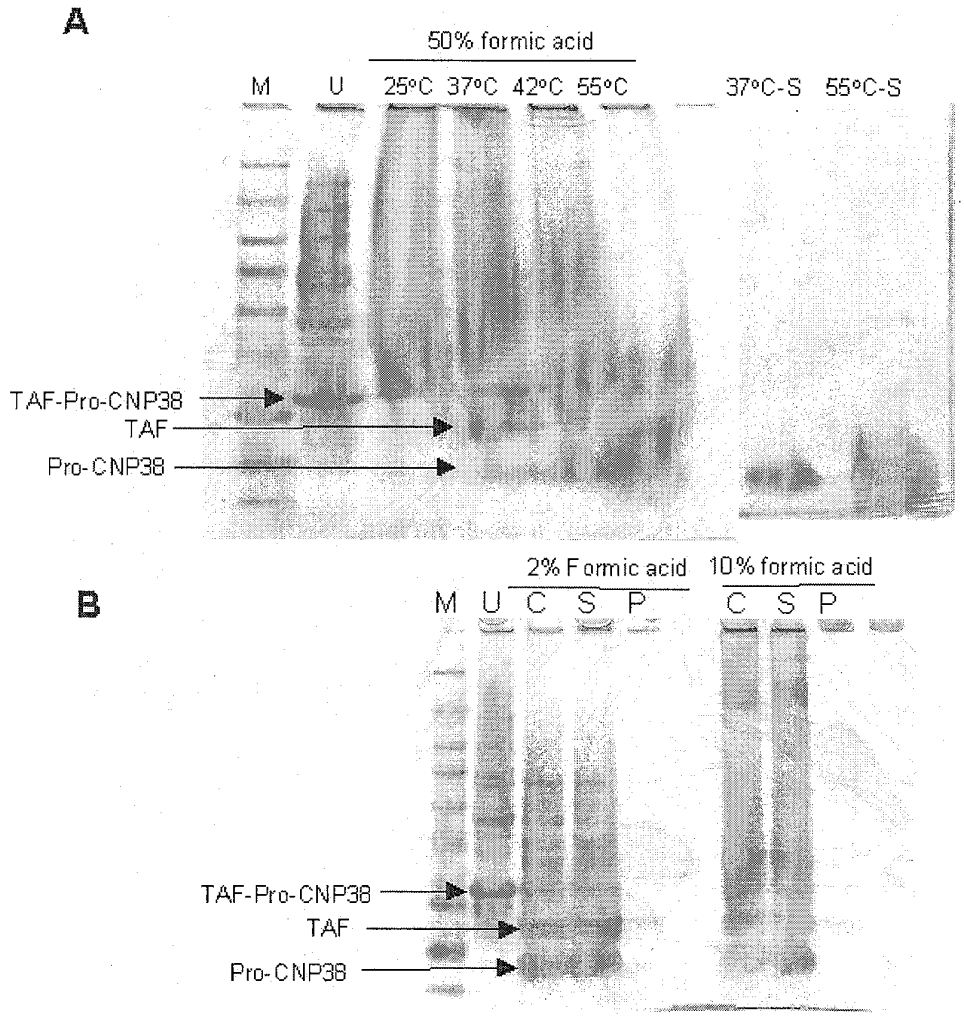

A
50% formic acid
M
U
25°C
37°C
42°C
55°C
37°C-S
55°C-S
TAF-Pro-CNP38
TAF
Pro-CNP38
B
2% Formic acid
10% formic acid
M
U
C
S
P
C
S
P
TAF-Pro-CNP38
TAF
Pro-CNP38

FIGURE 4C

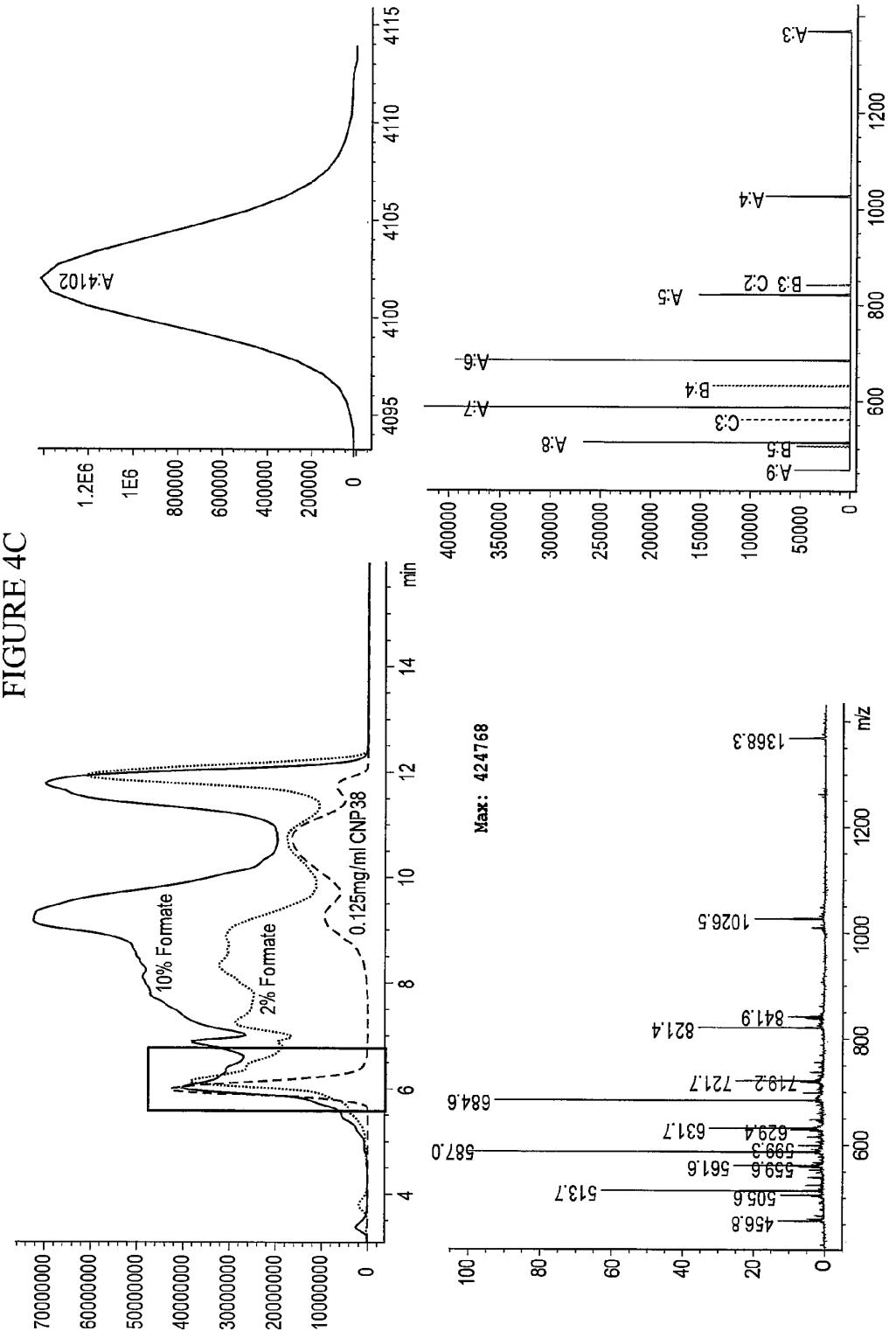

10% Formate
2% Formate
0.125mg/ml CNP38
min
A:4102
Max: 424768
m/z
A:3
A:4
B:3 C:2
A:5
A:6
A:7
B:4
C:3
A:8
B:5
A:9
1368.3
1026.5
841.9
821.4
719.2
721.7
684.6
687.0
629.4
631.7
599.3
559.5
561.6
513.7
505.6
456.8

FIGURE 5

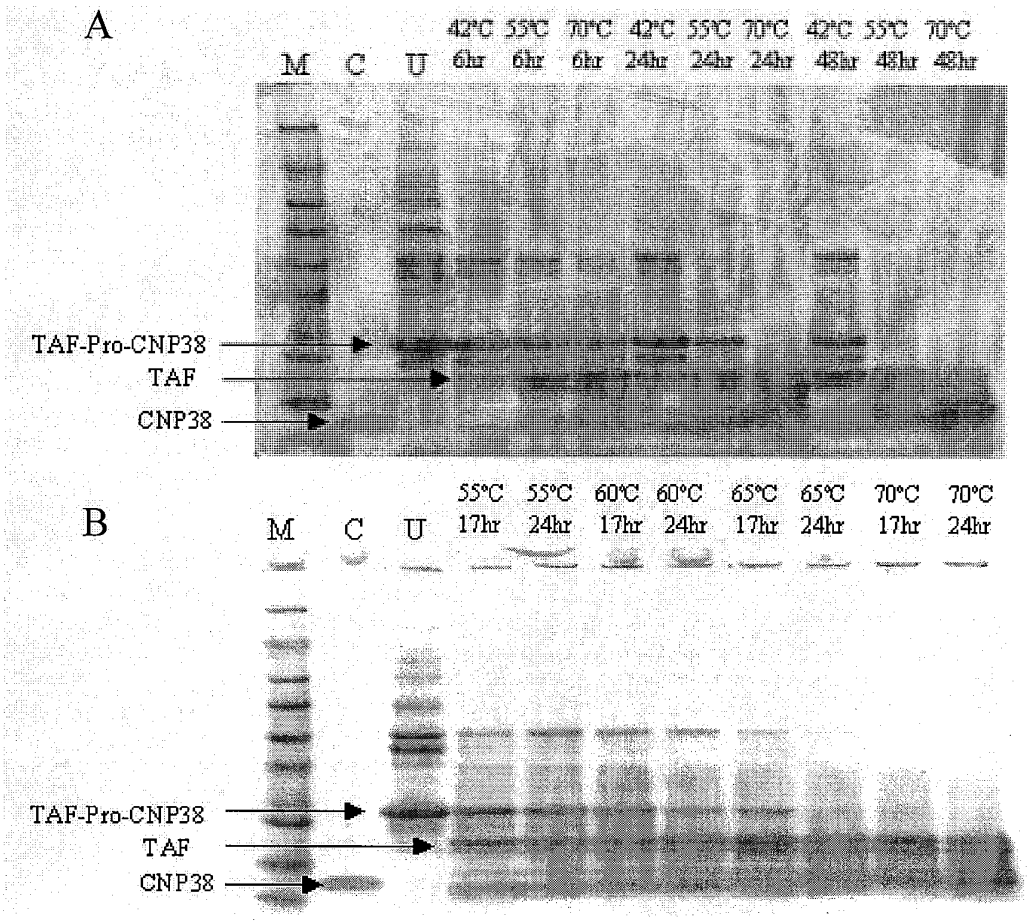

A
M
C
U
42°C 6hr
55°C 6hr
70°C 6hr
42°C 24hr
55°C 24hr
70°C 24hr
42°C 48hr
55°C 48hr
70°C 48hr
TAF-Pro-CNP38
TAF
CNP38
B
M
C
U
55°C 17hr
55°C 24hr
60°C 17hr
60°C 24hr
65°C 17hr
65°C 24hr
70°C 17hr
70°C 24hr
TAF-Pro-CNP38
TAF
CNP38

FIGURE 5C

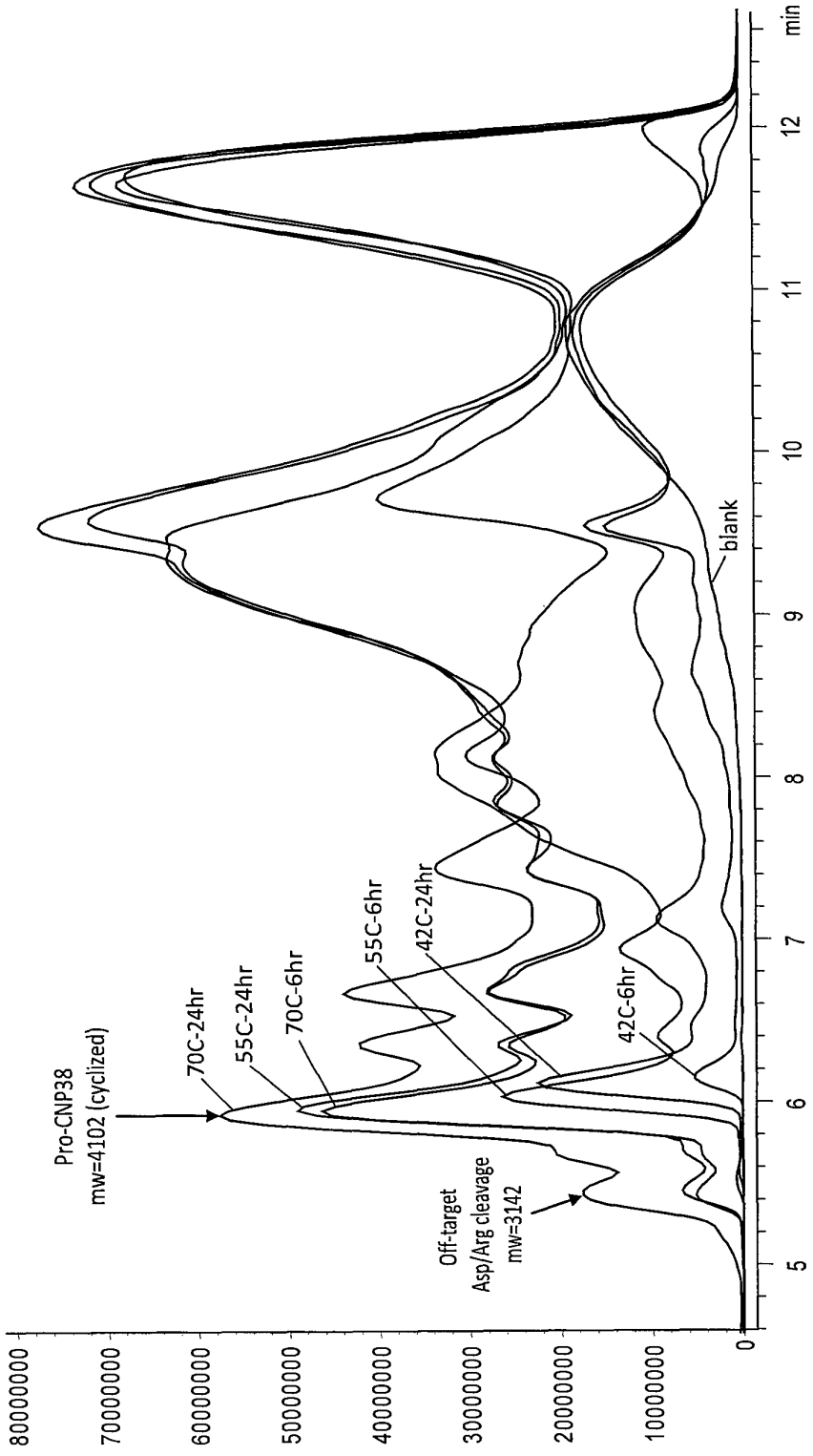

Pro-CNP38
mw=4102 (cyclized)
70C-24hr
55C-24hr
70C-6hr
55C-6hr
42C-24hr
42C-6hr
blank
Off-target
Asp/Arg cleavage
mw=3142
80000000
70000000
60000000
50000000
40000000
30000000
20000000
10000000
0
5
6
7
8
9
10
11
12
min

FIGURE 6

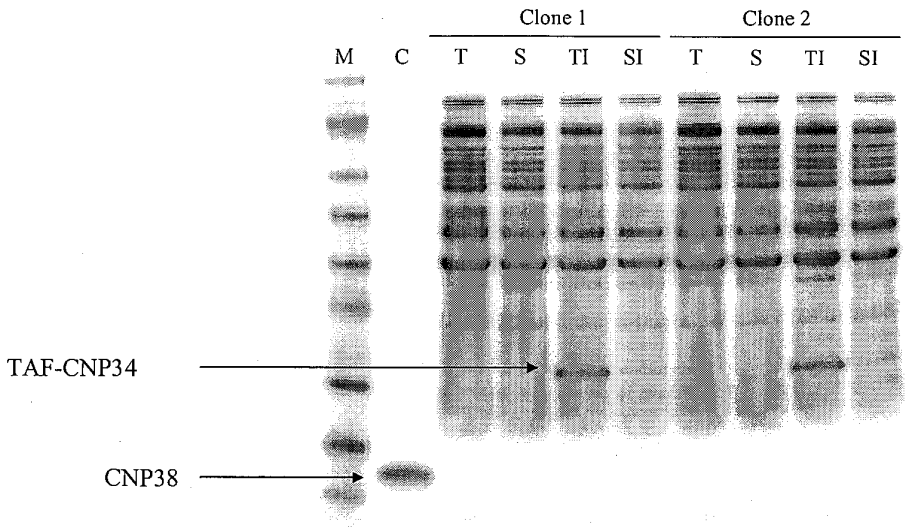


FIGURE 7

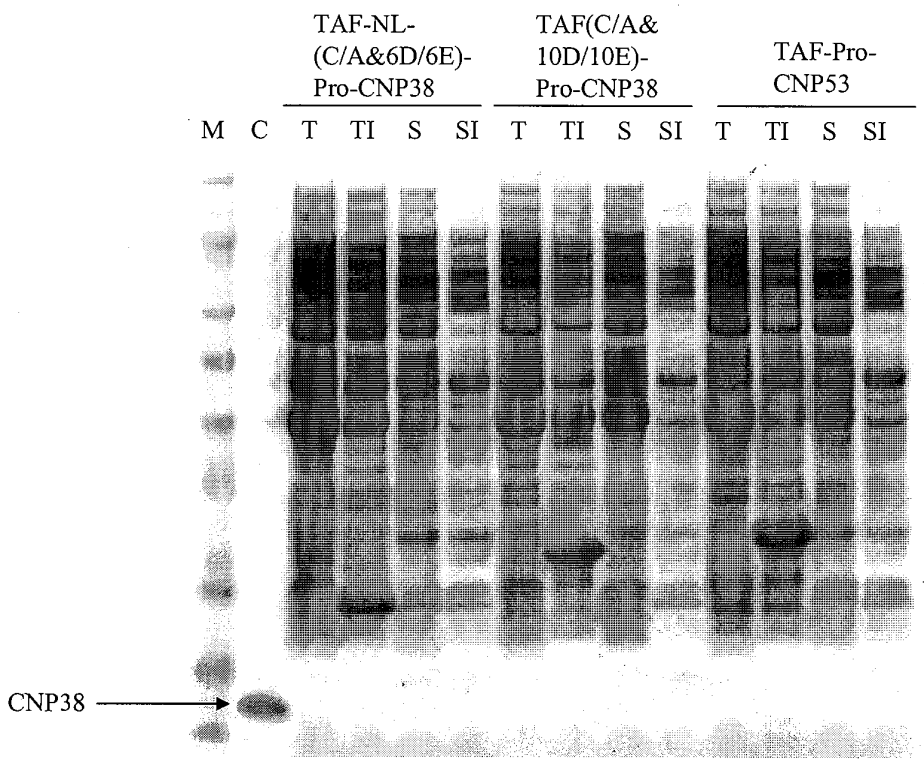


FIGURE 8

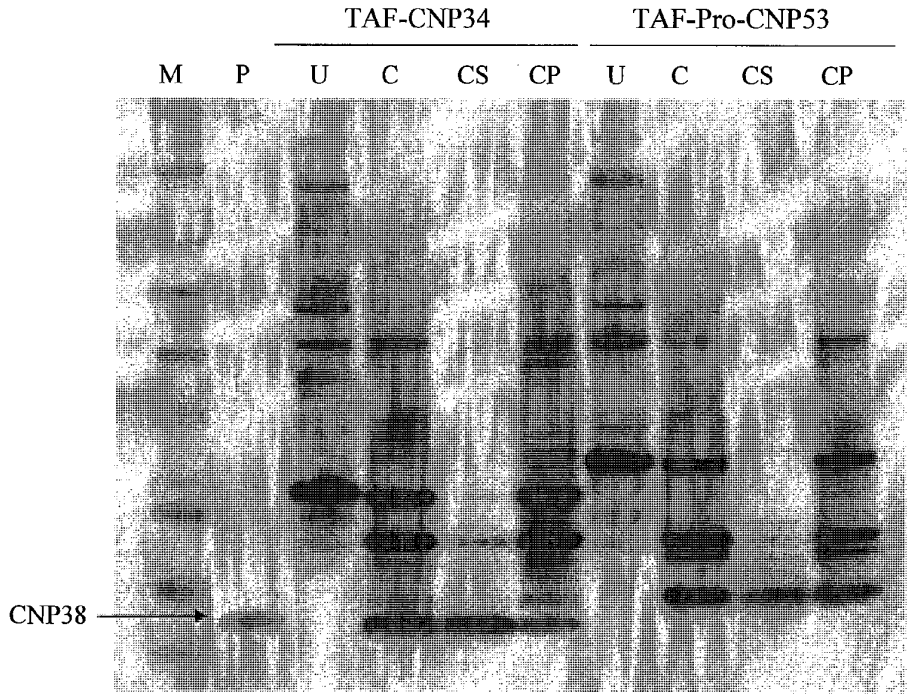


FIGURE 9

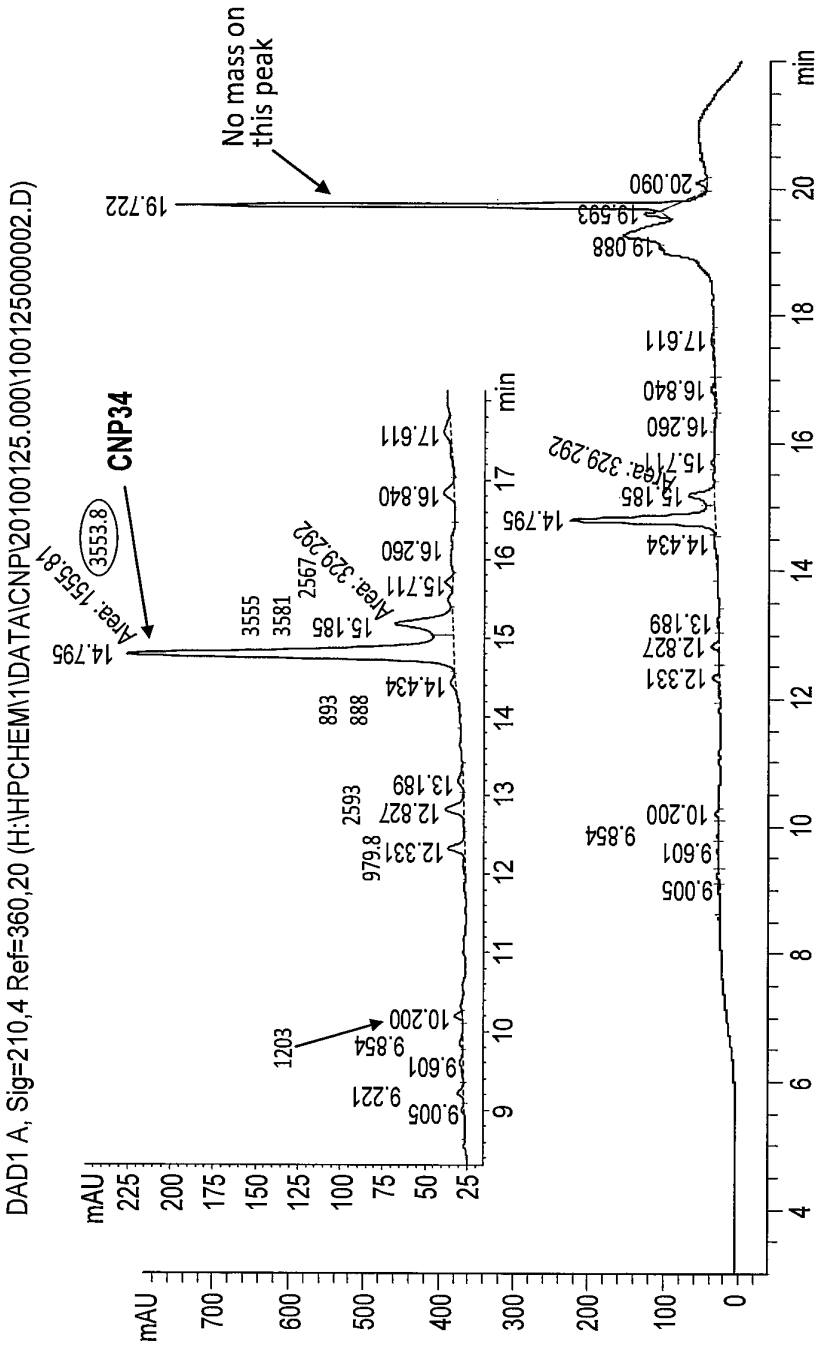

Calc. CNP34 monoisotopic mass = 3553.88
DAD1 A, Sig=210,4 Ref=360,20 (H:\HPCHEM\1\DATA\CNP\20100125.000\100125000002.D)
No mass on this peak
CNP34
3553.8
Area: 1555.81
Area: 329.292
mAU
min

FIGURE 10

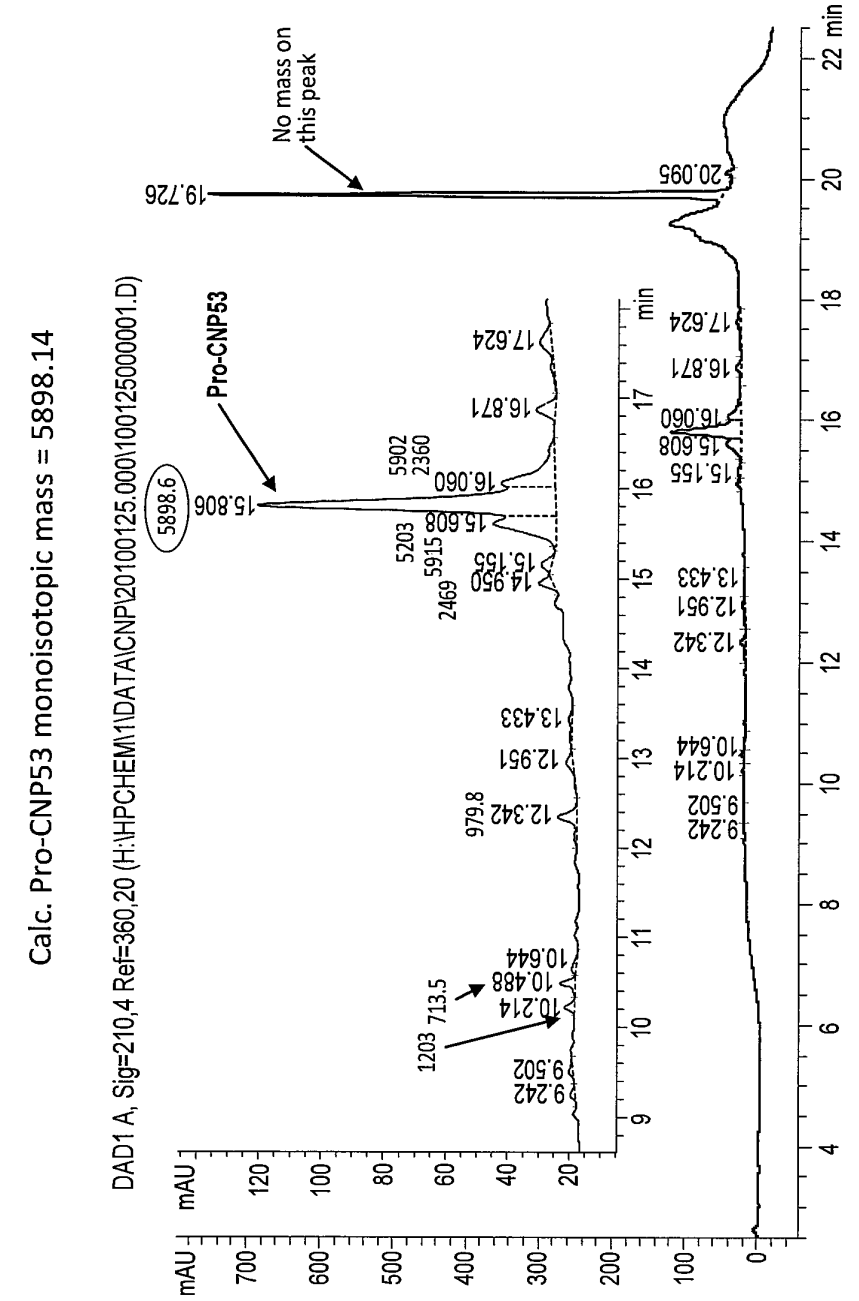

Calc. Pro-CNP53 monoisotopic mass = 5898.14
DAD1 A, Sig=210,4 Ref=360,20 (H:\HPCHEM\1\DATA\CNP\20100125.000\100125000001.D)
mAU
min
No mass on this peak
Pro-CNP53
5898.6
19.726
15.806
16.060
16.871
17.624
20.095
22 min

FIGURE 11

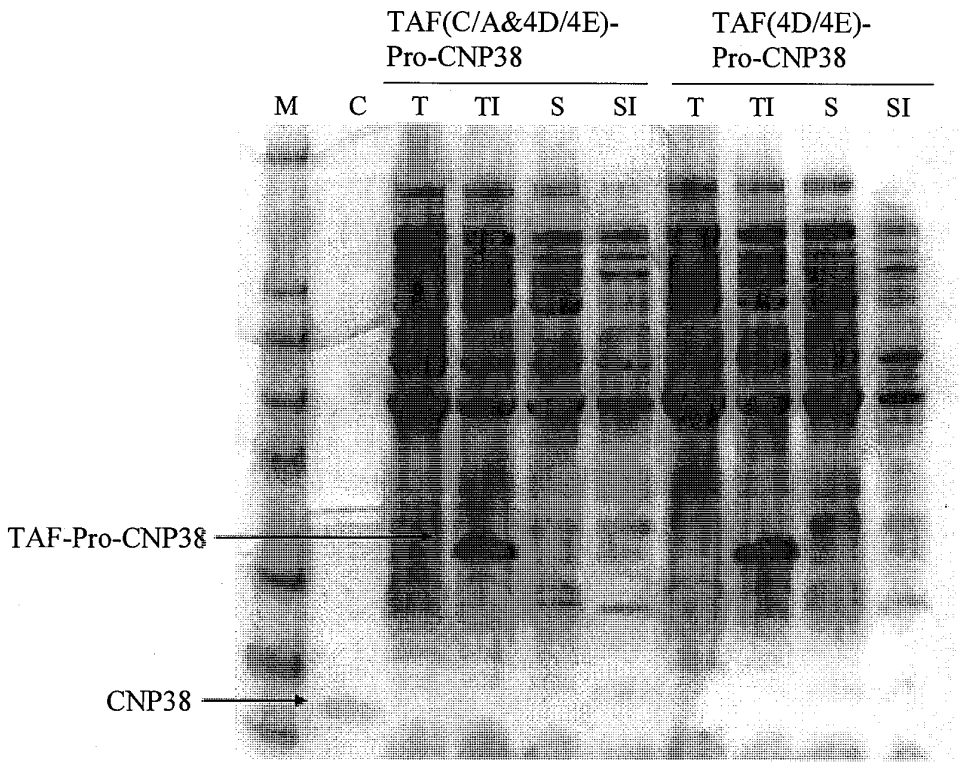

TAF(C/A&4D/4E)-Pro-CNP38
TAF(4D/4E)-Pro-CNP38
M
C
T
TI
S
SI
T
TI
S
SI
TAF-Pro-CNP38
CNP38

FIGURE 12

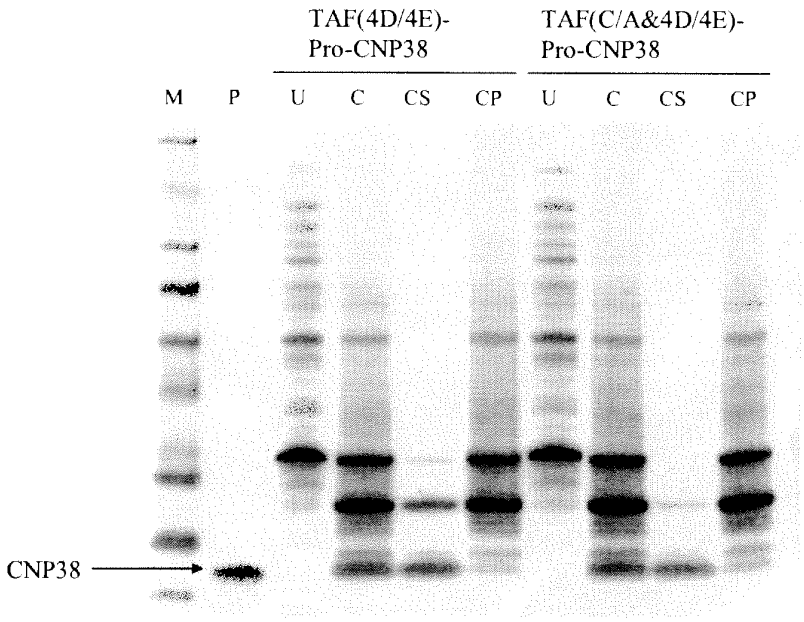


FIGURE 13

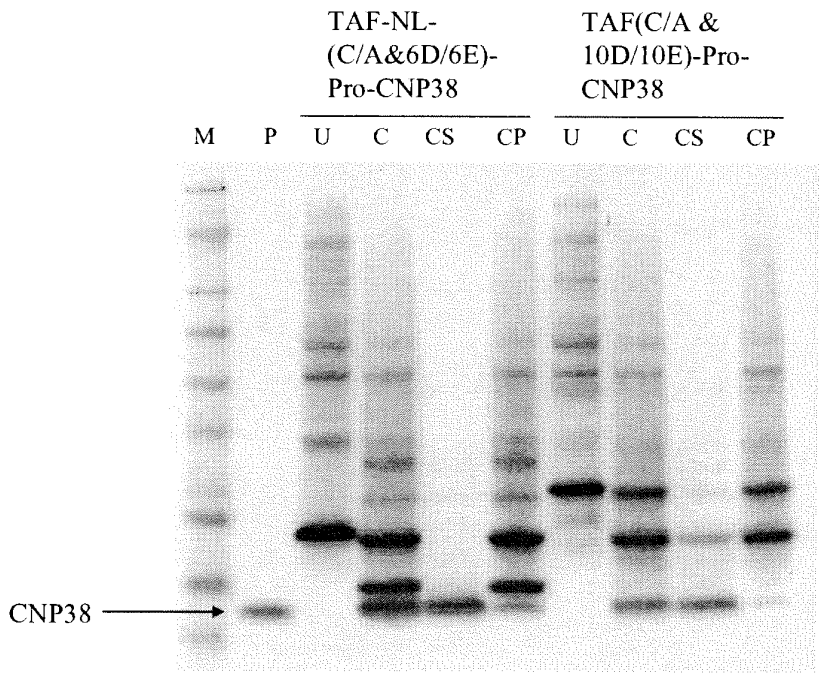


FIGURE 14

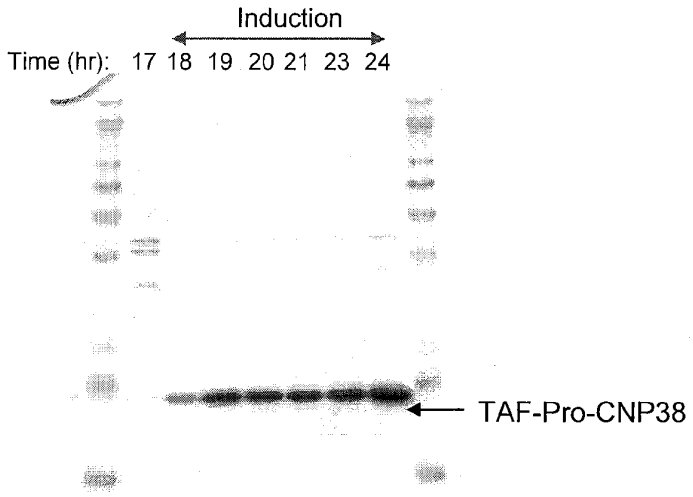


FIGURE 15

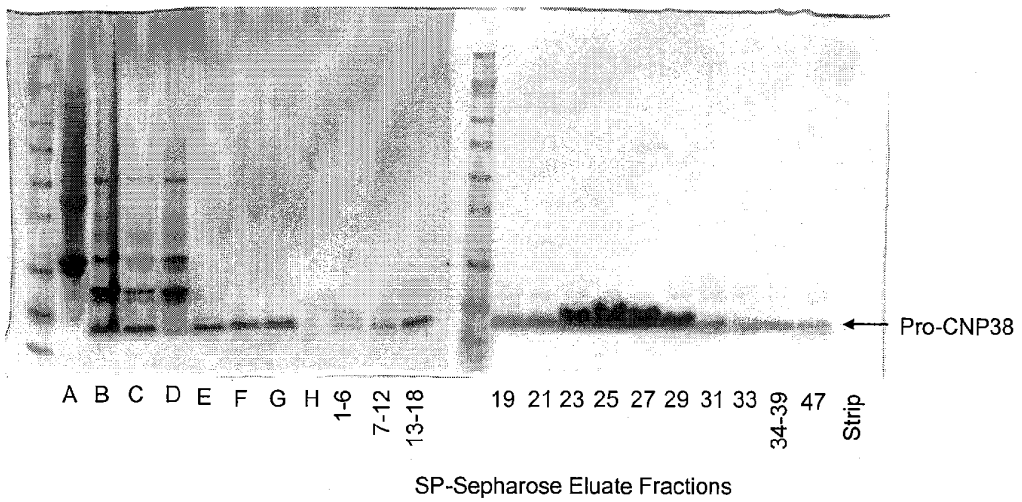


FIGURE 16

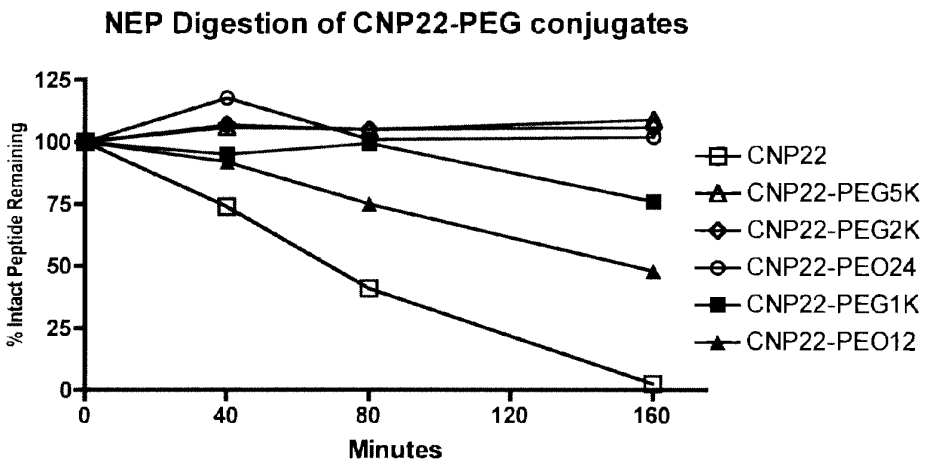


FIGURE 17

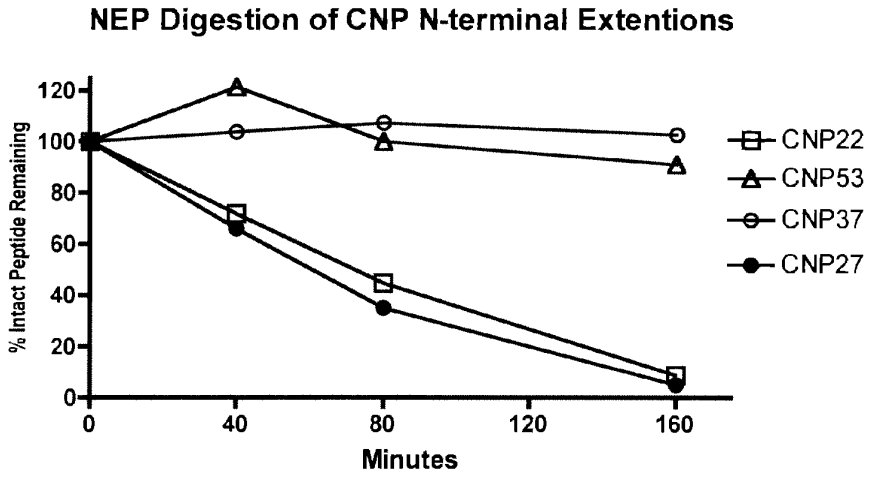


FIGURE 18

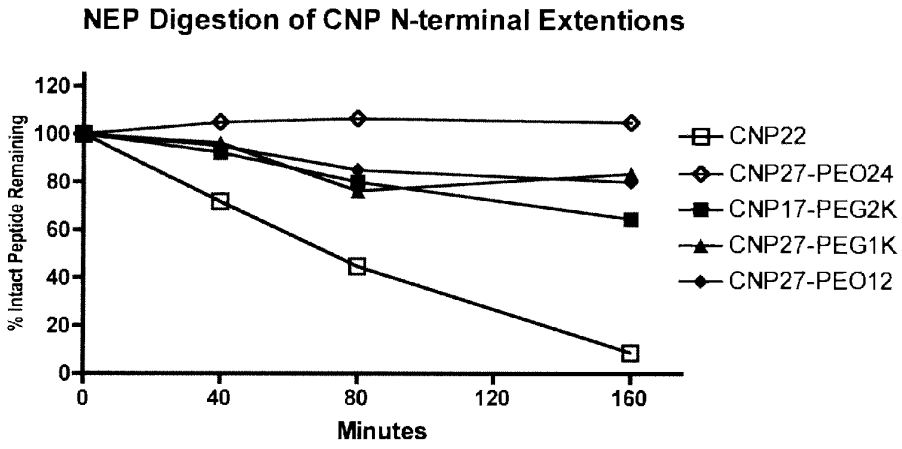


FIGURE 19

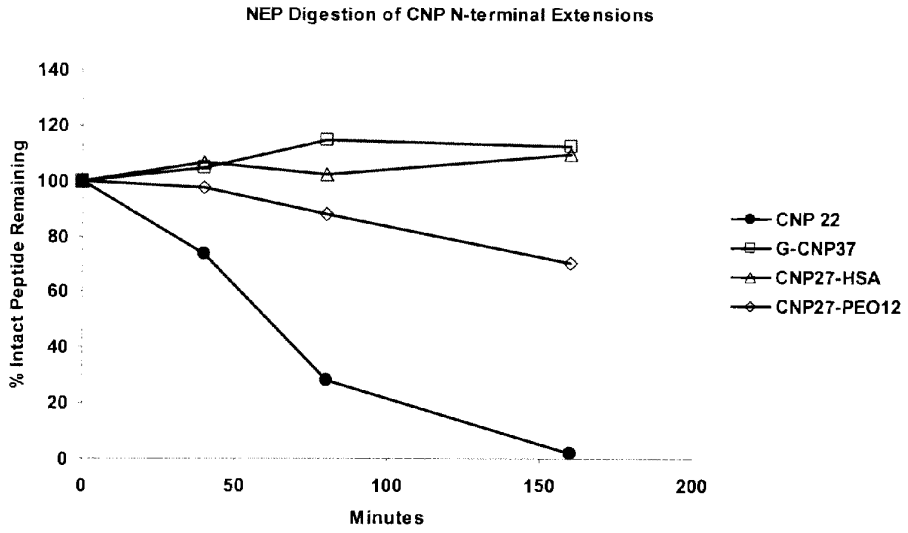


FIGURE 20

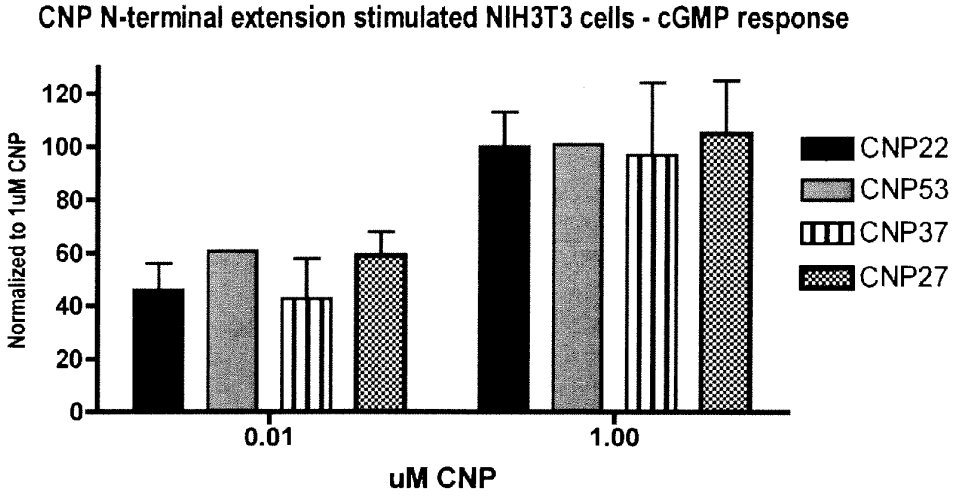


FIGURE 21

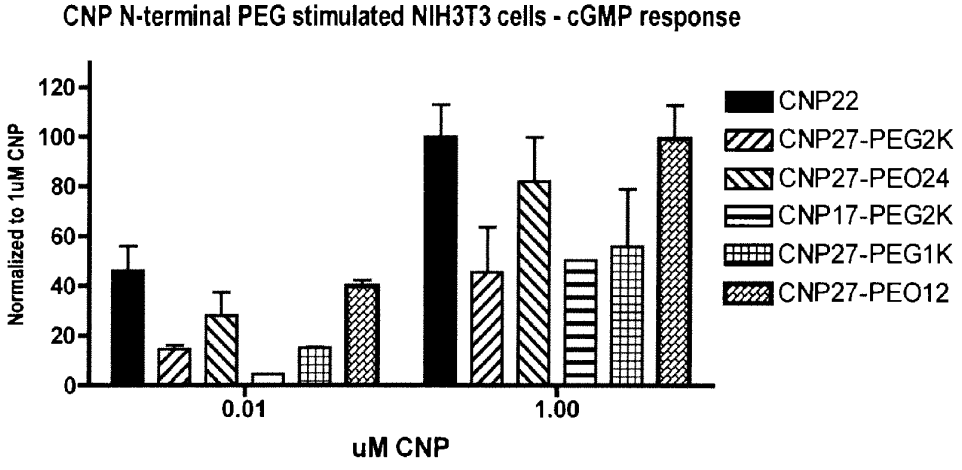


FIGURE 22

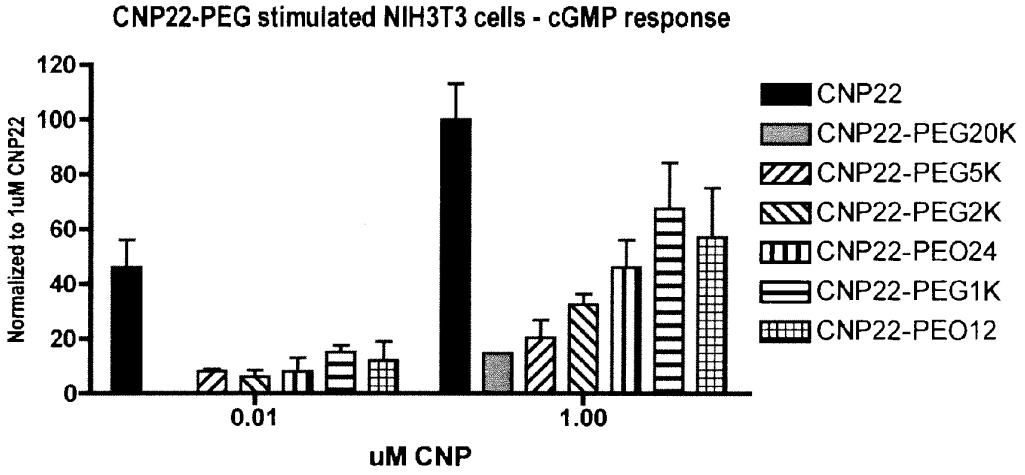


FIGURE 23

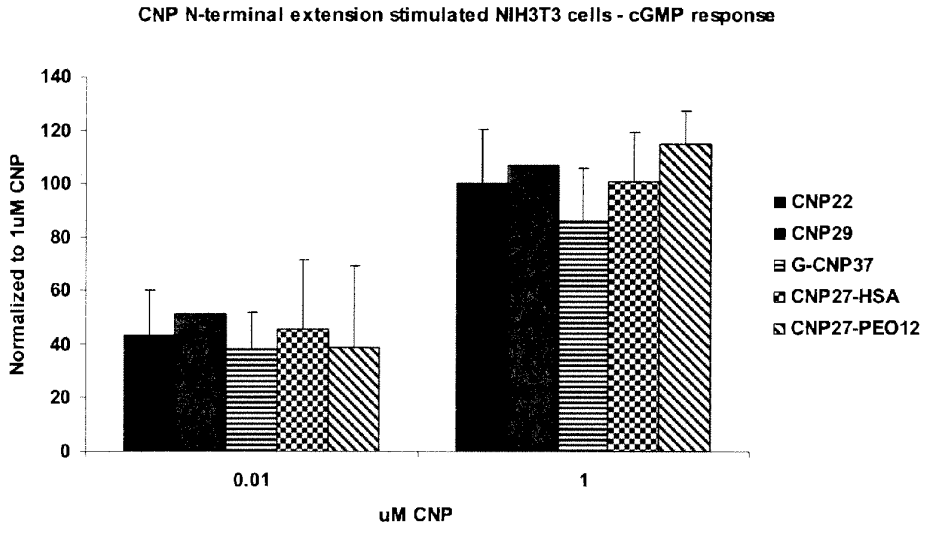
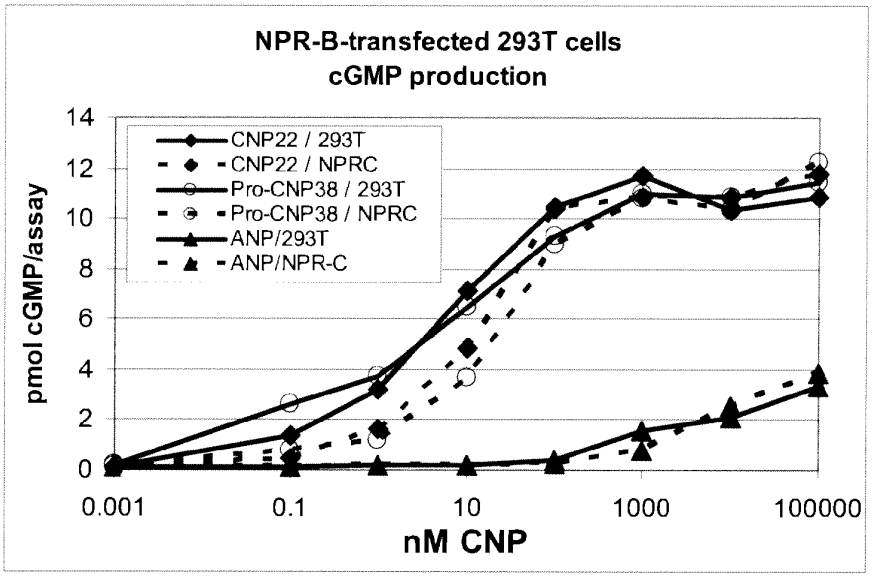


FIGURE 24

A.


NPR-B-transfected 293T cells
cGMP production
pmol cGMP/assay
nM CNP
CNP22 / 293T
CNP22 / NPRC
Pro-CNP38 / 293T
Pro-CNP38 / NPRC
ANP/293T
ANP/NPR-C
0
2
4
6
8
10
12
14
0.001
0.1
10
1000
100000

B.

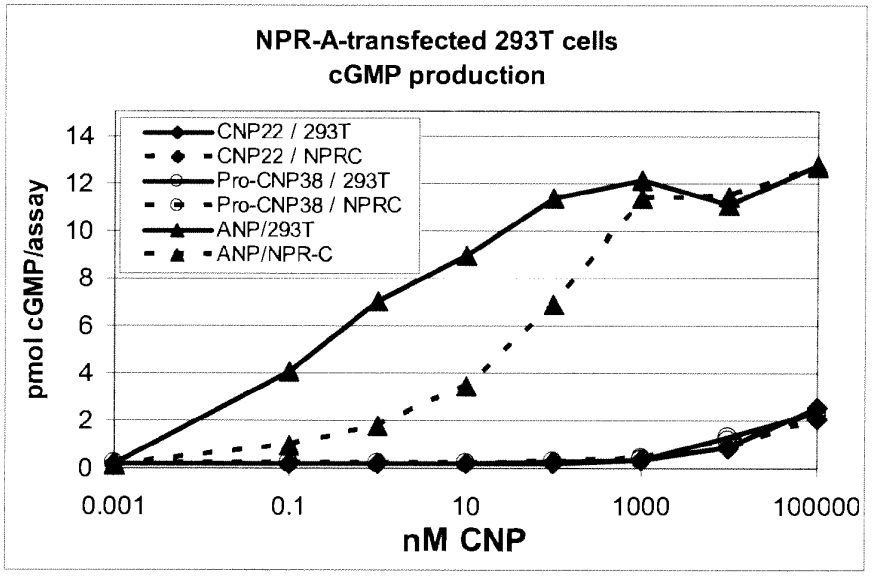
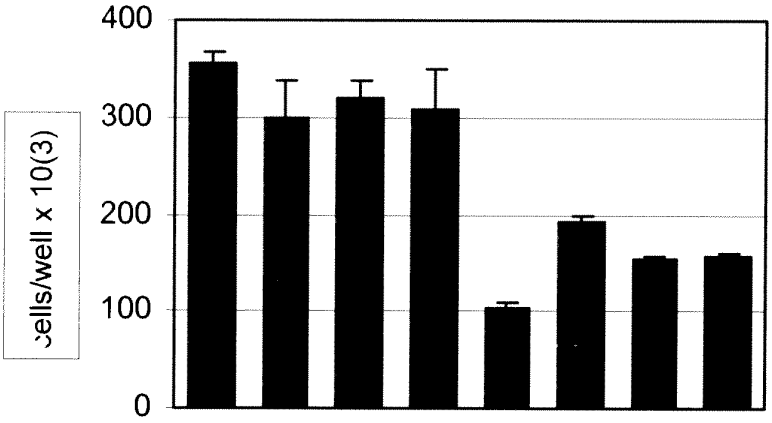

NPR-A-transfected 293T cells
cGMP production
pmol cGMP/assay
nM CNP
CNP22 / 293T
CNP22 / NPRC
Pro-CNP38 / 293T
Pro-CNP38 / NPRC
ANP/293T
ANP/NPR-C
0
2
4
6
8
10
12
14
0.001
0.1
10
1000
100000

FIGURE 25



Bars from left to right:

1	control (no CNP22, no FGF2)
2	CNP22 (0.2 uM) continuous
3	CNP22 (0.2 uM) 1 hr once daily
4	CNP22 (0.2 uM) 2 hr once daily
5	FGF2 (5 ng/mL) continuous
6	FGF2 + CNP22 (0.2 uM) continuous
7	FGF2 + CNP22 (0.2 uM) 1 hr daily
8	FGF2 + CNP22 (0.2 uM) 2 hr daily

FIGURE 26

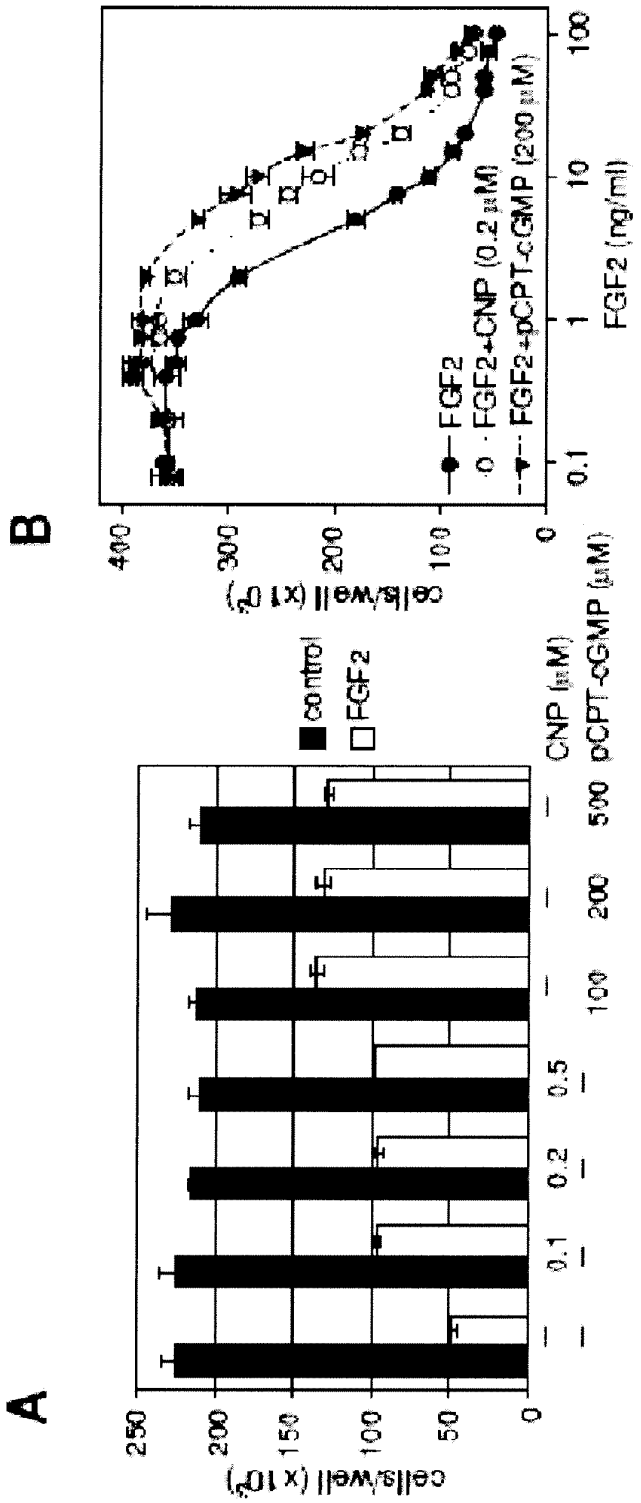

A
cells/well (x 10^2)
250
200
150
100
50
0
control
FGF2
—
0.1
0.2
0.5
—
100
200
500
CNP (μM)
pCPT-cGMP (μM)
B
cells/well (x 10^2)
400
300
200
100
0
FGF2
FGF2+CNP (0.2 μM)
FGF2+pCPT-cGMP (200 μM)
0.1
1
10
100
FGF2 (ng/ml)

FIGURE 27

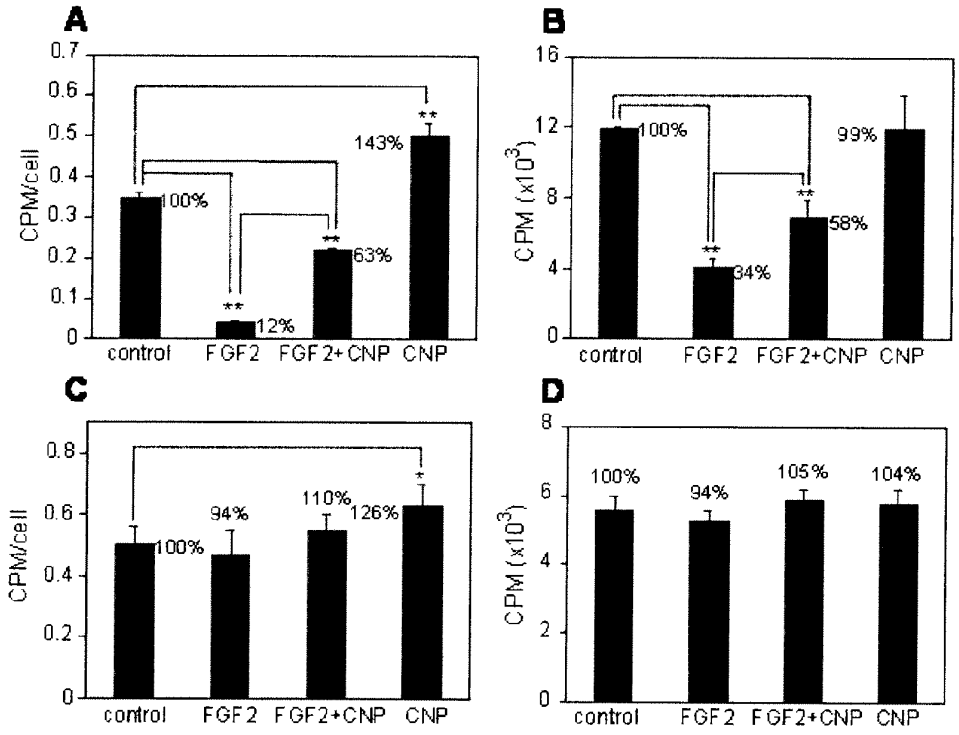

A
CPM/cell
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
100%
12%
63%
143%
**
control
FGF2
FGF2+CNP
CNP
B
CPM (x10^3)
16
12
8
4
0
100%
34%
58%
99%
**
control
FGF2
FGF2+CNP
CNP
C
CPM/cell
0.8
0.6
0.4
0.2
0
100%
94%
110%
126%
*
control
FGF2
FGF2+CNP
CNP
D
CPM (x10^3)
8
6
4
2
0
100%
94%
105%
104%
control
FGF2
FGF2+CNP
CNP

FIGURE 28

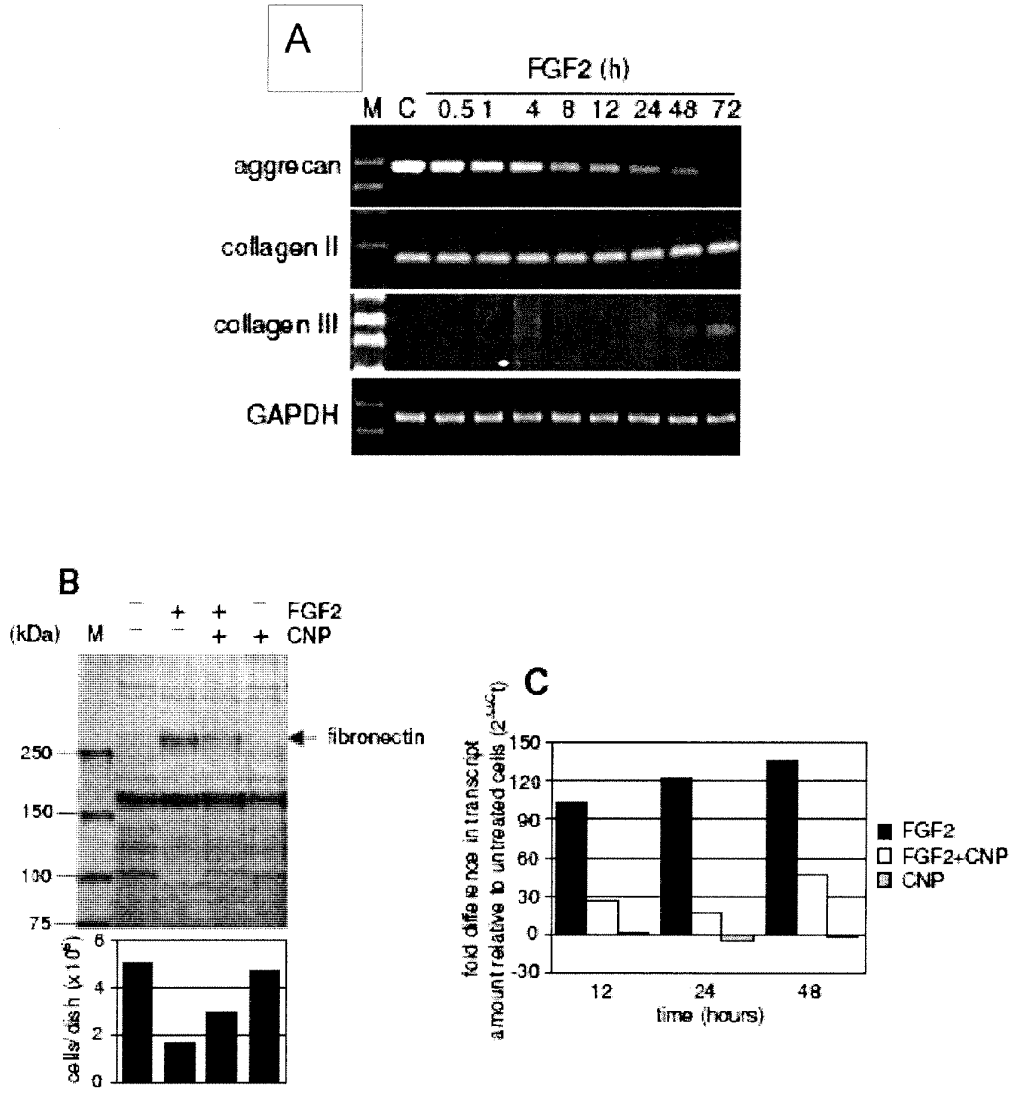

A
FGF2 (h)
M C 0.5 1 4 8 12 24 48 72
aggrecan
collagen II
collagen III
GAPDH
B
(kDa) M
FGF2
CNP
fibronectin
250
150
100
75
cells/dish (x10⁵)
C
fold difference in transcript amount relative to untreated cells (2^-ΔΔCt)
time (hours)
FGF2
FGF2+CNP
CNP

FIGURE 29

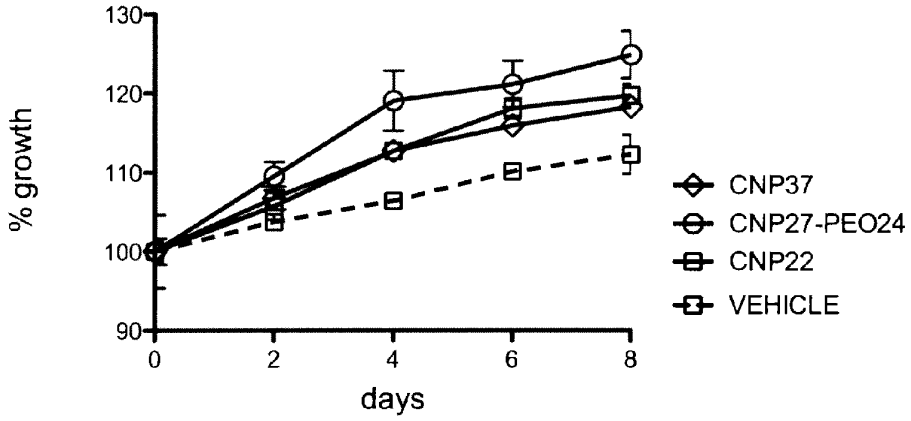


FIGURE 30

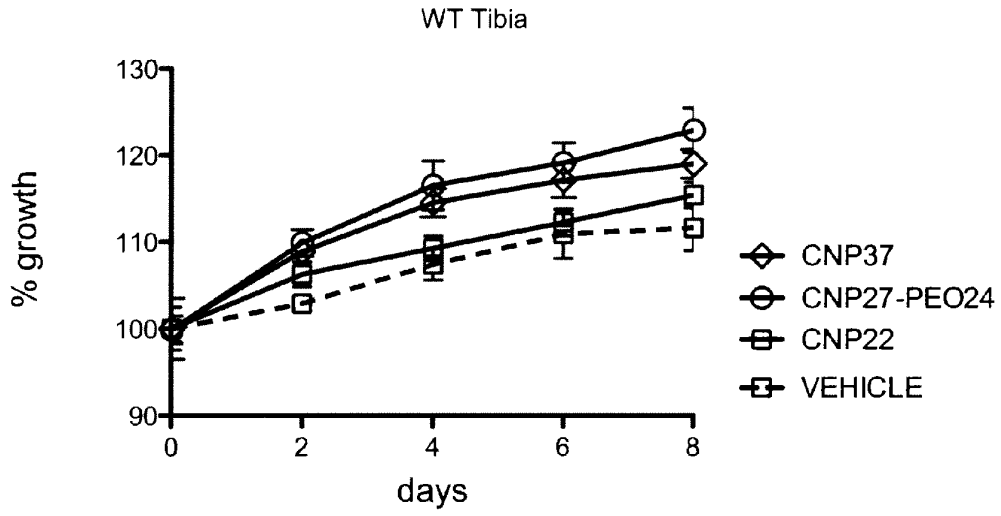


FIGURE 31

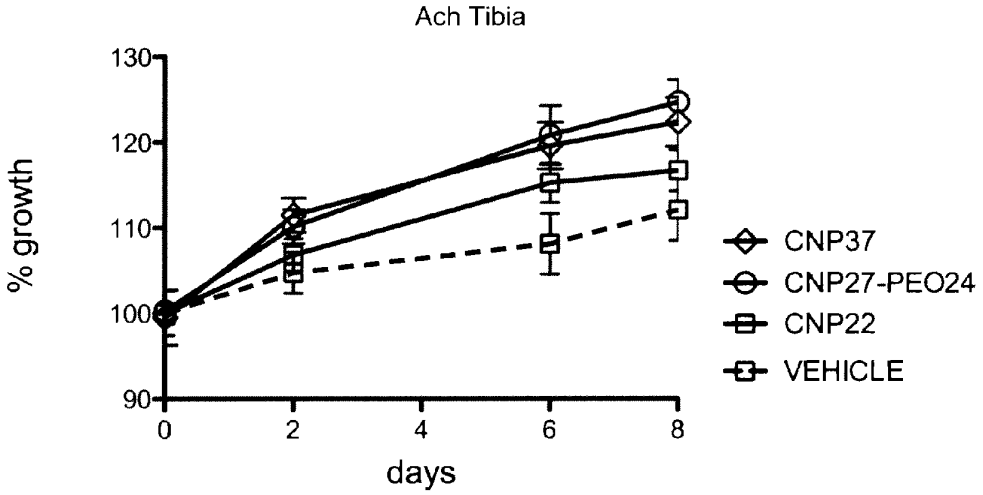


FIGURE 32

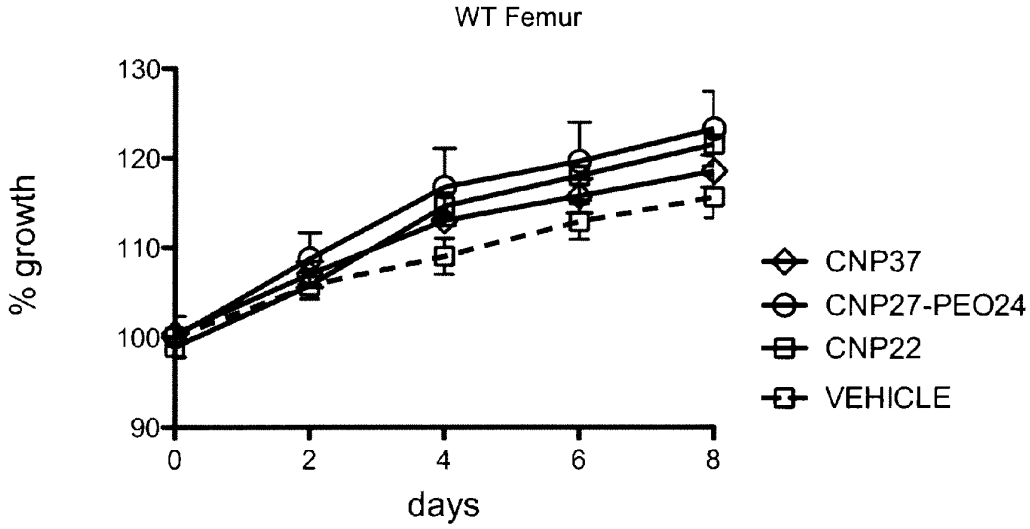


FIGURE 33

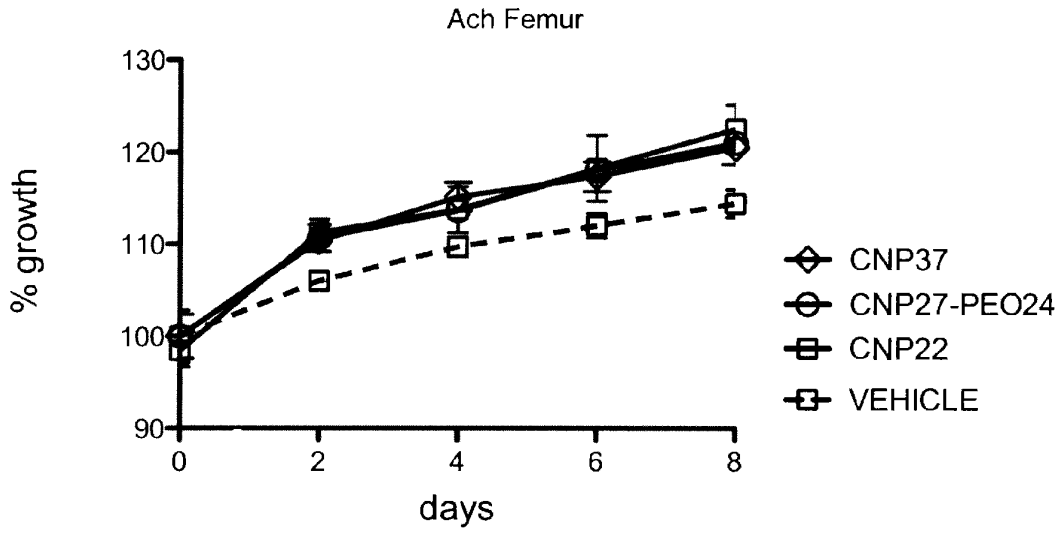

Ach Femur
% growth
130
120
110
100
90
0
2
4
6
8
days
CNP37
CNP27-PEO24
CNP22
VEHICLE

FIGURE 34

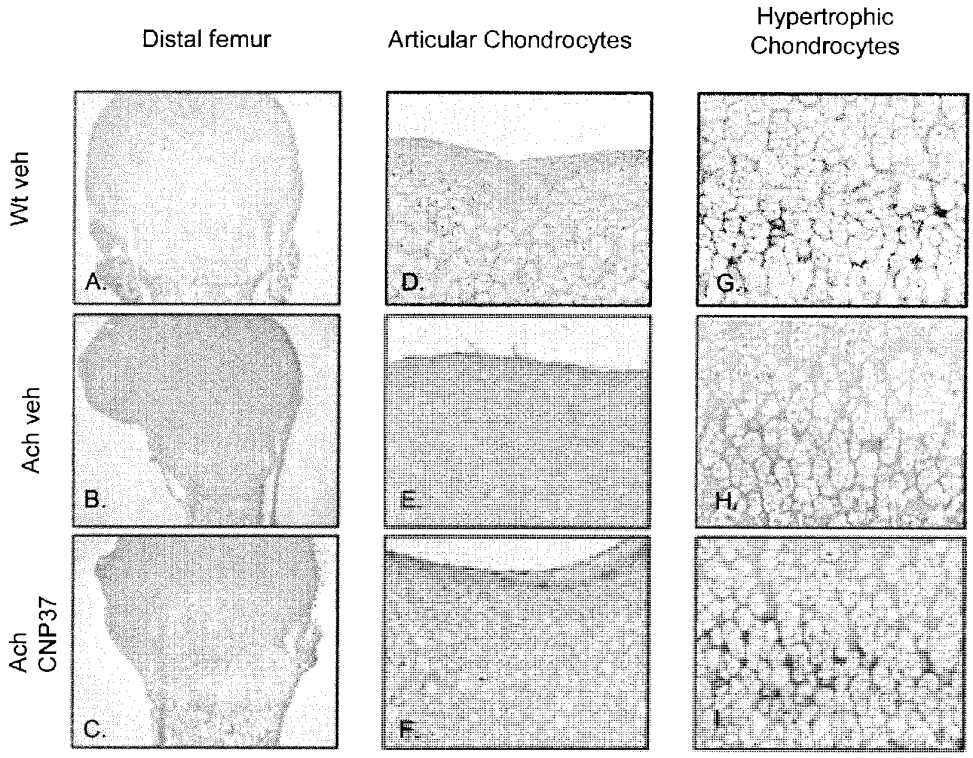

Distal femur
Articular Chondrocytes
Hypertrophic Chondrocytes
Wt veh
Ach veh
Ach CNP37
A.
B.
C.
D.
E.
F.
G.
H.
I.

FIGURE 35

Cellularity of Proliferating Columns

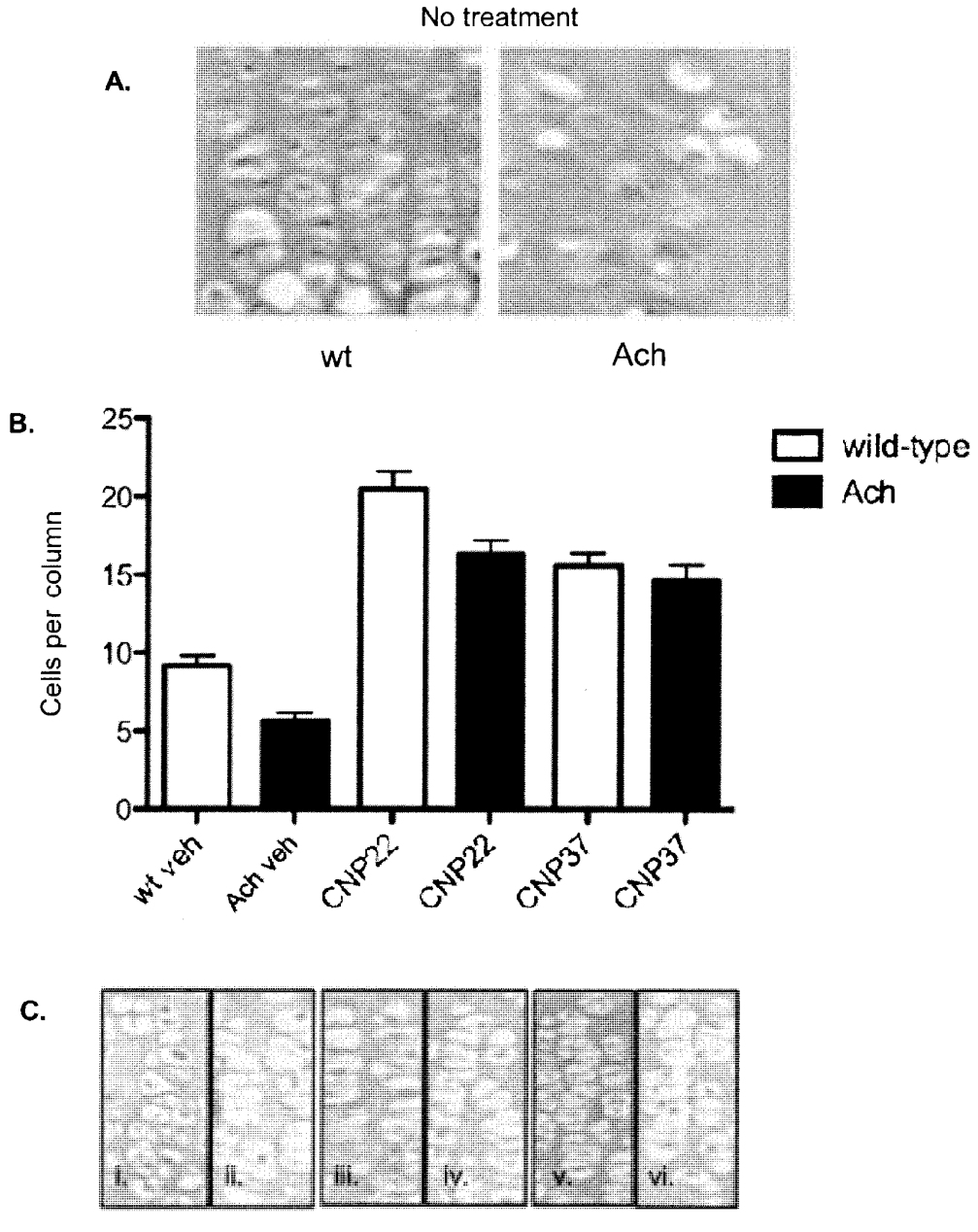


FIGURE 36

Chondrocyte Hypertrophy

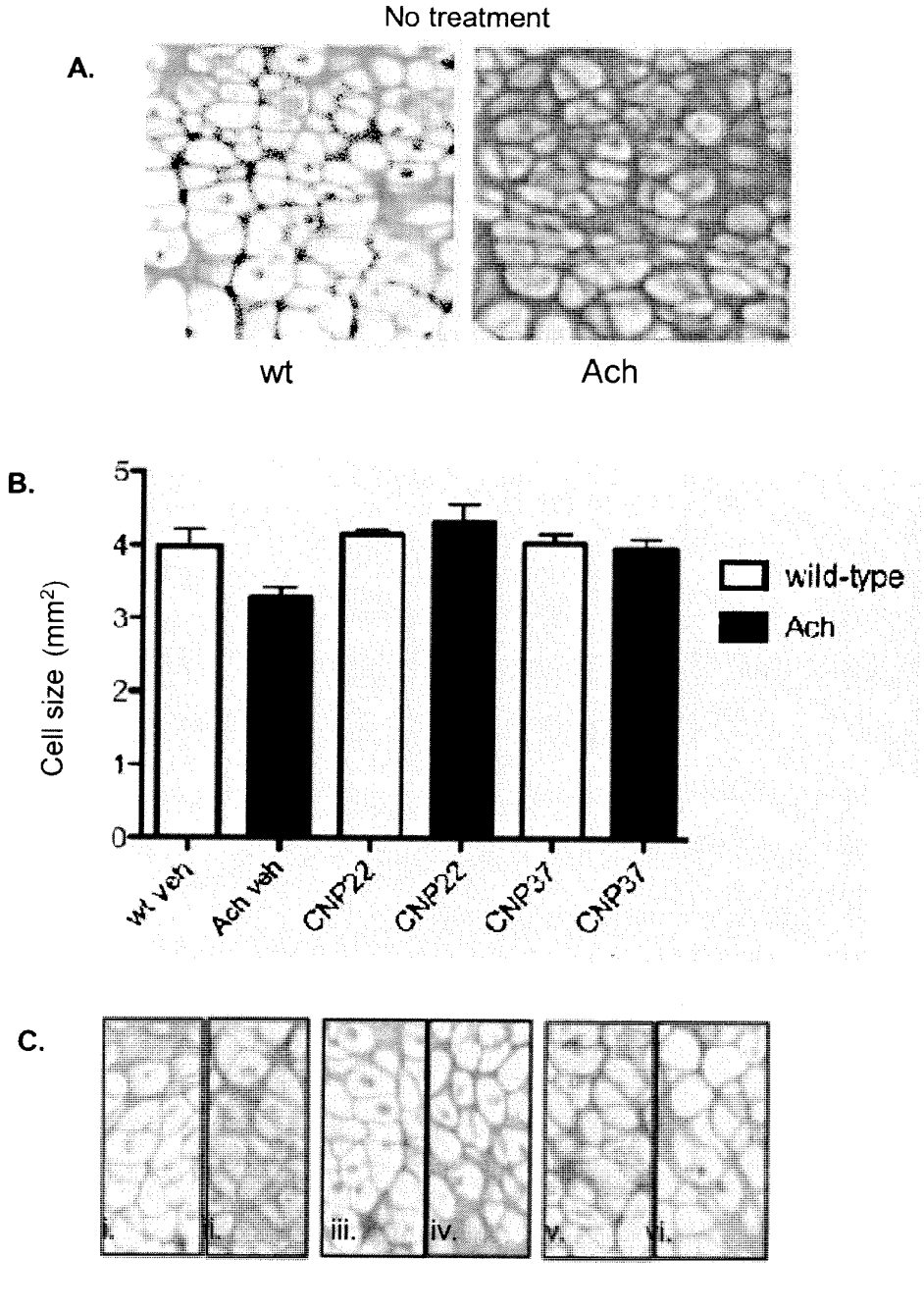


FIGURE 37

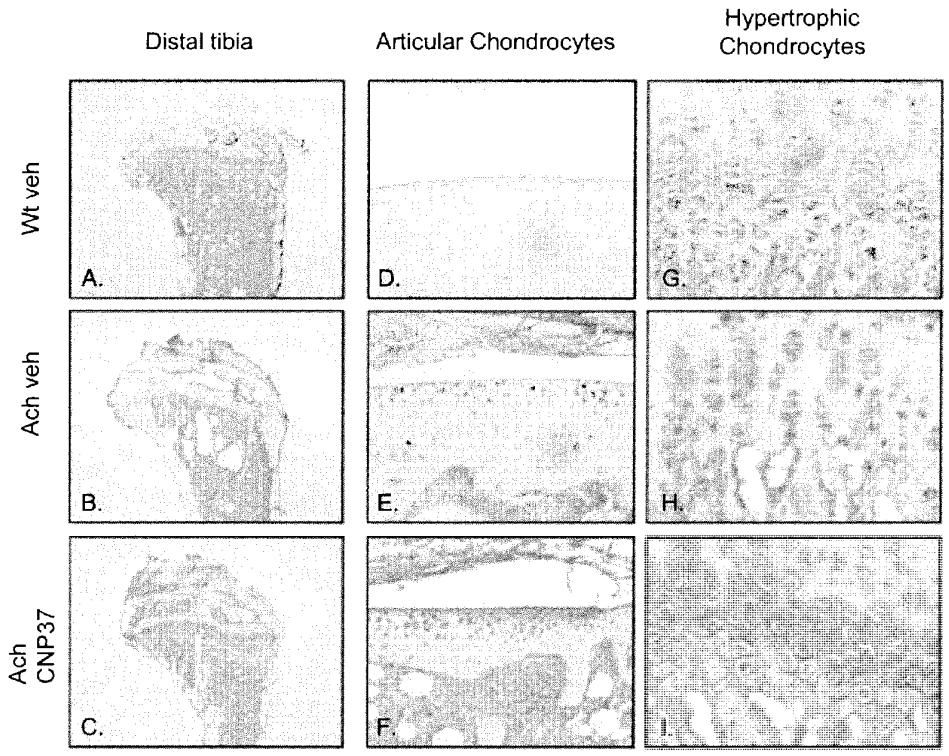
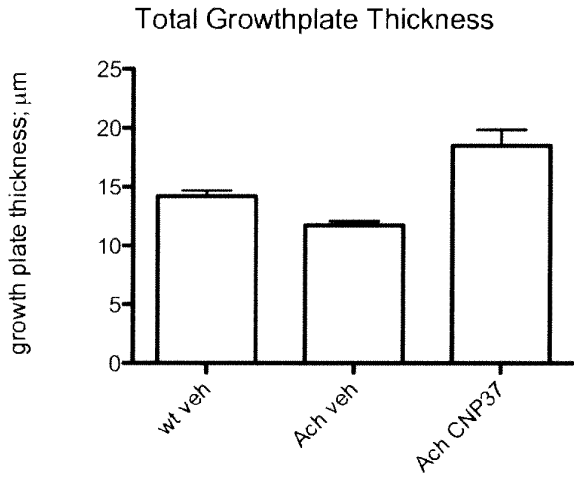
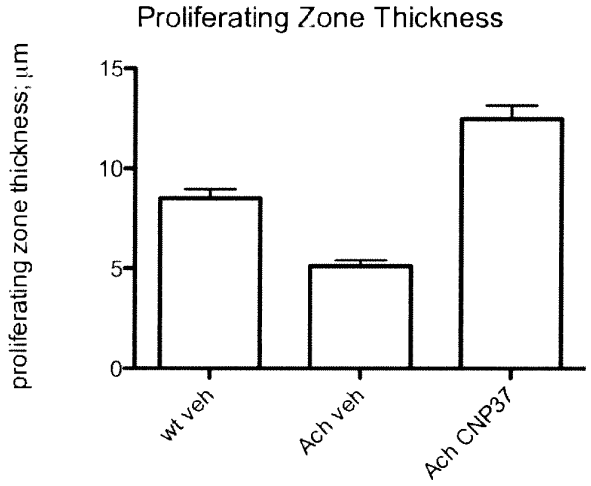

Distal tibia
Articular Chondrocytes
Hypertrophic Chondrocytes
Wt veh
Ach veh
Ach CNP37
A.
B.
C.
D.
E.
F.
G.
H.
I.

FIGURE 38

A.



B.



C.

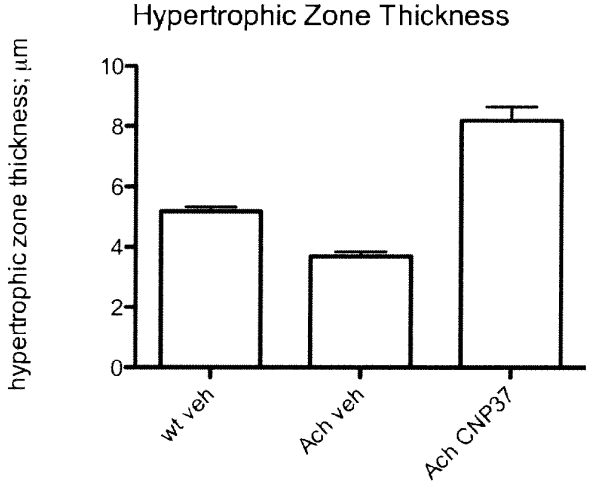


FIGURE 39

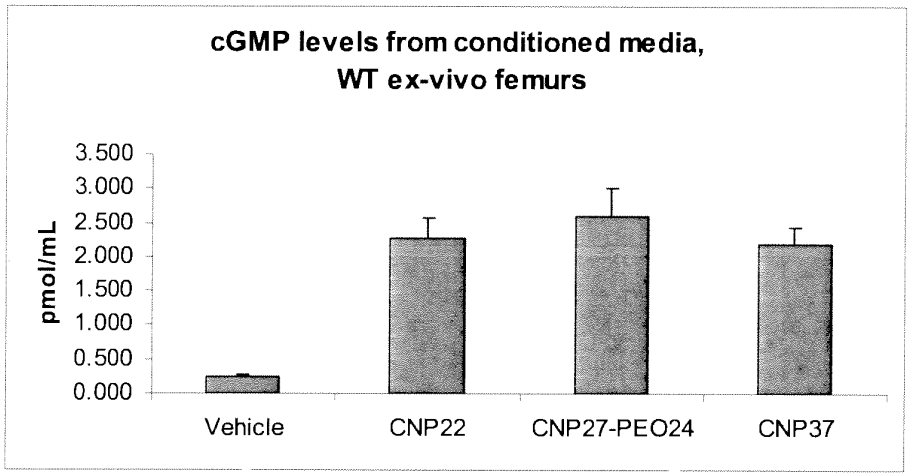


FIGURE 40

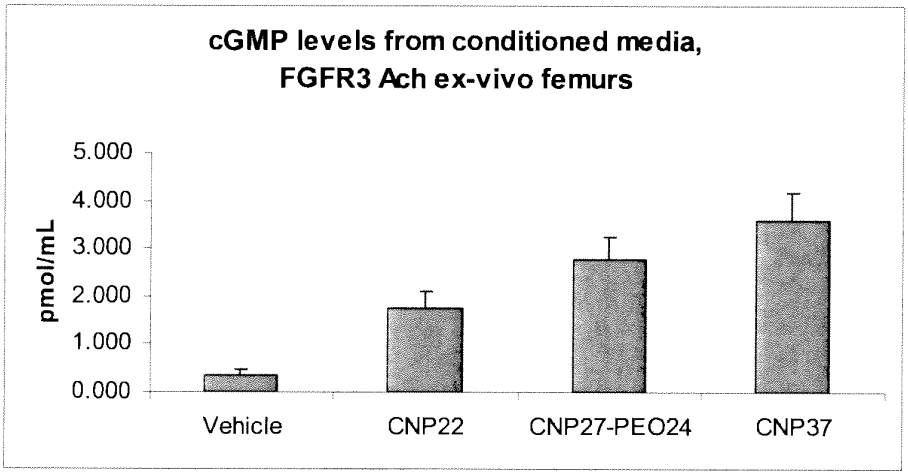


FIGURE 41

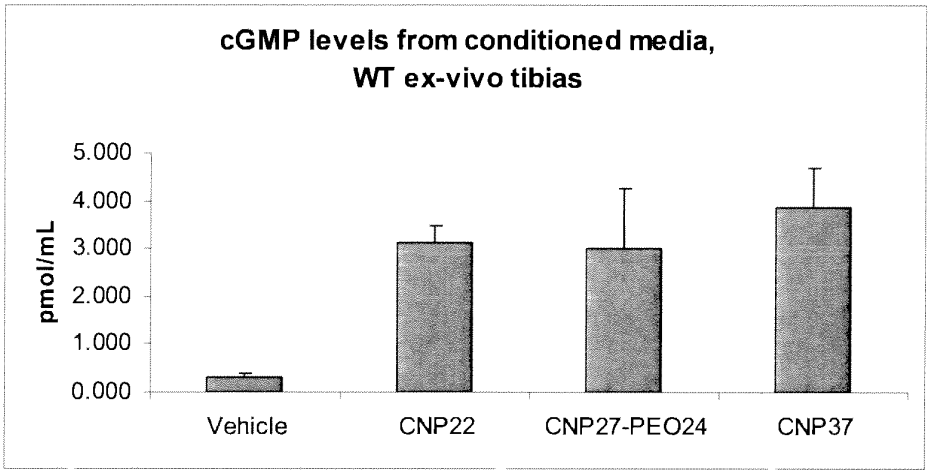


FIGURE 42

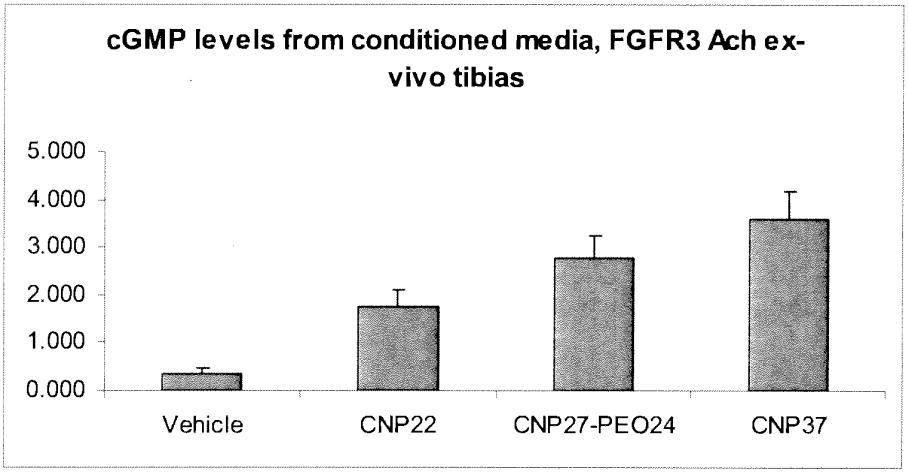


FIGURE 43

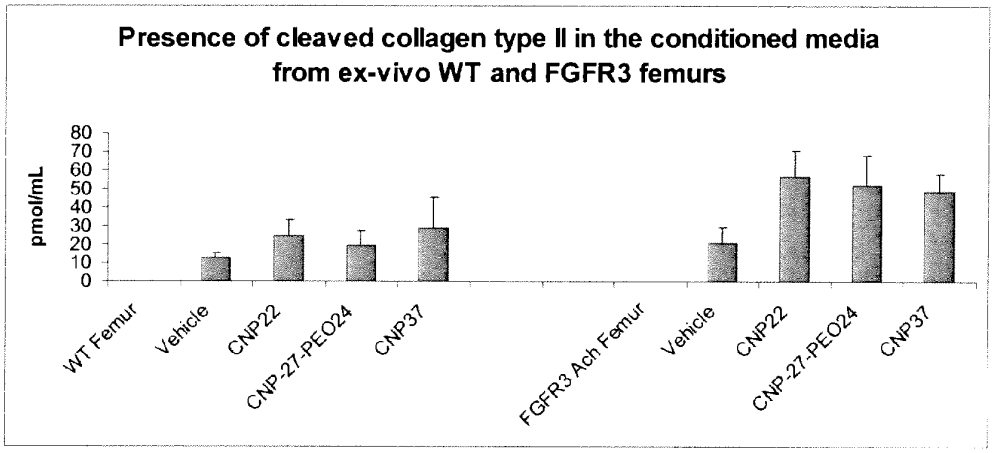


FIGURE 44

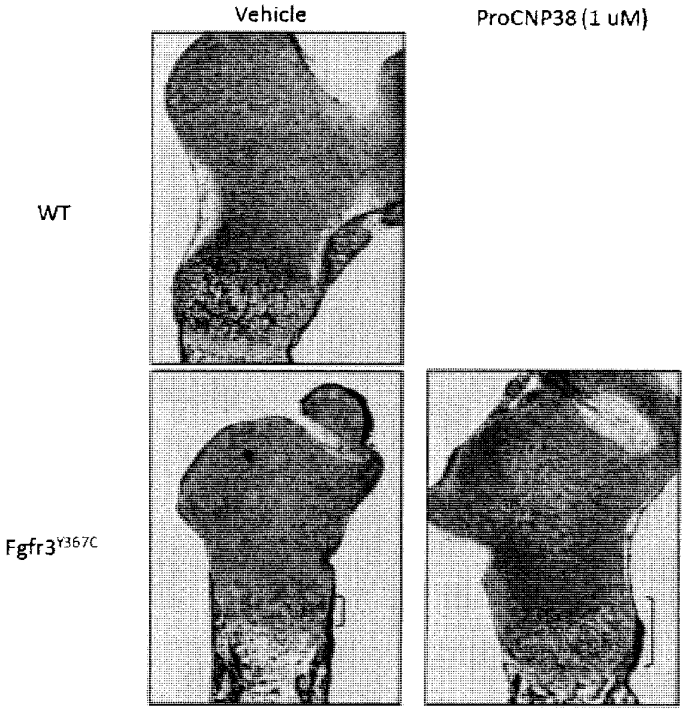


FIGURE 45

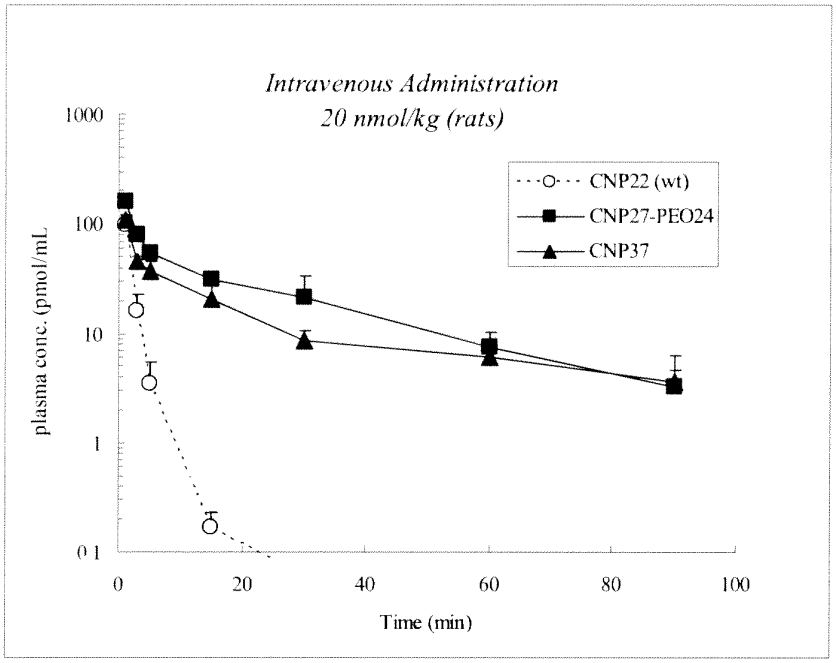


FIGURE 46

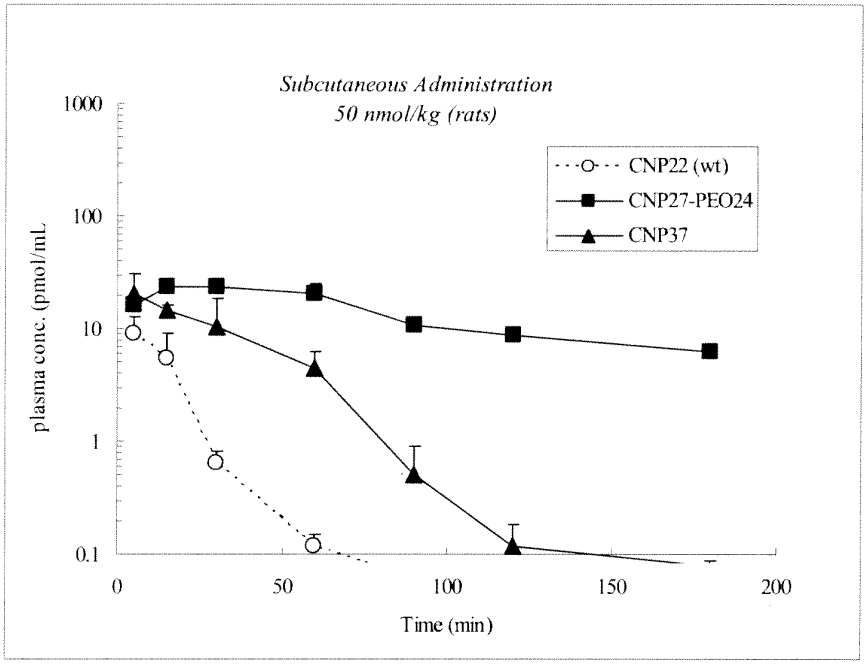


FIGURE 47

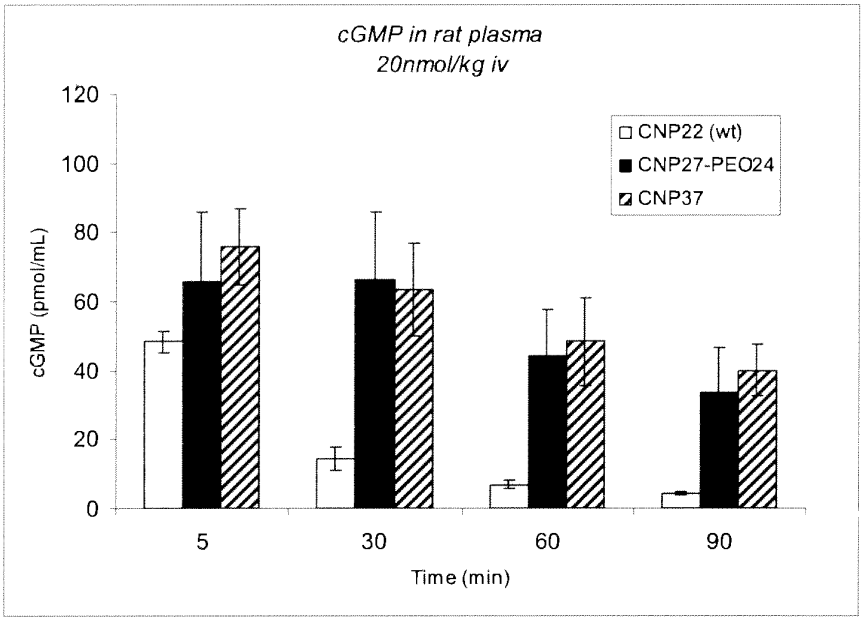


FIGURE 48

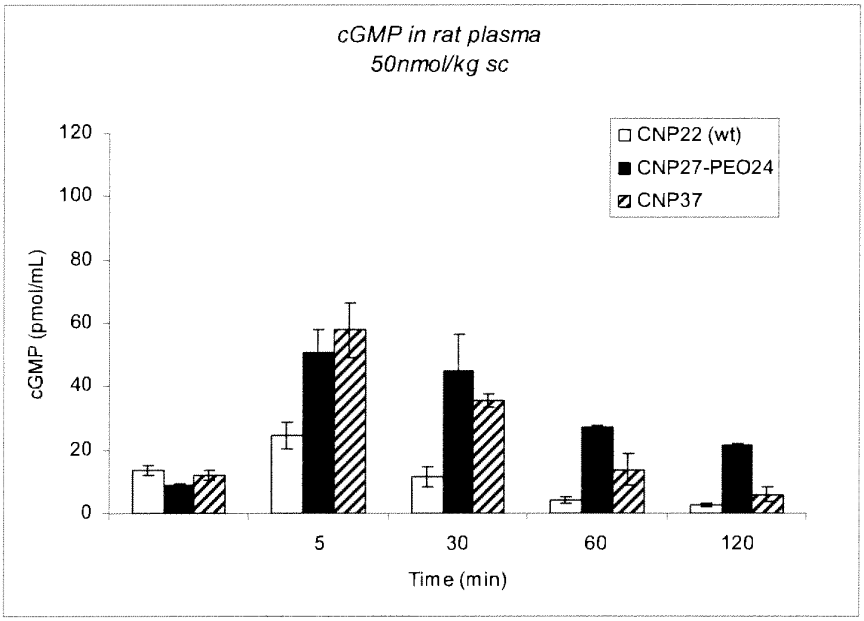


FIGURE 49

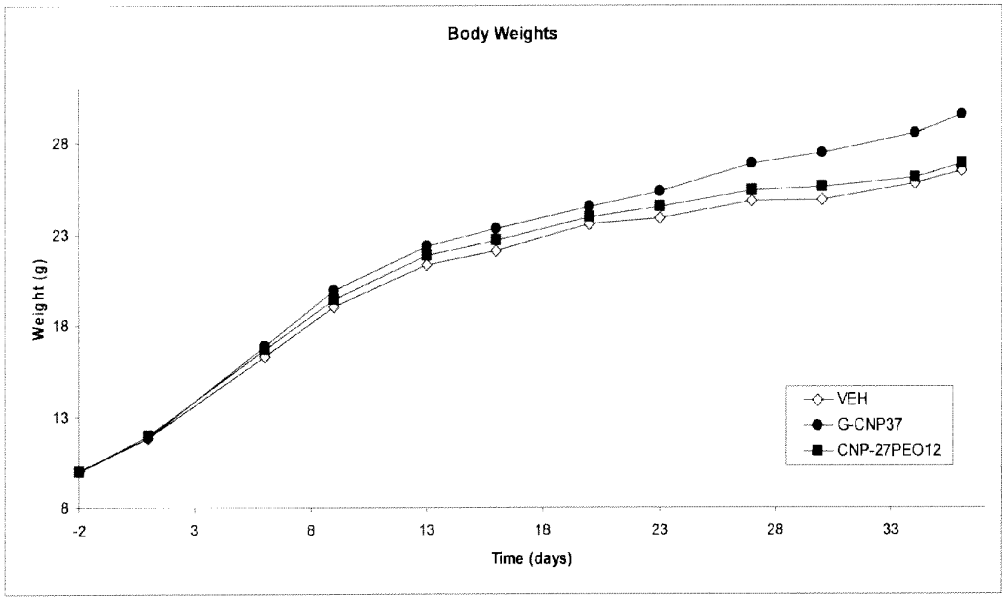


FIGURE 50

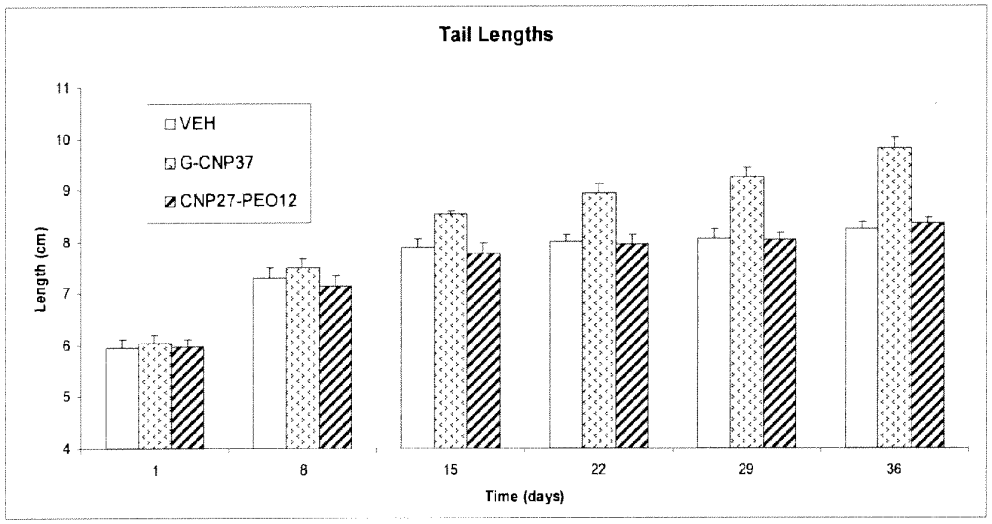


FIGURE 51

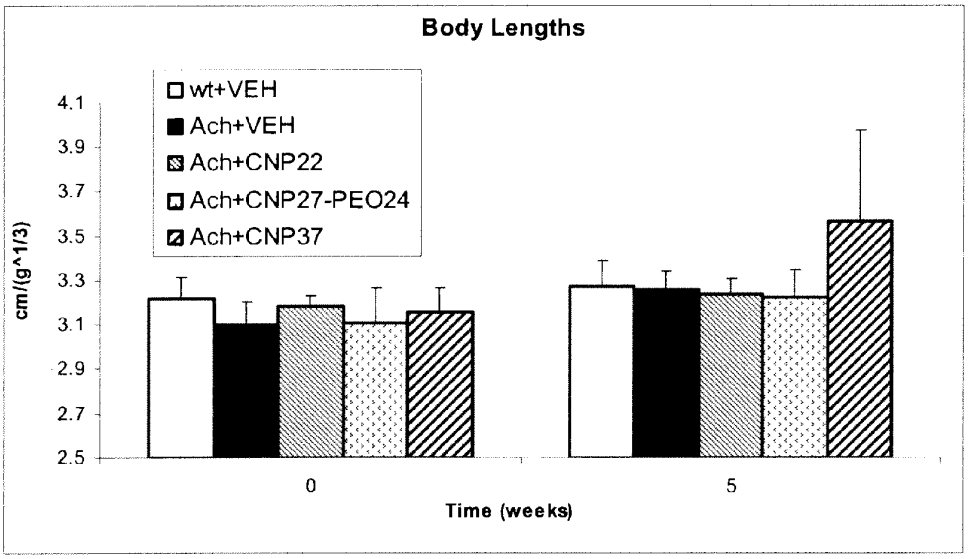


FIGURE 52

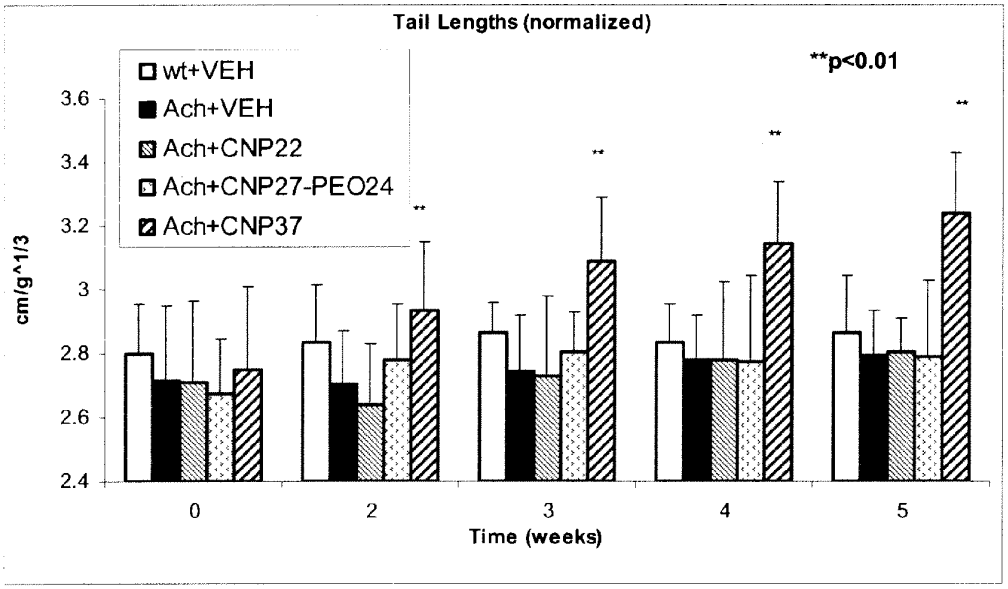
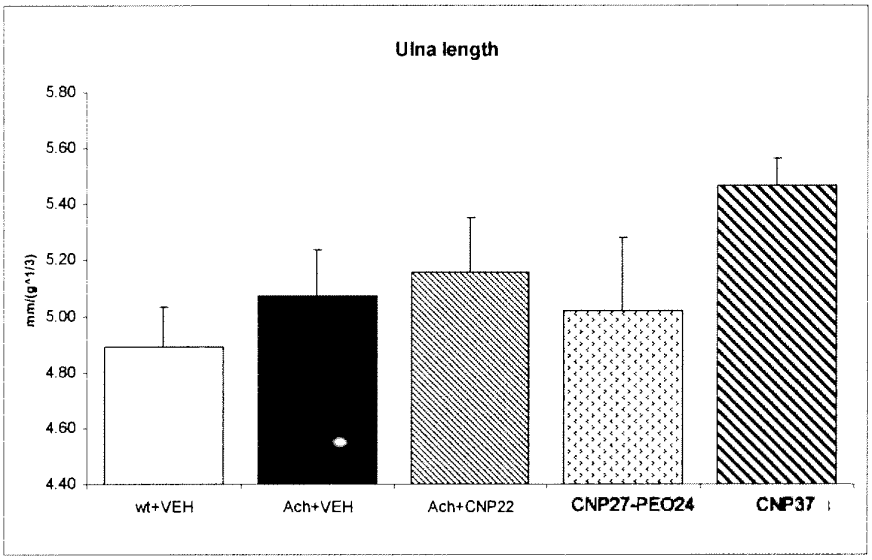


FIGURE 53

A.


Ulna length
mm/(g^1/3)
5.80
5.60
5.40
5.20
5.00
4.80
4.60
4.40
wt+VEH
Ach+VEH
Ach+CNP22
CNP27-PEO24
CNP37

B.

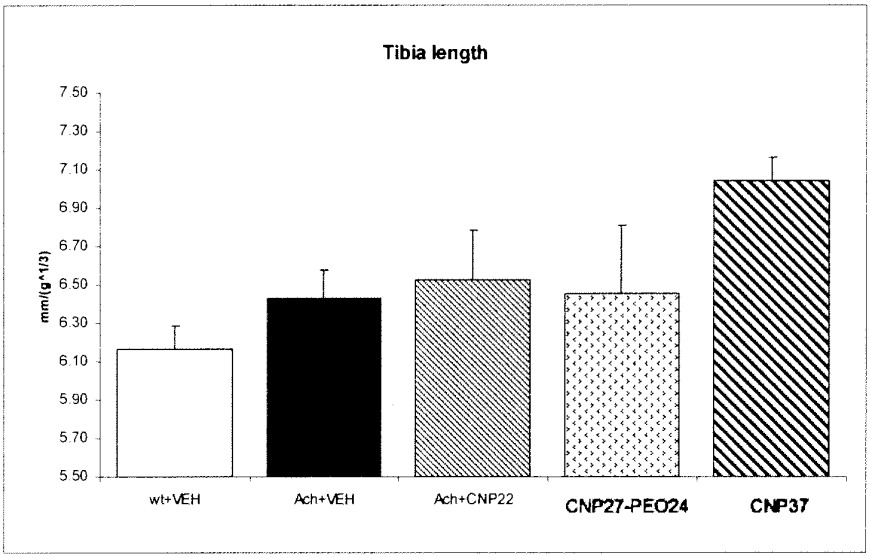
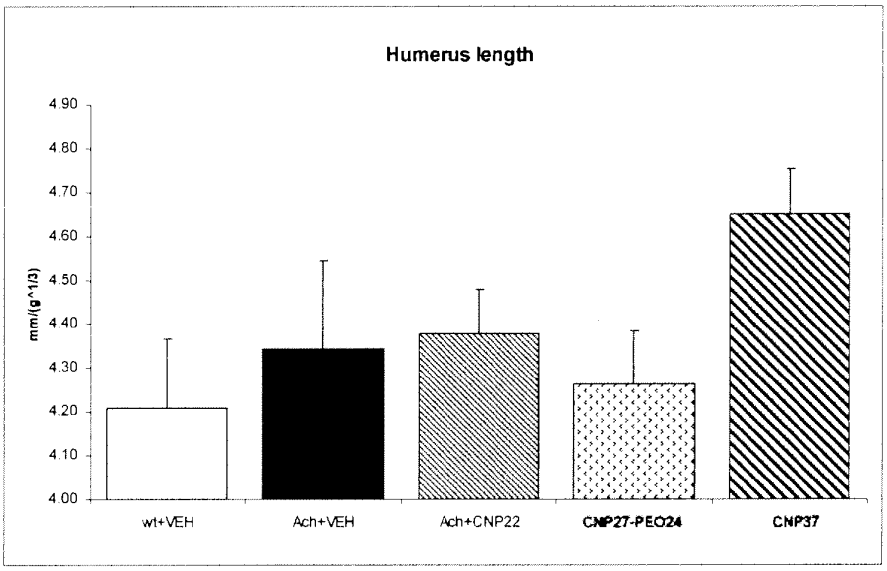

Tibia length
mm/(g^1/3)
7.50
7.30
7.10
6.90
6.70
6.50
6.30
6.10
5.90
5.70
5.50
wt+VEH
Ach+VEH
Ach+CNP22
CNP27-PEO24
CNP37

FIGURE 54

A.


Humerus length
mm/(g^1/3)
4.90
4.80
4.70
4.60
4.50
4.40
4.30
4.20
4.10
4.00
wt+VEH
Ach+VEH
Ach+CNP22
CNP27-PEO24
CNP37

B.

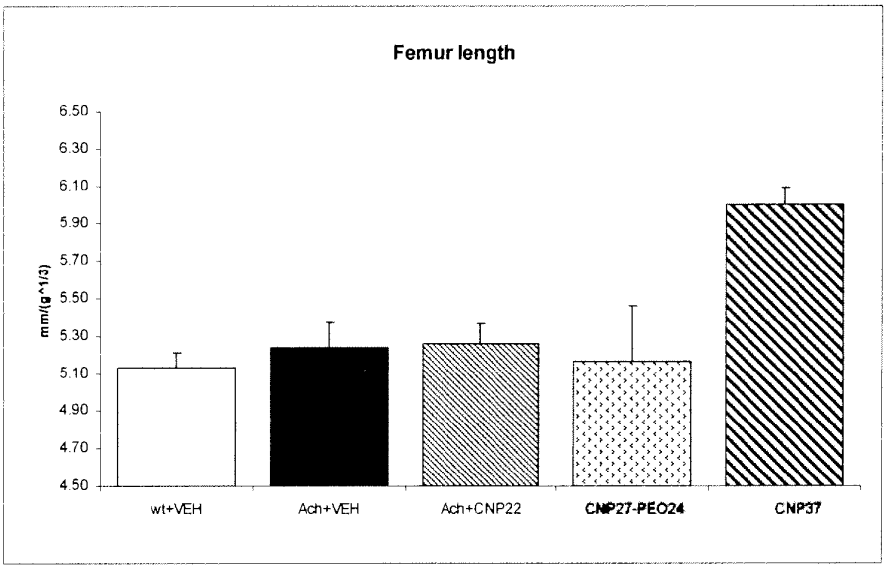

Femur length
mm/(g^1/3)
6.50
6.30
6.10
5.90
5.70
5.50
5.30
5.10
4.90
4.70
4.50
wt+VEH
Ach+VEH
Ach+CNP22
CNP27-PEO24
CNP37

FIGURE 55

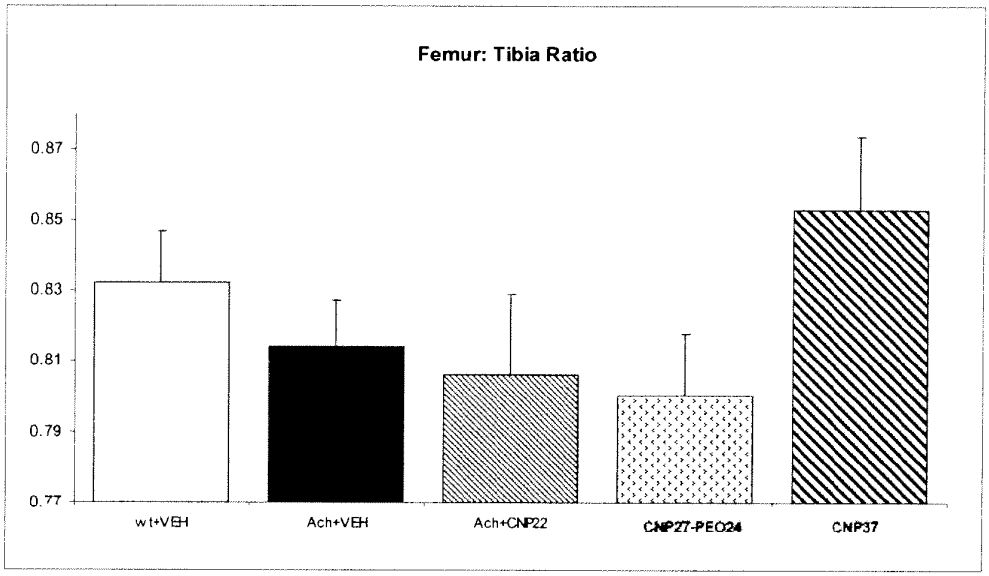


FIGURE 56

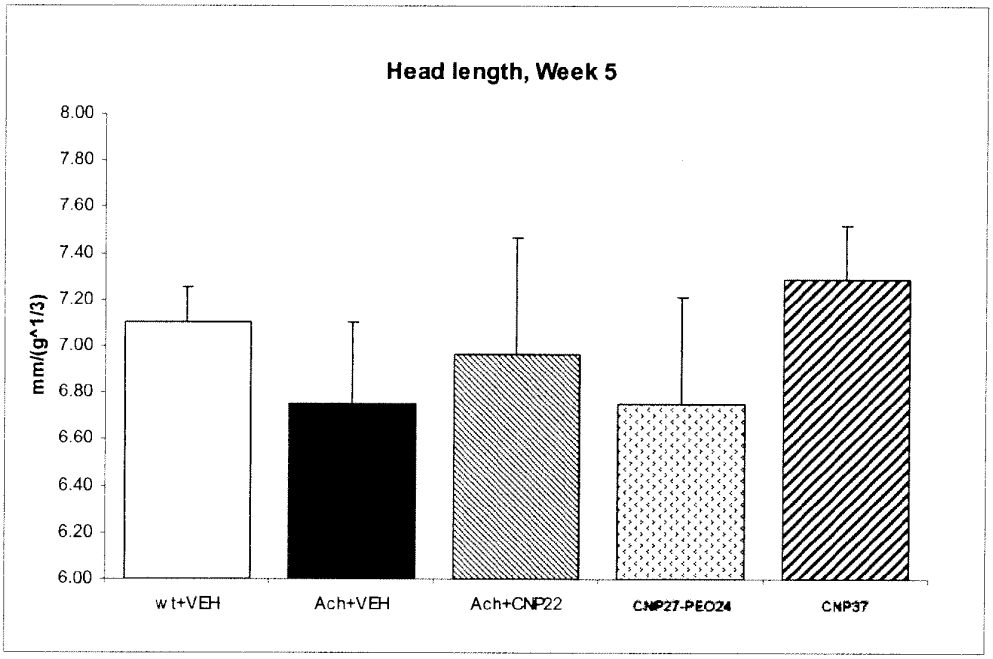


FIGURE 57

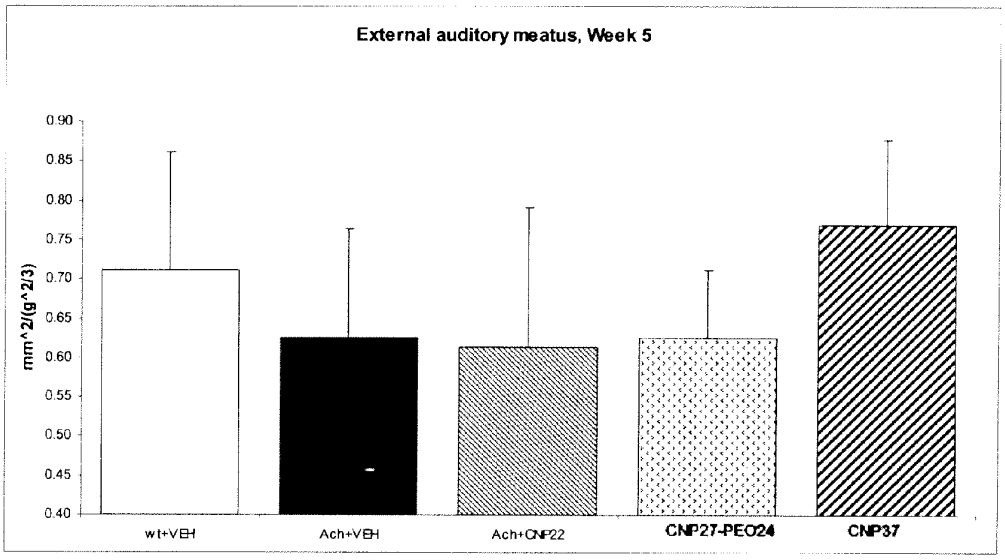


FIGURE 58

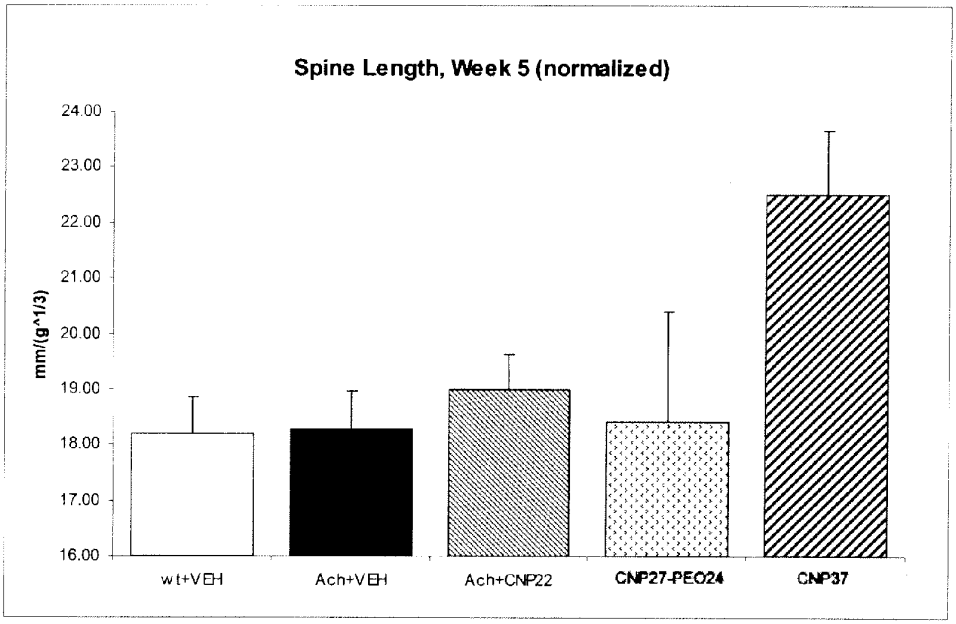


FIGURE 59

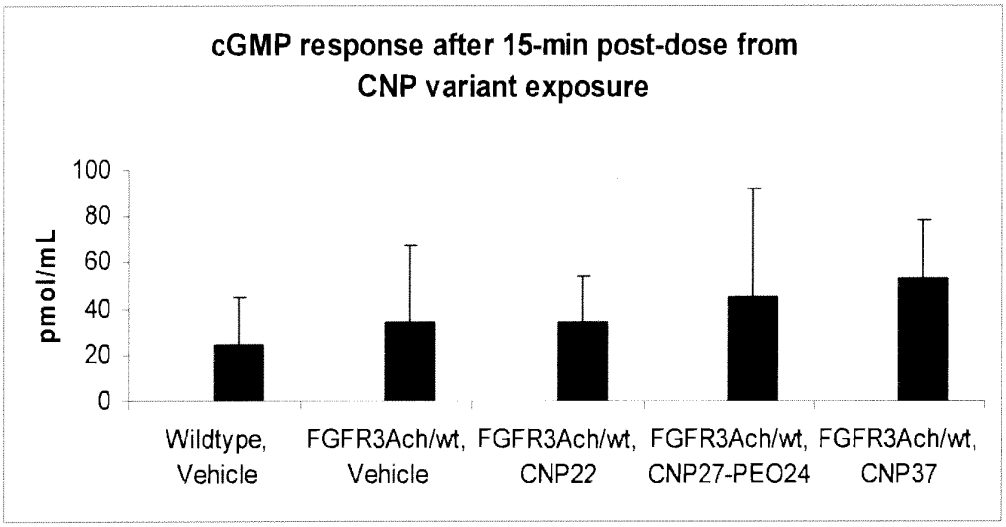


FIGURE 60

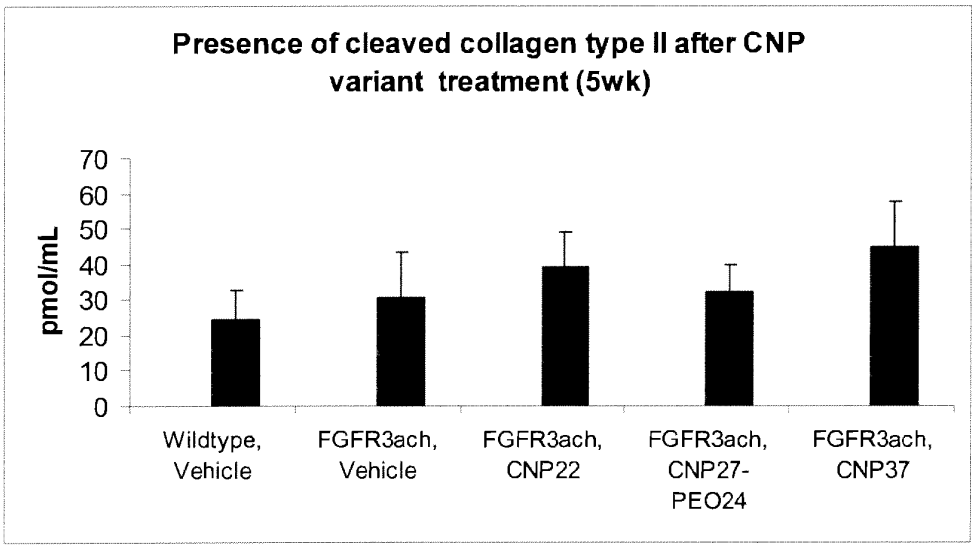


FIGURE 61

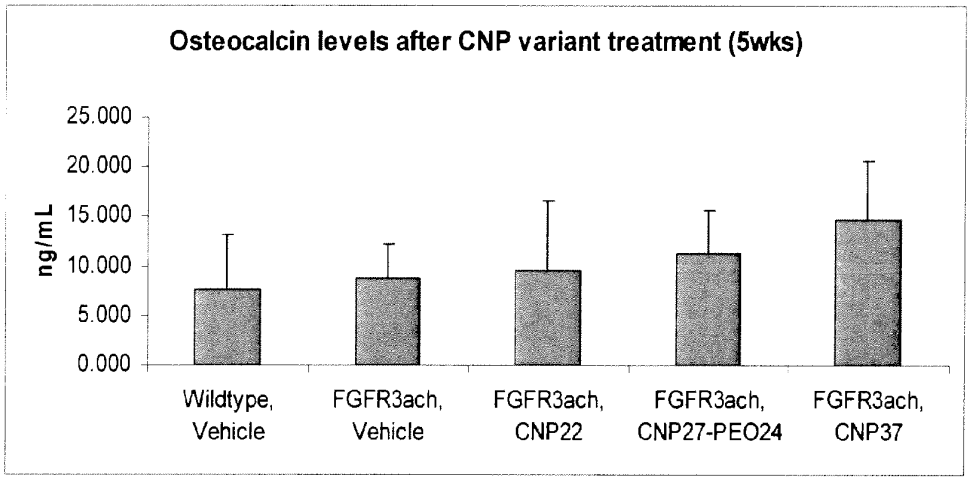


FIGURE 62

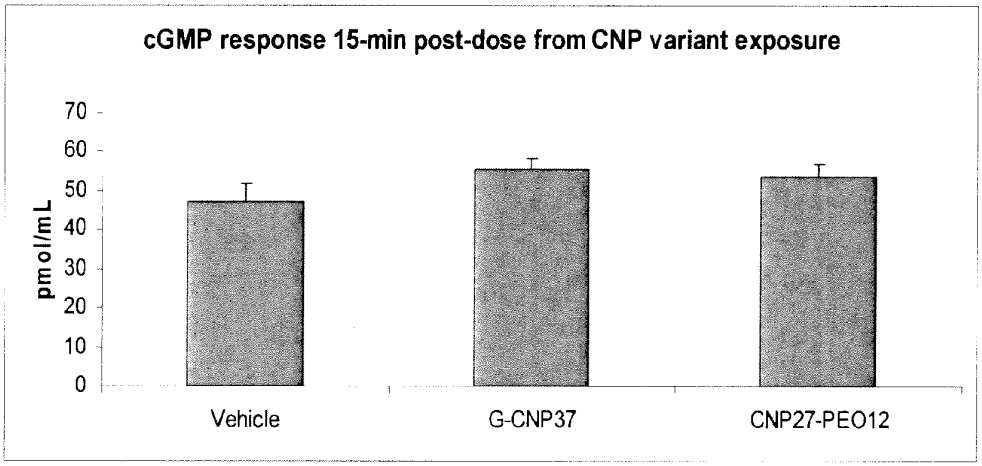


FIGURE 63

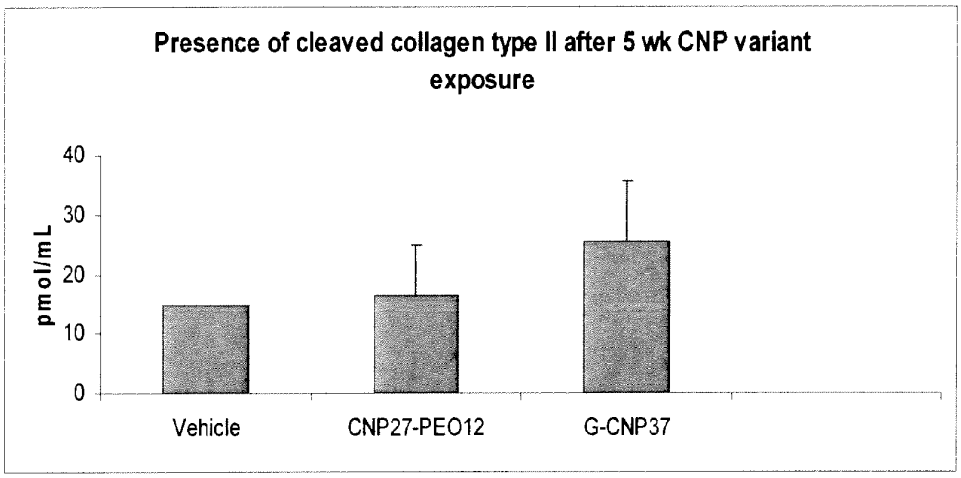
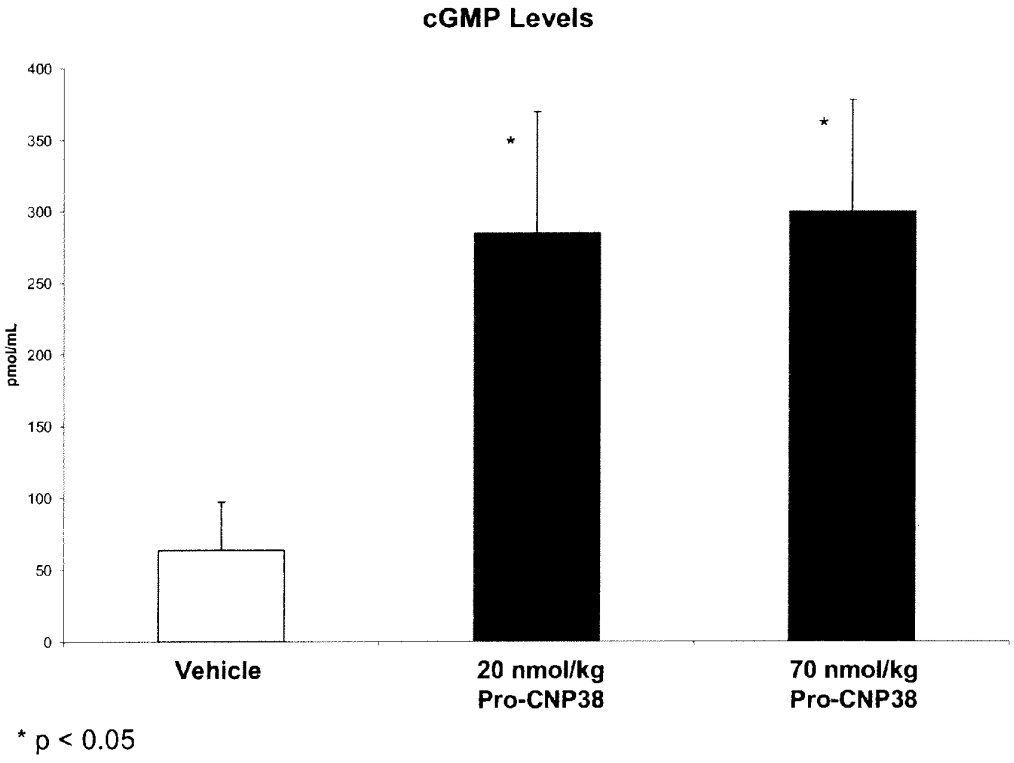


FIGURE 64



* $p < 0.05$

FIGURE 65

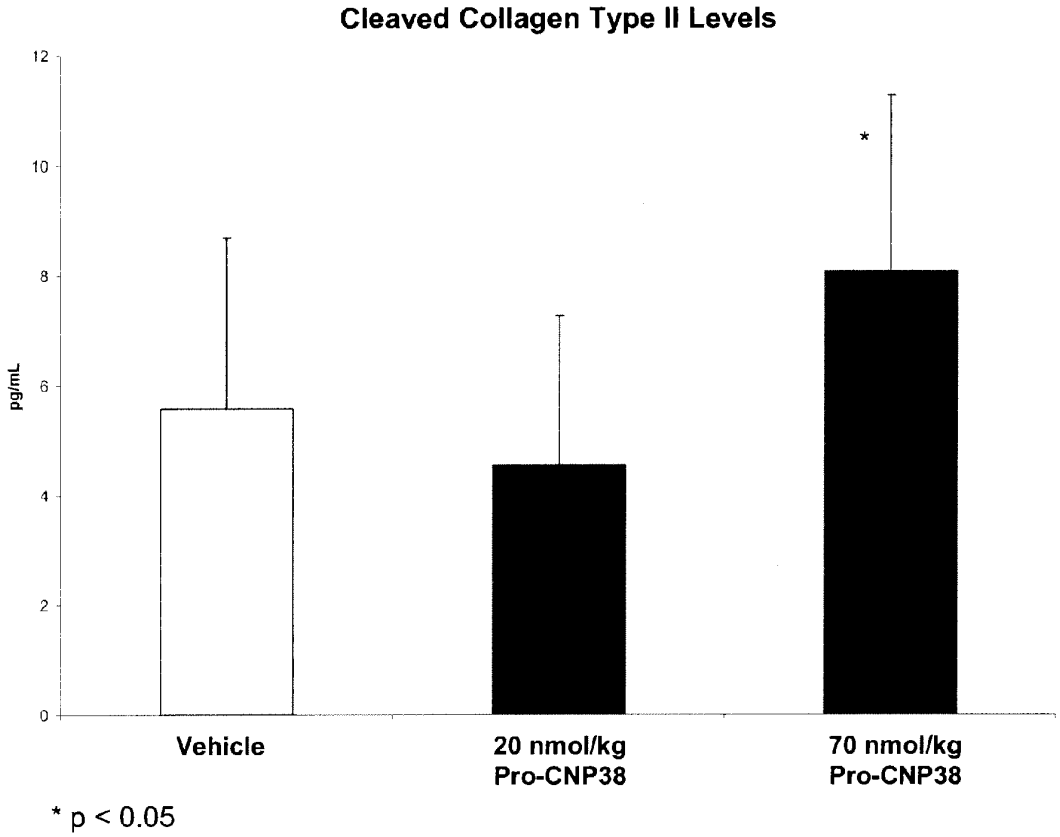

Cleaved Collagen Type II Levels
pg/mL
12
10
8
6
4
2
0
*
Vehicle
20 nmol/kg Pro-CNP38
70 nmol/kg Pro-CNP38
* p < 0.05

FIGURE 66

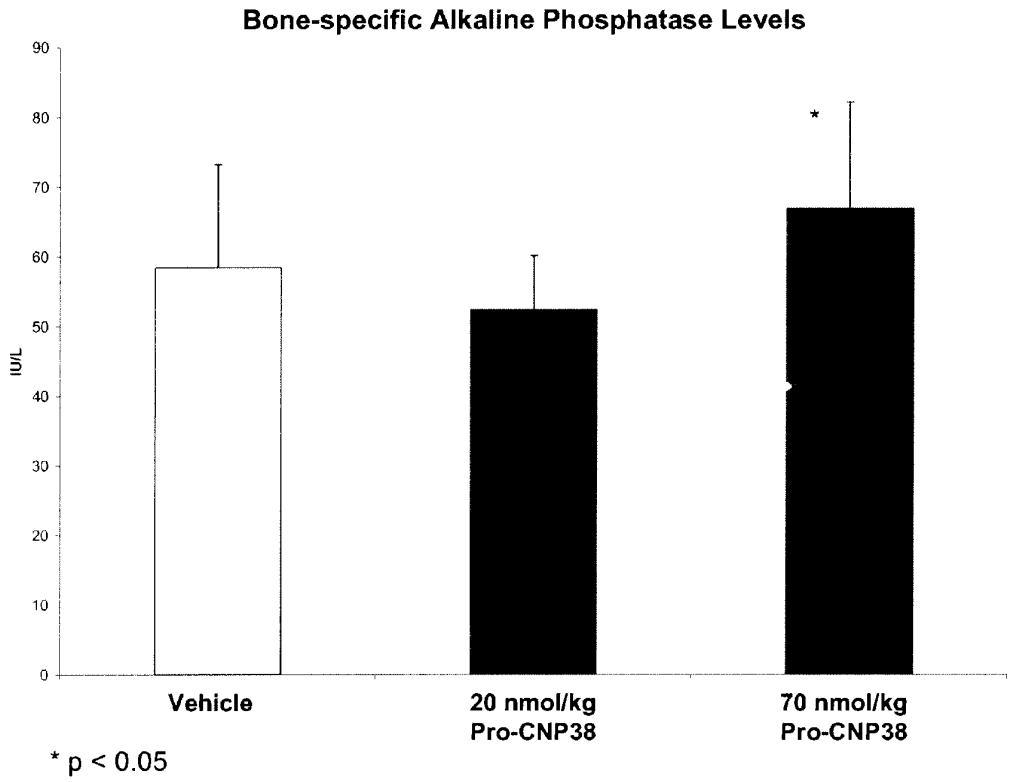

Bone-specific Alkaline Phosphatase Levels
IU/L
90
80
70
60
50
40
30
20
10
0
*
Vehicle
20 nmol/kg Pro-CNP38
70 nmol/kg Pro-CNP38
* p < 0.05

FIGURE 67

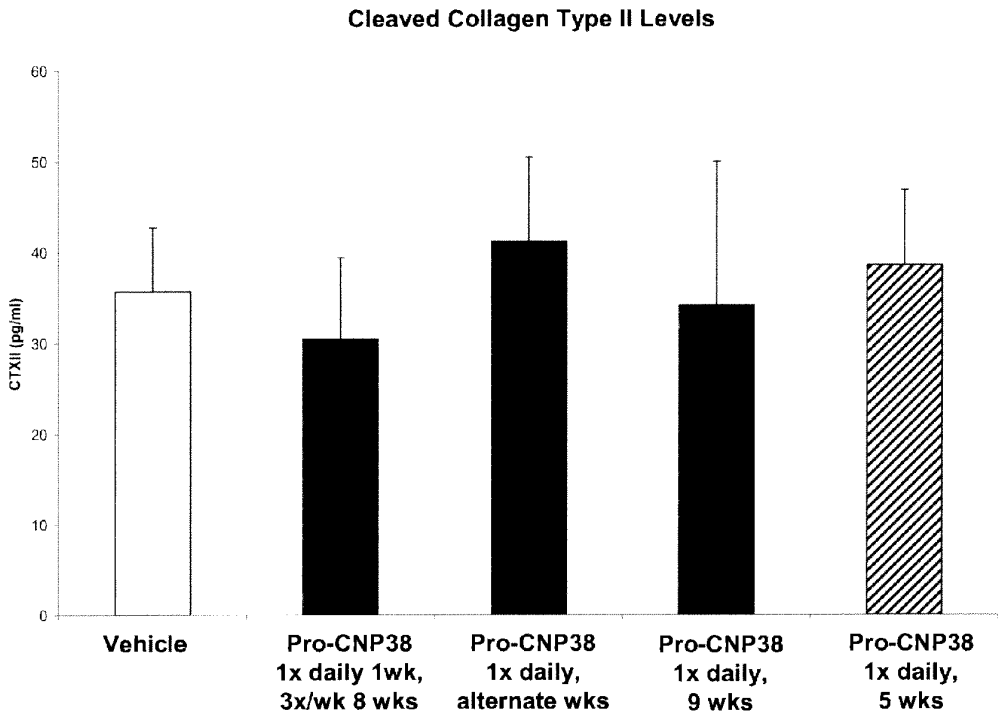

Cleaved Collagen Type II Levels
CTXII (pg/ml)
60
50
40
30
20
10
0
Vehicle
Pro-CNP38 1x daily 1wk, 3x/wk 8 wks
Pro-CNP38 1x daily, alternate wks
Pro-CNP38 1x daily, 9 wks
Pro-CNP38 1x daily, 5 wks

FIGURE 68

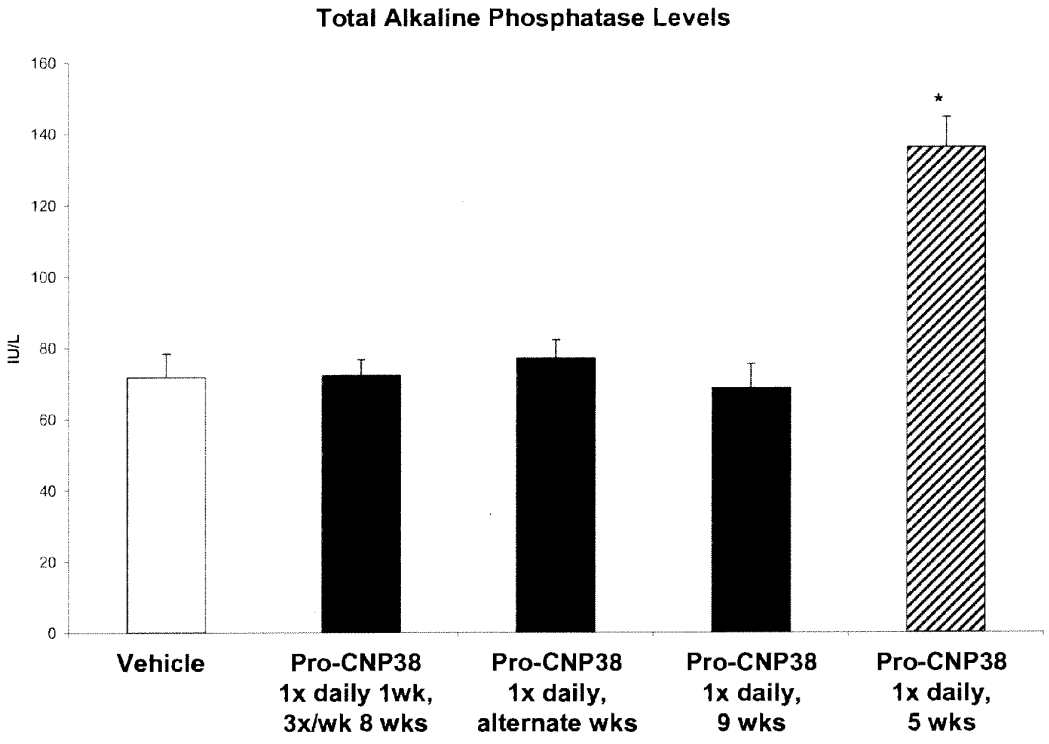

Total Alkaline Phosphatase Levels
IU/L
160
140
120
100
80
60
40
20
0
*
Vehicle
Pro-CNP38 1x daily 1wk, 3x/wk 8 wks
Pro-CNP38 1x daily, alternate wks
Pro-CNP38 1x daily, 9 wks
Pro-CNP38 1x daily, 5 wks
* p < 0.001

FIGURE 69

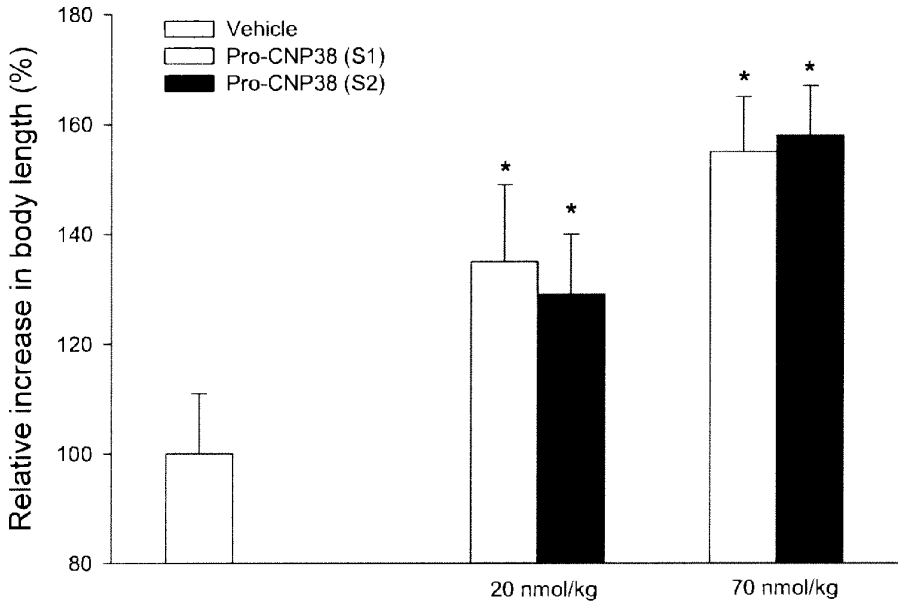
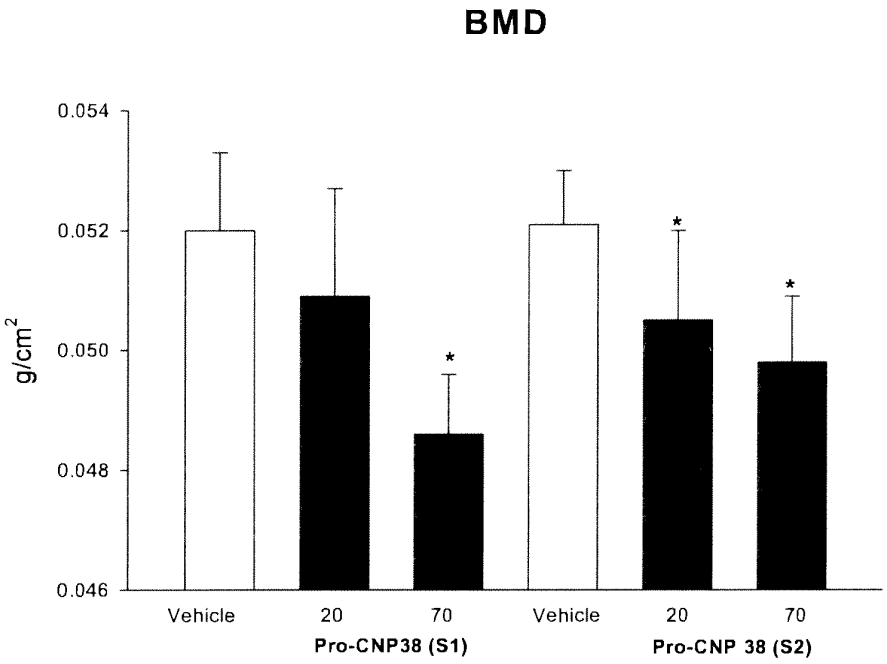

Vehicle
Pro-CNP38 (S1)
Pro-CNP38 (S2)
Relative increase in body length (%)
180
160
140
120
100
80
*
*
*
*
20 nmol/kg
70 nmol/kg

FIGURE 70

A.


BMD
0.054
0.052
0.050
0.048
0.046
g/cm2
*
*
*
Vehicle
20
70
Vehicle
20
70
Pro-CNP38 (S1)
Pro-CNP 38 (S2)

B.

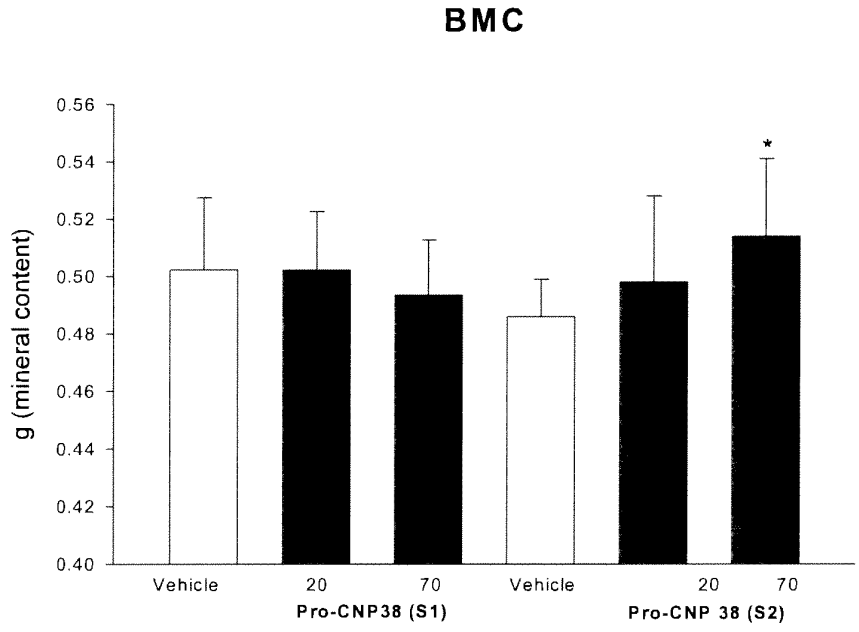

BMC
0.56
0.54
0.52
0.50
0.48
0.46
0.44
0.42
0.40
g (mineral content)
*
Vehicle
20
70
Vehicle
20
70
Pro-CNP38 (S1)
Pro-CNP 38 (S2)

FIGURE 71

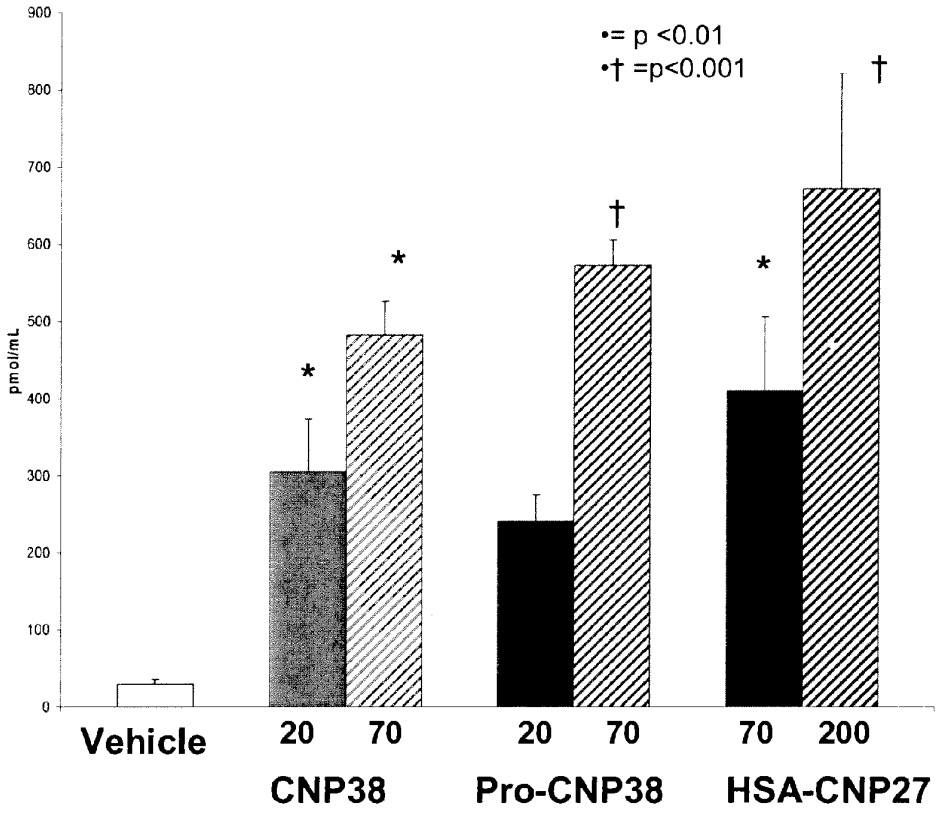

•= p <0.01
•† =p<0.001
pmol/mL
900
800
700
600
500
400
300
200
100
0
*
*
*
†
†
Vehicle
20
70
20
70
70
200
CNP38
Pro-CNP38
HSA-CNP27

FIGURE 72

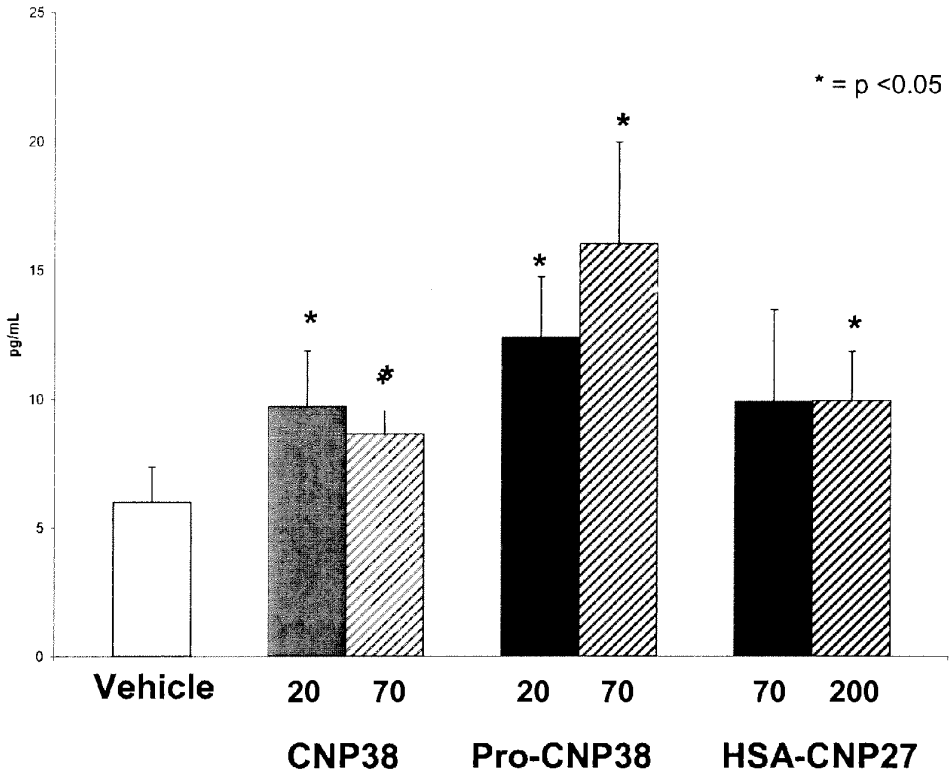

* = p <0.05
pg/mL
25
20
15
10
5
0
Vehicle
20
70
20
70
70
200
CNP38
Pro-CNP38
HSA-CNP27

FIGURE 73

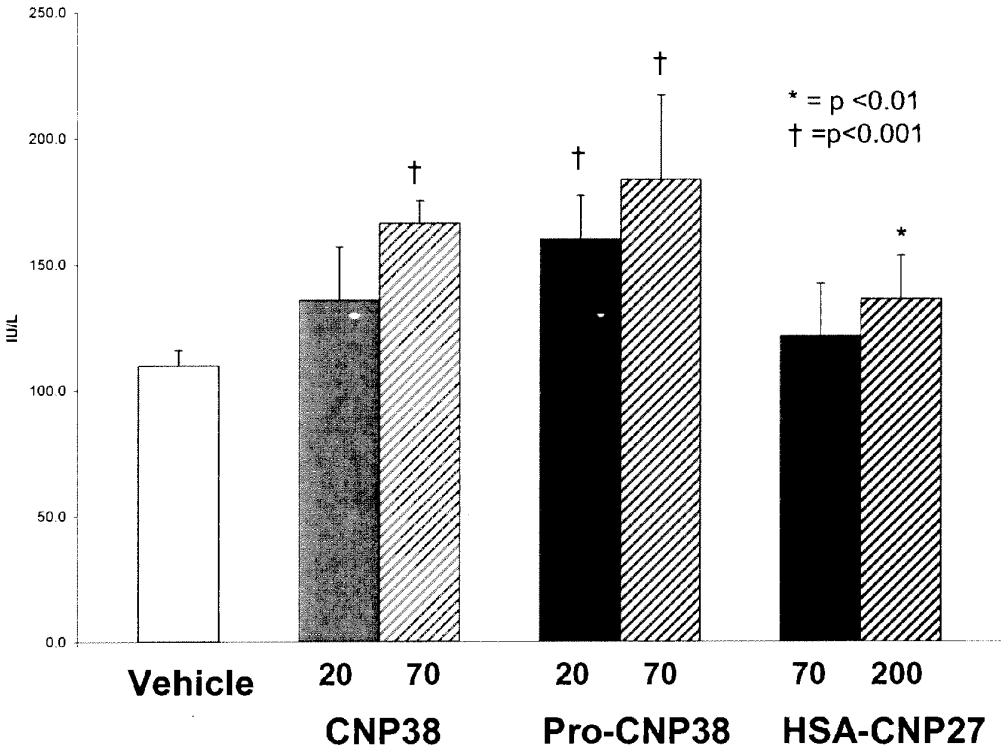
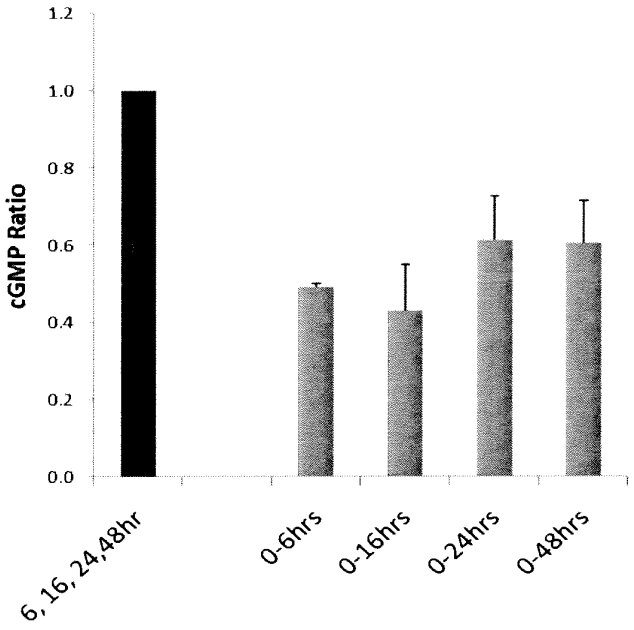

250.0
200.0
150.0
100.0
50.0
0.0
IU/L
* = p <0.01
† =p<0.001
†
†
†
*
Vehicle
20
70
20
70
70
200
CNP38
Pro-CNP38
HSA-CNP27

FIGURE 74

A.


1.2
1.0
0.8
0.6
0.4
0.2
0.0
cGMP Ratio
6, 16, 24, 48hr
0-6hrs
0-16hrs
0-24hrs
0-48hrs

B.

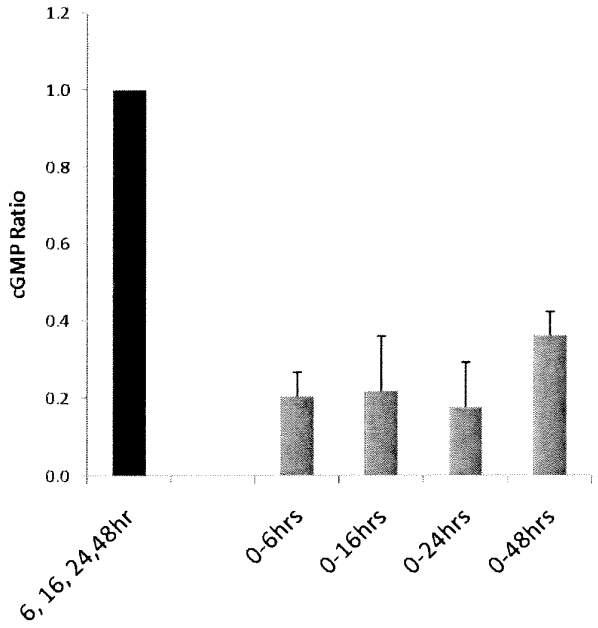

1.2
1.0
0.8
0.6
0.4
0.2
0.0
cGMP Ratio
6, 16, 24, 48hr
0-6hrs
0-16hrs
0-24hrs
0-48hrs

FIGURE 75

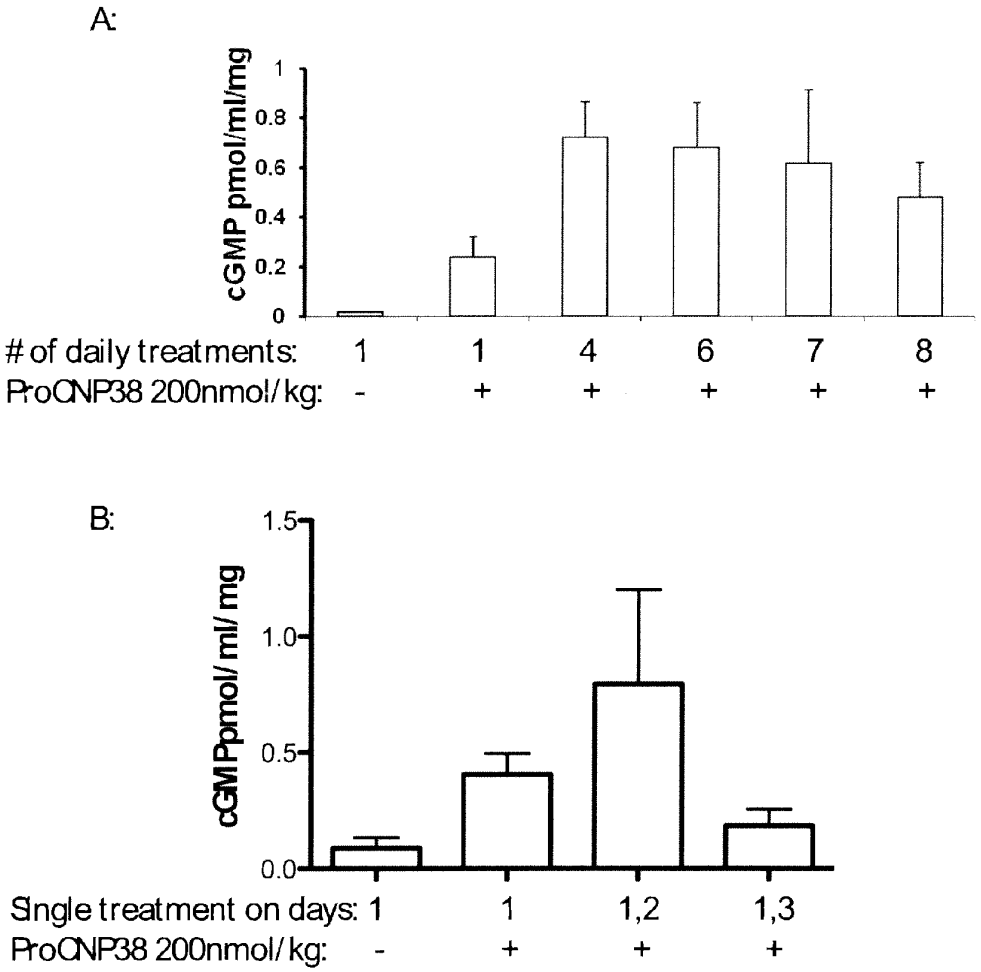

A:
cGMP pmol/ml/mg
1
0.8
0.6
0.4
0.2
0
# of daily treatments: 1 1 4 6 7 8
ProCNP38 200nmol/kg: - + + + + +
B:
cGMPpmol/ml/mg
1.5
1.0
0.5
0.0
Single treatment on days: 1 1 1,2 1,3
ProCNP38 200nmol/kg: - + + +

FIGURE 76

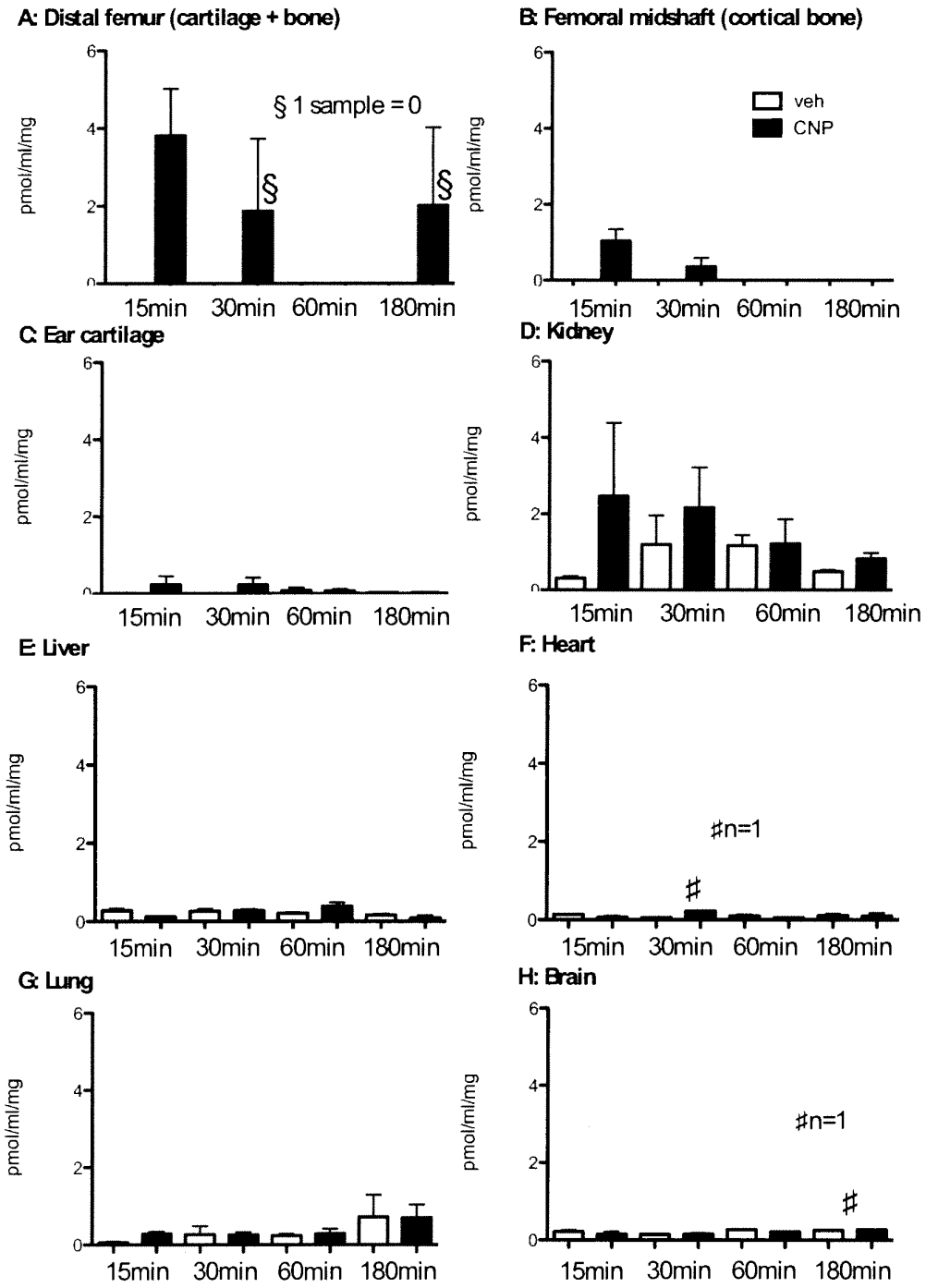

A: Distal femur (cartilage + bone)
§ 1 sample = 0
B: Femoral midshaft (cortical bone)
veh
CNP
C: Ear cartilage
D: Kidney
E: Liver
F: Heart
#n=1
G: Lung
H: Brain
#n=1
pmol/ml/mg
15min 30min 60min 180min

FIGURE 77

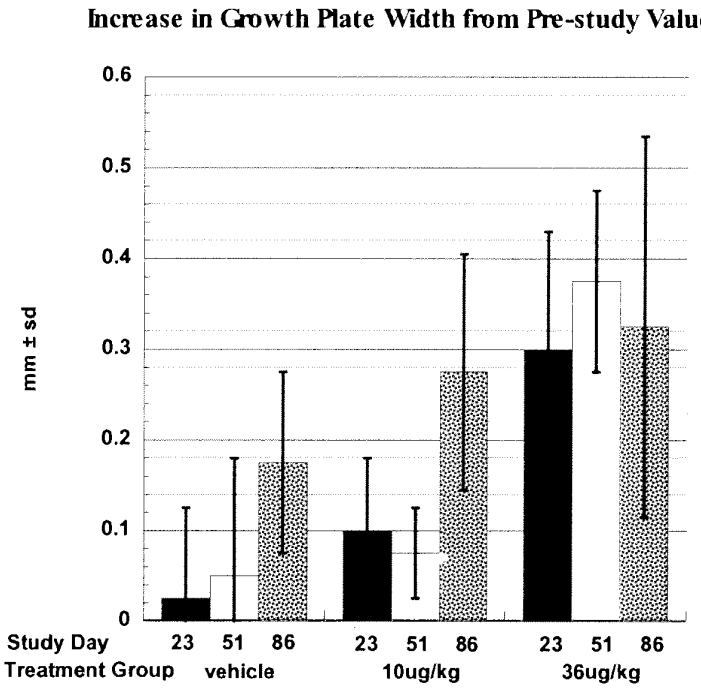


FIGURE 79

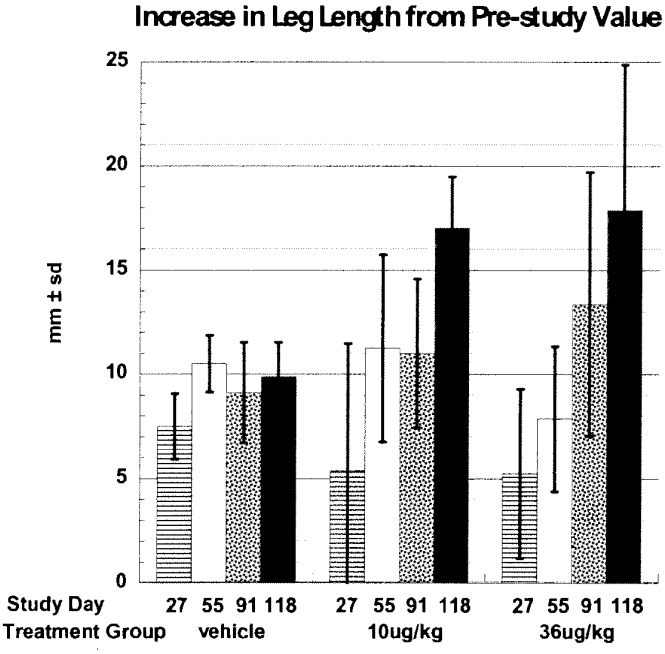
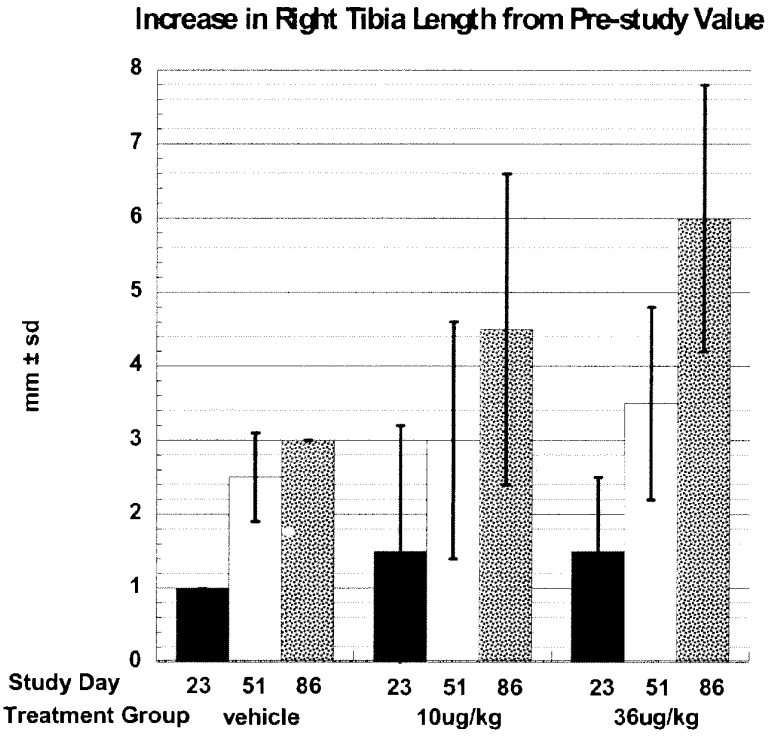


FIGURE 78

A.


Increase in Right Tibia Length from Pre-study Value
mm ± sd
0
1
2
3
4
5
6
7
8
Study Day
23 51 86 23 51 86 23 51 86
Treatment Group
vehicle
10ug/kg
36ug/kg

B.

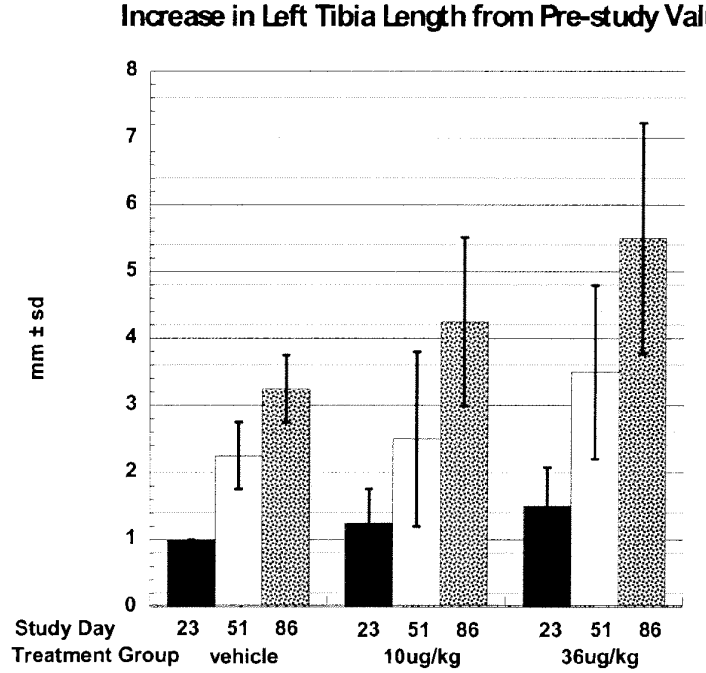

Increase in Left Tibia Length from Pre-study Value
mm ± sd
0
1
2
3
4
5
6
7
8
Study Day
23 51 86 23 51 86 23 51 86
Treatment Group
vehicle
10ug/kg
36ug/kg

FIGURE 80

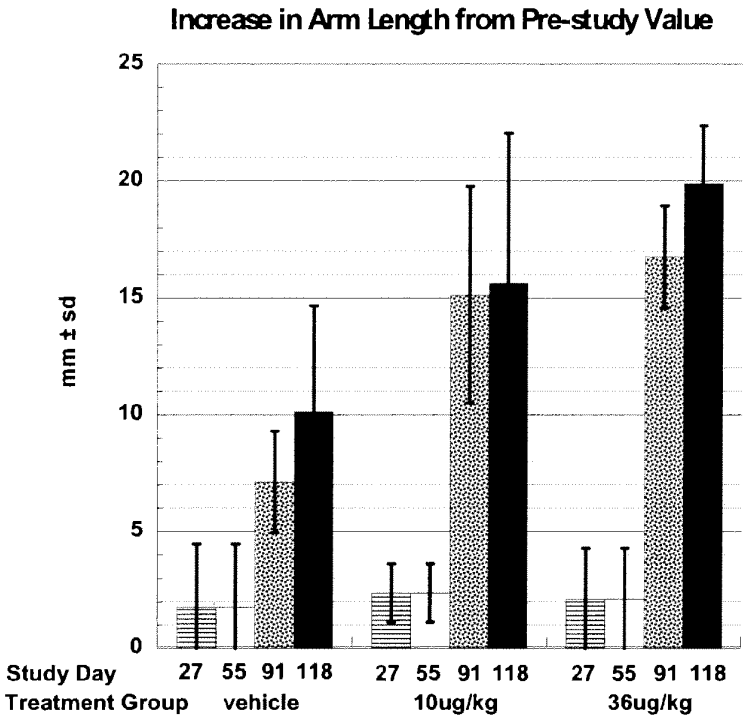


FIGURE 81

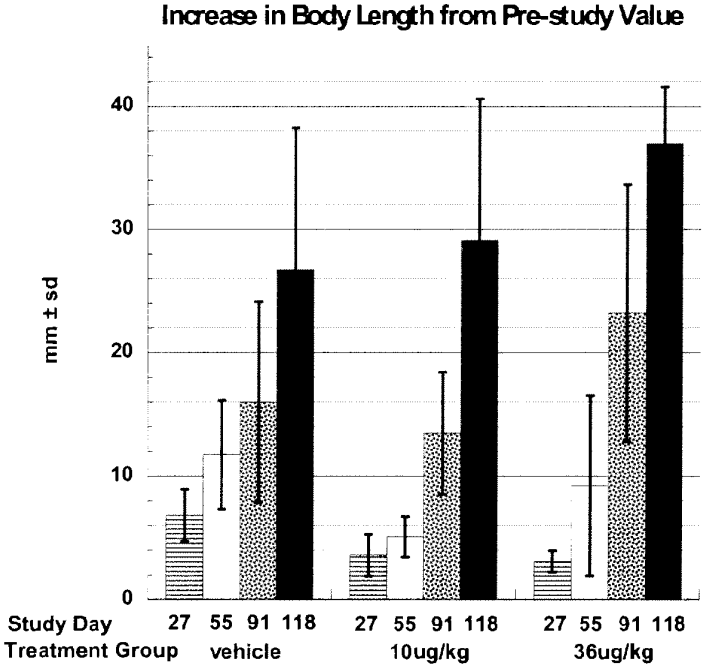


FIGURE 82

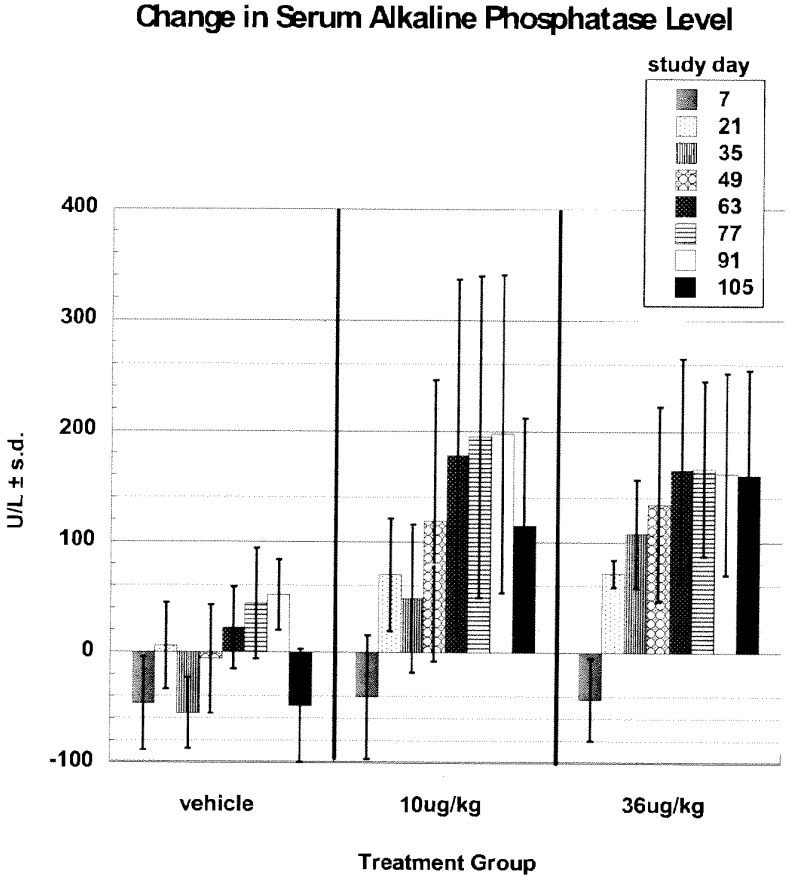
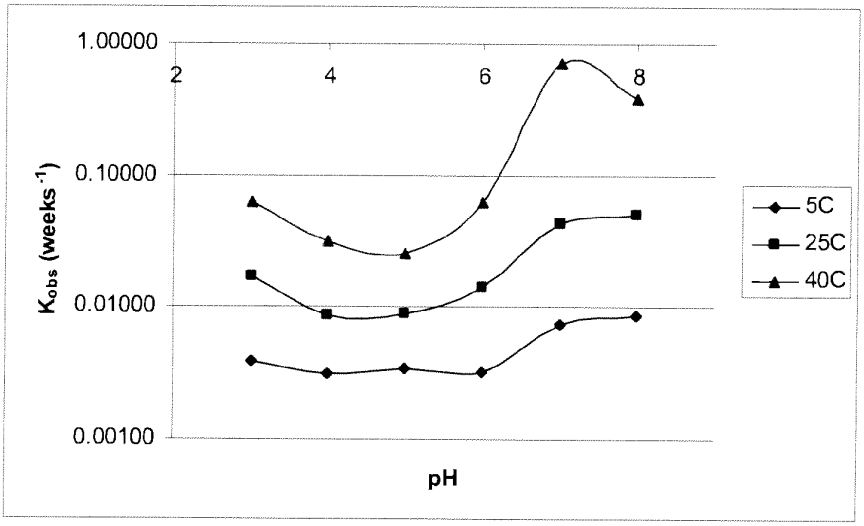


FIGURE 83



VARIANTS OF C-TYPE NATRIURETIC PEPTIDE

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application claims the priority and benefit of U.S. Provisional Application No. 61/254,563, filed on Oct. 23, 2009, and U.S. Provisional Application No. 61/180,112, filed on May 20, 2009, the disclosure of each of which is incorporated herein by reference in its entirety.

FIELD OF THE DISCLOSURE

The field of the disclosure relates, in general, to variants of C-type natriuretic peptide (CNP), compositions comprising CNP variants, methods of making CNP variants, and methods of using CNP variants to treat disorders responsive to CNP, including but not limited to bone-related disorders such as skeletal dysplasias (e.g., achondroplasia) and vascular smooth muscle disorders.

BACKGROUND OF THE DISCLOSURE

The natriuretic peptide family consists of three structurally related peptides: atrial natriuretic peptide (ANP) (Genbank Accession No. NP_006163, for the ANP precursor protein, NPPA), brain natriuretic peptide (BNP) (GenBank Accession No. NP_002512, for the BNP precursor protein, NPPB), and C-type natriuretic peptide (CNP) (Biochem. Biophys. Res. Commun., 168: 863-870 (1990) (GenBank Accession No. NP_077720, for the CNP precursor protein, NPPC) (J. Hypertens., 10: 907-912 (1992)). These small, single chain peptides (ANP, BNP, CNP) have a 17-amino acid loop structure (Levin et al., N. Engl. J. Med., 339: 863-870 (1998)) and have important roles in multiple biological processes. ANP and BNP bind to and activate the natriuretic peptide receptor A (NPR-A), also termed guanalyl cyclase A (GC-A), resulting in higher intracellular cyclic guanosine monophosphate (cGMP) levels. Likewise, CNP interacts with NPR-B (GC-B) to stimulate the generation of cGMP (J. Hypertens., 10: 1111-1114 (1992)). A third type of receptor, NPR-C, binds each of the natriuretic peptides with high affinity and functions primarily to capture the peptides from the extracellular compartment and deposit the peptides into lysosomes, where they are degraded (Science, 238: 675-678 (1987)). ANP and BNP are produced primarily within the muscle cells of the heart, and are believed to have important roles in cardiovascular homeostasis (Science, 252: 120-123 (1991)). CNP is expressed more widely, including in the central nervous system, reproductive tract, bone and endothelium of blood vessels (Hypertension, 49: 419-426 (2007)).

In humans, CNP is initially produced from the natriuretic peptide precursor C(NPPC) gene as a single chain 126-amino acid pre-pro polypeptide (Biochem. Biophys. Res. Commun., 168: 863-870 (1990)). Removal of the signal peptide yields pro-CNP, and further cleavage by the endoprotease furin generates an active 53-amino acid peptide (CNP-53), which is secreted and cleaved again by an unknown enzyme to produce the mature 22-amino acid peptide (CNP-22) (Wu, J. Biol. Chem. 278: 25847-852 (2003)). CNP-53 and CNP-22 differ in their distribution, with CNP-53 predominating in tissues, while CNP-22 is mainly found in plasma and cerebrospinal fluid (J. Alfonzo, Recept. Signal. Transduct. Res., 26: 269-297 (2006)). The predominant CNP form in cartilage is unknown. Both CNP-53 and CNP-22 bind similarly to NPR-B. Furthermore, they both induce cGMP production in a dose-dependent and similar fashion (V T Yeung, Peptides, 17: 101-106 (1996)).

Natural CNP gene and polypeptide have been previously described. U.S. Pat. No. 5,352,770 discloses isolated and purified CNP-22 from porcine brain identical in sequence to human CNP and its use in treating cardiovascular indications. U.S. Pat. No. 6,034,231 discloses the human gene and polypeptide of proCNP (126 amino acids) and the human CNP-53 gene and polypeptide.

Clearance of CNP from the extracellular space occurs through the action of membrane-bound neutral endopeptidase (NEP), which rapidly degrades CNP (Biochem. J., 291 (Pt 1): 83-88 (1993)), and through NPR-C, which binds to and deposits CNP into lysosomes, where CNP is degraded. CNP has been shown to have an in vivo half-life of 2.6 min in the normal human (J. Clin. Endocrinol. Metab., 78: 1428-35 (1994)). The low plasma concentration of CNP (J. Bone Moner. Res., 19 (Suppl. 1) S20 (2004)) and its co-expression with NPR-B in a number of tissues suggests that CNP functions primarily through an autocrine/paracrine mechanism.

As stated above, CNP binds to and activates natriuretic peptide receptor B (NPR-B), also termed guanylyl cyclase B (GC-B), resulting in higher intracellular cyclic guanosine monophosphate (cGMP) levels. Downstream signaling mediated by cGMP generation influences a diverse array of biological processes that include endochondral ossification. Accordingly, elevated or depressed levels of any of the components in this pathway may lead to aberrant bone growth. For example, knockout of either CNP or NPR-B in mouse models results in animals having a dwarfed phenotype with shorter long bones and vertebrae. Mutations in human NPR-B that block proper CNP signaling have been identified and result in dwarfism (Olney, et al., J. Clin. Endocrinol. Metab. 91(4): 1229-1232 (2006); Bartels, et al., Am. J. Hum. Genet. 75: 27-34 (2004)). In contrast, mice engineered to produce elevated levels of CNP display elongated long bones and vertebrae.

Achondroplasia is a result of an autosomal dominant mutation in the gene for fibroblast growth factor receptor 3 (FGFR-3), which causes an abnormality of cartilage formation. FGFR-3 normally has a negative regulatory effect on chondrocyte growth, and hence bone growth. In achondroplasia, the mutated form of FGFR-3 is constitutively active, which leads to severely shortened bones. Both chondrocyte proliferation and differentiation appear to be disturbed, leading to remarkably short growth plate cartilage (P. Krejci et al., J. Cell Sci. 118: 5089-5100 (2005)). Endochondral ossification is the process that governs longitudinal long-bone growth. There are four zones of the growth plate—resting, proliferative, hypertrophic and zone of calcification. In the growth plate, NPR-B is expressed by proliferative cells while NPR-C is expressed by hypertrophic cells (Yamashite et al., J. Biochem. 127: 177-179 (2000)). In normal endochondral bone growth, chondrocytes organize in columns and proliferate in the proliferative zone of the growth plate. These columns are disorganized in achondroplasia patients. Additionally, the hypertrophic zone is where the cells become large and eventually apoptose (lyse), leading to osteocyte invasion and mineralization. The hypertrophic chondrocytes and the overall size of the zone are much smaller in achondroplasia patients than in normal patients. CNP is an agonist for NPR-B, a positive regulator of chondrocyte and bone growth. Downstream signaling of CNP/NPR-B inhibits the FGFR-3 pathway at the level of mitogen-activated protein kinase (MAP K). Inhibition at MAP K promotes proliferation and differ-

entiation of the chondrocytes in the proliferative and hypertrophic zones of the growth plate, resulting in bone growth.

In humans activating mutations of FGFR-3 are the primary cause of genetic dwarfism. Mice having activated FGFR-3 serve as a model of achondroplasia, the most common form of the skeletal dysplasias, and overexpression of CNP rescues these animals from dwarfism. Accordingly, CNP and functional variants of CNP are potential therapeutics for treatment of the various skeletal dysplasias.

Therapeutic use of CNP is currently limited by its short plasma half-life, which has been shown to be 2.6 minutes in vivo in humans (J. Clin. Endocrinol. Metab., 78: 1428-35 (1994)). To increase CNP concentration above intrinsic levels (about 5 pM) typically found in human plasma, continuous infusion has been necessary in all human and animal studies using systemically administered CNP. A CNP variant having a longer in vivo serum half-life and exhibiting similar or improved activity to that of wild-type CNP is important for a sustainable therapeutic strategy. Two mechanisms by which the half-life of CNP is reduced in human plasma are degradation by neutral endopeptidase (NEP) and clearance by natriuretic peptide receptor C(NPR-C) (Growth Horm. & IGF Res., 16: S6-S14 (2006)). Modifications of peptides reportedly can improve resistance to endopeptidase and exopeptidase cleavage (Amino Acids, 30: 351-367 (2006); Curr. Opin. Biotech., 17: 638-642 (2006)).

The biological activities of various analogs and derivatives of CNP have been evaluated. By substituting S-methyl Cys in place of both Cys_6 and Cys_{22}, cyclization of the peptide via a Cys6-Cys22 disulfide linkage was reportedly shown to be important for the activity of CNP in stimulating cGMP formation (Biochem. Biophys. Res. Comm., 183: 964-969 (1992), also using alanine scanning to identify amino acids important for CNP functionality). A significant additional enhancement of activity reportedly results from the combined presence of the amino acids Leu_9, Lys_{10}, and Leu_{11}. U.S. Pat. No. 5,434,133 describes CNP analogs comprising CNP-22 with substitutions at amino acid position 6, 7, 9, 11, or 22, wherein the amino acid is selected from Cys or Pmp (pentacyclomercaptopropionic acid) at position 6, Phe, 4-chloro-Phe, 4-fluoro-Phe, 4-nitro-Phe, or Cha (3-cyclohexyl-Ala) at position 7, Gly, Val, Aib, or tLeu at position 9, Leu or Ile at position 11, and Cys or Pmp at position 22.

U.S. Patent Publication No. 2004/0138134 (now U.S. Pat. No. 7,276,481) describes CNP variants comprising amino acids Cys_6 to Cys_{22} of CNP-22 ("CNP-17") which include at least one substitution for another natural amino acid at position 9, 10, 11, 16, 17, 19, or 20, CNP variants with insertions and deletions, such as addition of a His residue at the reported primary site of NEP cleavage, between Cys_6 and Phe_7, and methods of using such variants for increasing the size of a bone growth plate in abnormal bone and elongation of an abnormal bone. However, no significant gains in activity as measured by cGMP production were obtained for these variants, and activity was diminished for nearly all of the variants, as observed in an in vitro cell-based method (Example 7). Further no supportive data, such as for example in vitro stability or in vivo determination of improved pharmacokinetics (PK) were provided to substantiate the asserted NEP resistance and NPR-C resistance of the CNP analogs. U.S. Pat. No. 6,743,425 discloses substances for treating achondroplasia which activate NPR-B/GC-B and are peptides or low molecular weight compounds, including the C-type natriuretic peptides CNP-22 and CNP-53. PCT Publication No. WO 94/20534 discloses a chimera of CNP-22 and the 5-amino acid C-terminus of ANP designated as the vasonatrin peptide (VNP), a limited number of amino acid substitutions and cyclic chimeric peptides that result from formation of a disulfide or double bond.

Approaches for improving the half-life of other natriuretic peptide family members include decreasing the affinity of ANP for NPR-C (U.S. Pat. No. 5,846,932), utilizing pentapeptide antagonists of NPR-C (WO 00/61631), and co-administering NEP inhibitors such as thiorphan and candoxatril (Clin. Exp. Pharma. Physiol., 25: 986-991 (1997), Hyperten., 30: 184-190 (1997)). WO 2004/047871 describes conjugates of BNP and BNP variants to polyalkylene glycol moieties, sugar moieties, polysorbate moieties, polycationic moieties, and other hydrophilic polymer moieties that reportedly exhibit improved half-life in circulation and reportedly are useful for the treatment of acute congestive heart failure.

There have been no published reports, however, on a successful strategy for making CNP resistant to NEP while retaining its functionality.

SUMMARY OF THE DISCLOSURE

The present disclosure relates to variants of C-type natriuretic peptide (CNP) which are useful in the treatment of bone-related disorders (e.g., achondroplasia) and vascular smooth muscle disorders. The disclosure encompasses CNP variants having increased serum half-life, e.g. as a result of reduced ability to bind to neutral endopeptidase (NEP), greater resistance to proteolysis by NEP and/or reduced affinity to the clearance natriuretic peptide receptor C(NPR-C), while retaining the functionality of CNP.

The wild-type sequence of human CNP-22 (referred to herein as "hCNP22", "wtCNP22" or "CNP22") is set forth below:

(SEQ ID NO: 1)
(N-terminus) Gly_1-Leu_2-Ser_3-Lys_4-Gly_5-Cys_6-Phe_7-Gly_8-Leu_9-Lys_{10}-Leu_{11}-Asp_{12}-Arg_{13}-Ile_{14}-Gly_{15}-Ser_{16}-Met_{17}-Ser_{18}-Gly_{19}-Leu_{20}-Gly_{21}-Cys_{22}.

Positions 6 to 22 of CNP22 form a cyclic domain by means of a disulfide bond between Cys6 and Cys22. The 17-amino acid cyclic structure has been shown to be important for binding of CNP to NPR-B (Schiller, Biochem. Biophys. Res. Commun., 138: 880-886 (1986)). The amino acid sequence of positions 6 to 22 of CNP22 is referred to herein as "CNP17" (SEQ ID NO: 2).

CNP is susceptible to NEP cleavage at a number of sites: Cys6-Phe7, Gly8-Leu9, Lys10-Leu11, Arg13-Ile14, Ser16-Met17 and Gly19-Leu20. In one embodiment, the disclosure encompasses a CNP variant that is (1) modified to increase its overall size or molecular weight, e.g., to a range from about 2.6 kDa or 2.8 kDa to about 4 kDa, 4.2 kDa, 4.4 kDa, 4.6 kDa, 4.8 kDa, 5 kDa, 5.2 kDa, 5.4 kDa, 5.6 kDa, 5.8 kDa, 6 kDa, 6.2 kDa, 6.4 kDa, or to about 7 kDa, 7.2 kDa or about 8.2 kDa, and/or (2) modified at certain amino acid positions to reduce its susceptibility to NEP cleavage at 1, 2, 3, 4, 5 or all 6 of the sites listed above. The size or molecular weight of the CNP variant can be increased by various means, e.g., by conjugating additional amino acids and/or other kinds of chemical (e.g., natural or synthetic polymeric) groups to the peptide sequence at, e.g., the N-terminus, the C-terminus and/or side chain(s), and/or by using natural amino acids, unnatural amino acids, and/or peptidomimetics with bulkier side chains. The CNP variant is optionally further conjugated to other functional or structural moieties. Optionally in combination with any of the embodiments described herein, mutation(s) (e.g., substitution(s), addition(s), and/or deletion(s))

may be introduced to certain position(s) of CNP22 to reduce the CNP variants' affinity to NPR-C. Further modifications may be made without affecting NEP resistance or CNP activity, e.g., conservative substitutions, or other modifications known in the art.

In one embodiment, the CNP variant is represented by the general formula: (x)-Gly_1-Leu_2-Ser_3-Lys_4-Gly_5-Cys_6-Phe_7-Gly_8-Leu_9-Lys_{10}-Leu_{11}-Asp_{12}-Arg_{13}-Ile_{14}-Gly_{15}-Ser_{16}-Met_{17}-Ser_{18}-Gly_{19}-Leu_{20}-Gly_{21}-Cys_{22}-(z) (SEQ ID NO: 5), wherein:

the CNP variant comprises one or more modified amino acids, which may result in modified peptide bonds (e.g., through use of peptide bond isosteres), at a position corresponding to one or more of the following CNP residues: Gly1, Lys4, Gly5, Cys6, Phe7, Gly8, Leu9, Lys10, Leu11, Ile14, Gly15, Ser16, Met17, Gly19, Leu20 and Gly21; and

(x) and (z) independently may be absent or may be an amino acid sequence derived from a natriuretic polypeptide (e.g., NPPC, ANP, BNP) or a non-natriuretic polypeptide (e.g., human serum albumin (HSA), IgG, etc.).

In an embodiment, the CNP variant includes: (1) a modification at an amino acid position corresponding to one of positions 6, 7 or 8 (Cys6, Phe7 or Gly8) of CNP22, (2) optionally deletion, addition and/or substitution of any or all of the amino acids at positions 1-5 (Gly1, Leu2, Ser3, Lys4, and Gly5) and (3) optionally up to 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 further modifications (deletions, additions and/or substitutions) at positions corresponding to positions 6-22, of which 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 may be conservative substitutions or other substitutions described herein or known in the art.

It is understood that a reference to a particular amino acid position by number (e.g. position 7 of CNP22) refers to the corresponding amino acid position in any CNP variant, even if the number of the position in that CNP variant has changed due to preceding insertions or deletions. For example, a reference to "position 7" or "Phe7" would mean the corresponding position 2 for a CNP variant in which the first five amino acids had been deleted. Similarly, a reference to "position 7" would mean the corresponding position 8 for a CNP variant in which one amino acid had been added to the N-terminus.

In any of the embodiments described herein, the CNP variant may be cyclized through a covalent bond between positions corresponding to 6 and 22 of CNP22. It is contemplated that the covalent bond is formed using any methods known in the art. In another embodiment, the CNP variant may be cyclized through a covalent bond formed between an amino acid at or toward the N-terminus and an amino acid at or toward the C-terminus (referred to as "terminal" amino acids for this purpose) of the peptide. In one embodiment, the covalent bond is formed between the side chains of the two terminal amino acids or the amino acids at positions corresponding to 6 and 22 of CNP22. In another embodiment, the covalent bond is formed between the side chain of one terminal amino acid and the terminal group of the other terminal amino acid, or between the terminal groups of each terminal amino acid. For example, head-to-tail, side chain-to-side chain, side chain-to-head, or side chain-to-tail bonds are possible for the covalent bond formed between the terminal amino acids or between the amino acids at positions corresponding to 6 and 22 of CNP22.

In one embodiment, the disclosure provides a CNP variant having reduced affinity to NEP, and/or greater resistance to cleavage by NEP and/or increased in vivo serum half-life, while retaining functionality of CNP (e.g., stimulation of cGMP production). NEP preferably recognizes substrates smaller than about 3 kDa, due to the limited size of its active site cavity (Oefner, J. Mol. Biol., 296: 341-349 (2000)). In an embodiment, the CNP variants are modified to increase their overall molecular weight to a range from about 2.6 or 2.8 kDa to about 4, 4.6, 5, 5.2, 5.8, 6, 6.4 or 7 kDa, e.g., by adding about 0.6 to about 5 kDa of amino acids, hydrophilic or water-soluble polymers, hydrophobic acids (including fatty acids), and/or carbohydrates. In specific exemplary embodiments, the CNP variants have a molecular weight between about 2.6 kDa and about 7 kDa, or between about 2.8 kDa and 6 kDa, or between about 2.8 kDa and about 5.8 kDa. In certain embodiments, at least about 0.6, 0.8, 1, 1.2, 1.4, 1.6 or 1.8 kDa, or up to 2, 2.2, 2.4, 2.6, 2.8, 3, 3.2, 3.4, 3.6, 3.8, 4, 4.2, 4.4, 4.6, 4.8 or 5 kDa are added, to increase the total molecular weight of the CNP variant, e.g., to a range from about 2.6 or 2.8 kDa up to about 4 kDa, 4.2 kDa, 4.4 kDa, 4.6 kDa, 4.8 kDa, 5 kDa, 5.2 kDa, 5.4 kDa, 5.6 kDa, 5.8 kDa, 6 kDa, 6.2 kDa, 7.2 kDa, 8.2 kDa or higher. In some embodiments, such CNP variants comprise an amino acid sequence at least about 70%, 75%. 80%, 85%, 90%, or 95% identical or homologous to amino acids 6-22 of CNP22. In other embodiments, such CNP variants comprise a substitution, insertion or deletion of 1, 2, 3, 4, 5, 6 or 7 amino acids with another natural or unnatural amino acid or peptidomimetic. While both conservative and non-conservative substitutions or insertions are envisioned at any position, introduction of modifications may commence, e.g., by conservative substitutions in regions that have been identified in the art as involved in CNP activity or NPR-B binding, while non-conservative substitutions may be made in those regions that have been previously shown to be tolerant of modification.

In another embodiment, the CNP variants comprise a CNP having an intact cyclized portion between Cys6 and Cys22, and N-terminal and/or C-terminal tails that contain about 1-40, 1-20, 5-40, 5-35, 10-35, 15-35, 5-31, 10-31, or 15-31 amino acids and are fragments derived from a CNP polypeptide and/or a non-CNP polypeptide. In an embodiment, such CNP variants have a molecular weight in a range from about 2.8 kDa to about 4, 4.6, 5, 5.2, 5.8, 6, 6.4 or 7 kDa. Non-limiting examples of such CNP variants include wild-type CNP22 or CNP22 with one or more amino acid substitutions (e.g., a K4R substitution), having an N-terminal and/or C-terminal extension derived from natriuretic peptide precursor sequences (e.g., ANP, BNP or CNP) from human or other species, a natriuretic peptide precursor C (NPPC) variant with amino acid substitutions, additions and/or deletions (e.g., the CNP variants may be truncations of CNP-53 which result in peptides with a molecular weight between about 2.8 kDa and 5.8 kDa), or other non-CNP polypeptides such as, e.g., serum albumin or IgG protein (e.g., the CNP variants may be CNP chimeras containing fragments of serum albumin or IgG from human or other species).

In one embodiment, CNP variants having a total mass characterized by the ranges described generally herein, e.g., from about 2.6 kDa or 2.8 kDa to about 6 or 7 kDa, designed for increased resistance to NEP degradation, are represented by the general formula:

(x)-Gly_1-Leu_2-Ser_3-(b)$_4$-Gly_5-Cys_6-Phe_7-Gly_8-Leu_9-(h)$_{10}$-Leu_{11}-Asp_{12}-Arg_{13}-Ile_{14}-Gly_{15}-Ser_{16}-Met_{17}-Ser_{18}-Gly_{19}-Leu_{20}-Gly_{21}-Cys_{22}-(z) (SEQ ID NO: 6), wherein:

(x) is a synthetic or natural polymeric group, or a combination thereof, wherein a non-limiting example of a synthetic polymeric group is polyethylene glycol (PEG, also called polyethylene oxide (PEO)), and a non-limiting example of a natural polymeric group is an amino acid sequence containing from 1 to 35 amino acids and derived from NPPC or variants thereof with substitutions and/or deletions, ANP, BNP, or other non-CNP (poly)peptides such as, e.g., serum albumin, IgG, histidine-rich glycoproteins, fibronectin,

fibrinogen, zinc finger-containing polypeptides, osteocrin or fibroblast growth factor 2 (FGF2);

(z) may be absent or may be a synthetic or natural polymeric group, or a combination thereof, wherein a non-limiting example of a synthetic polymeric group is PEG, and a non-limiting example of a natural polymeric group is an amino acid sequence derived from a natriuretic polypeptide (e.g., NPPC, CNP, ANP or BNP) or non-natriuretic polypeptide (e.g., serum albumin or IgG); and

(b) and (h) independently may each be the wild type Lys at that position or may be replaced with a conservative amino acid substitution or any natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amine on a side chain, including but not limited to Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Glu or Ser. In one embodiment, (b) is Arg. In another embodiment, for improved NEP resistance, (b) is not Gly. In yet another embodiment, (h) is not Arg.

Non-limiting examples of amino acid sequences derived from NPPC or variants thereof include:

Arg,

Glu-Arg,

(SEQ ID NO: 7)
Gly-Ala-Asn-Lys-Lys,

(SEQ ID NO: 8)
Gly-Ala-Asn-Arg-Arg,

(SEQ ID NO: 9)
Gly-Ala-Asn-Pro-Arg,

(SEQ ID NO: 10)
Gly-Ala-Asn-Gln-Gln,

(SEQ ID NO: 11)
Gly-Ala-Asn-Ser-Ser,

(SEQ ID NO: 12)
Gly-Ala-Asn-Arg-Gln,

(SEQ ID NO: 13)
Gly-Ala-Asn-Arg-Met,

(SEQ ID NO: 14)
Gly-Ala-Asn-Arg-Thr,

(SEQ ID NO: 15)
Gly-Ala-Asn-Arg-Ser,

(SEQ ID NO: 16)
Ala-Ala-Trp-Ala-Arg-Leu-Leu-Gln-Glu-His-Pro-Asn-Ala,

(SEQ ID NO: 17)
Ala-Ala-Trp-Ala-Arg-Leu-Leu-Gln-Glu-His-Pro-Asn-Ala-Arg,

(SEQ ID NO: 18)
Asp-Leu-Arg-Val-Asp-Thr-Lys-Ser-Arg-Ala-Ala-Trp-Ala-Arg,

(SEQ ID NO: 19)
Gln-Glu-His-Pro-Asn-Ala-Arg-Lys-Tyr-Lys-Gly-Ala-Asn-Lys-Lys,

(SEQ ID NO: 20)
Gln-Glu-His-Pro-Asn-Ala-Arg-Lys-Tyr-Lys-Gly-Ala-Asn-Arg-Arg,

(SEQ ID NO: 21)
Asp-Leu-Arg-Val-Asp-Thr-Lys-Ser-Arg-Ala-Ala-Trp-Ala-Arg-Leu-Leu-Gln-Glu-His-Pro-Asn-Ala-Arg-Lys-Tyr-Lys-Gly-Ala-Asn-Lys-Lys,

-continued

and

(SEQ ID NO: 22)
Asp-Leu-Arg-Val-Asp-Thr-Lys-Ser-Arg-Ala-Ala-Trp-Ala-Arg-Leu-Leu-Gln-Glu-His-Pro-Asn-Ala-Arg-Lys-Tyr-Lys-Gly-Ala-Asn-Arg-Arg.

Non-limiting examples of amino acid sequences derived from non-CNP polypeptides such as, e.g., ANP, BNP, serum albumin and IgG include:

(SEQ ID NO: 23)
Ser-Leu-Arg-Arg-Ser-Ser;

(SEQ ID NO: 24)
Asn-Ser-Phe-Arg-Tyr;

(SEQ ID NO: 25)
Ser-Pro-Lys-Met-Val-Gln-Gly-Ser-Gly;

(SEQ ID NO: 26)
Met-Val-Gln-Gly-Ser-Gly;

(SEQ ID NO: 27)
Lys-Val-Leu-Arg-Arg-Tyr;

(SEQ ID NO: 28)
Lys-Val-Leu-Arg-Arg-His;

(SEQ ID NO: 29)
Gly-Gln-His-Lys-Asp-Asp-Asn-Pro-Asn-Leu-Pro-Arg;

(SEQ ID NO: 30)
Gly-Val-Pro-Gln-Val-Ser-Thr-Ser-Thr;

(SEQ ID NO: 31)
Gly-Glu-Arg-Ala-Phe-Lys-Ala-Trp-Ala-Val-Ala-Arg-Leu-Ser-Gln;

and

(SEQ ID NO: 32)
Gly-Gln-Pro-Arg-Glu-Pro-Gln-Val-Tyr-Thr-Leu-Pro-Pro-Ser.

In an embodiment, the N-terminus and/or C-terminus of CNP22 or a variant thereof independently may be conjugated to an amino acid extension containing 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39 or 40 amino acids. In one embodiment, the amino acid extension is derived from NPPC, CNP53, ANP or BNP. In a specific embodiment, the amino acid extension is Gln-Glu-His-Pro-Asn-Ala-Arg-Lys-Tyr-Lys-Gly-Ala-Asn-Lys-Lys (SEQ ID NO: 33). In a related embodiment, this 15-amino acid extension is added to the N-terminus to provide a CNP variant of the formula Gln-Glu-His-Pro-Asn-Ala-Arg-Lys-Tyr-Lys-Gly-Ala-Asn-Lys-Lys-Gly_1-Leu_2-Ser_3-$(b)_4$-Gly_5-Cys_6-Phe_7-Gly_8-Leu_9-$(h)_{10}$-Leu_{11}-Asp_{12}-Arg_{13}-Ile_{14}-Gly_{15}-Ser_{16}-Met_{17}-Ser_{18}-Gly_{19}-Leu_{20}-Gly_{21}-Cys_{22}-(z) (SEQ ID NO: 34).

In one embodiment, the CNP variants comprise wtCNP22 or a variant thereof (e.g., one having addition(s), deletion(s), and/or substitution(s) such as, e.g., a K4R substitution) (SEQ ID NO: 35) conjugated at the N-terminus and/or C-terminus to a hydrophilic polymer (e.g., PEG) to increase their overall molecular size to a range from about 2.6 kDa or 2.8 kDa to about 4, 5, 6 or 7 kDa. Such CNP variants are optionally further conjugated at the N-terminus and/or C-terminus to a polymeric group comprising, e.g., amino acids, carbohydrates, hydrophobic acids and/or phospholipids, a non-limiting example of which is an N-terminal amino acid extension containing 1 to 35, or 5 to 31, amino acids. In an embodiment, a hydrophilic polymeric (e.g., PEG) moiety of at least about

0.4, 0.6, 0.8, 1, 1.2, 1.4, 1.6 or 1.8 kDa, or up to about 2, 2.2, 2.4, 2.6, 2.8, 3, 3.2, 3.4, 3.6, 3.8, 4, 4.2, 4.4, 4.6, 4.8 or 5 kDa, is added to the N-terminus and/or C-terminus of wtCNP22 or a variant thereof.

As shown herein, conjugation of a hydrophilic or water-soluble PEG (or PEO) polymer of about 0.6 kDa or more to CNP22 or variants thereof generally increases resistance to NEP cleavage markedly. However, addition of PEG, even as small as 0.6 kDa, to wtCNP22 may reduce CNP functionality (e.g., stimulation of cGMP signaling), and addition of greater than about 2 or 3 kDa of PEG to CNP22 or variants thereof may reduce CNP functional activity in a size-dependent manner. But CNP functionality (at least comparable to that of wtCNP22) is retained when a PEG (or PEO) polymer of about 0.6 kDa to about 1.2 kDa, or potentially to about 2 kDa, is conjugated to a CNP variant having an N-terminal amino acid extension in which at least one relatively large amino acid that may potentially be positively charged under physiological conditions (e.g., arginine) immediately precedes the position corresponding to Gly1 of CNP22, such as, e.g., GANRR-CNP22(K4R) (SEQ ID NO: 36), GANPR-CNP22(K4R) (SEQ ID NO: 37), ER-CNP22 (SEQ ID NO: 38), ER-CNP22 (K4R) (SEQ ID NO: 39), R-CNP22 (SEQ ID NO: 40) and R-CNP22(K4R) (SEQ ID NO: 41).

Accordingly, in one embodiment, PEGylated CNP variants comprise at the N-terminus of CNP22 or a variant thereof (e.g., one having a K4R substitution) an amino acid extension containing at least 1, 2, 3, 4 or 5 amino acids, wherein the PEG polymer is conjugated to the N-terminus of the amino acid-extended CNP variant to result in a total mass characterized by the ranges described generally herein, e.g., from about 2.6 kDa or 2.8 kDa to about 6 or 7 kDa for increased resistance to NEP cleavage. In an embodiment, for enhanced CNP functionality, such pegylated, amino acid-extended CNP variants contain at least one relatively large natural or unnatural amino acid that may potentially be positively charged under physiological conditions, immediately preceding the position corresponding to Gly1 of CNP22. In a specific embodiment, the pegylated, amino acid-extended CNP variants contain at least one arginine residue immediately preceding the position corresponding to Gly1 of CNP22.

In addition to CNP variants conjugated at the N-terminus and/or C-terminus to a hydrophilic or water-soluble polymer such as, e.g., PEG (or PEO), the disclosure encompasses CNP variants conjugated to such a polymer at an internal site. For purposes of brevity here, PEG (or PEO) will be used as a representative example of a hydrophilic or water-soluble polymer. Various sites of PEGylation of a CNP variant are possible, including but not limited to: (1) PEGylation only at the N-terminus; (2) PEGylation only at the C-terminus; (3) PEGylation only at an internal site (e.g., Lys4); (4) PEGylation at both the N-terminus and the C-terminus; (5) PEGylation at the N-terminus and an internal site; and (6) PEGylation at the C-terminus and an internal site. For increased resistance to NEP degradation and retention of CNP functionality, in certain embodiments the total mass of PEGylatedCNP variants is characterized by the ranges described generally herein, e.g., in the range from about 2.6 kDa or 2.8 kDa to about 4, 5, 6 or 7 kDa. In a particular embodiment, CNP17, CNP22, CNP37 (defined below) or variants thereof (including those having amino acid additions, substitutions and/or deletions) are PEGylated only at the N-terminus. In another embodiment, the CNP variants are PEGylated only at an internal site (e.g., Lys4). In yet another embodiment, the CNP variants are PEGylated at the N-terminus and an internal site (e.g., Lys4). In still another embodiment, for better functionality the CNP variants are not PEGylated at a site (e.g., Lys10) within the cyclic domain (corresponding to Cys6 to Cys22 of CNP22). To prevent PEGylation at an internal site, Lys4 and/or Lys10 can be substituted with a natural or unnatural amino acid or peptidomimetic that does not contain a reactive primary amino group on a side chain, such as, e.g., Gly, Ser, Arg, Asn, Gln, Asp, Glu or citrulline (Cit). In a particular embodiment, Lys4 and/or Lys10 are replaced with Arg. In another embodiment, Lys10 is not replaced with Arg.

The disclosure contemplates use of hydrophilic or water soluble polymers (e.g., PEG molecules) that may vary in type (e.g., homopolymer or copolymer; random, alternating or block copolymer; linear or branched; monodispersed or polydispersed), linkage (e.g., hydrolysable or stable linkage such as, e.g., amide, imine, aminal, alkylene, or ester bond), conjugation site (e.g., at the N-terminus and/or C-terminus, preferably not at any of the residues in the cyclized region of CNP (corresponding to residues 6-22 of CNP22)), and length (e.g., from about 0.2, 0.4 or 0.6 kDa to about 2, 3, 4 or 5 kDa). The hydrophilic or water-soluble polymer can be conjugated to the CNP peptide by means of N-hydroxy succinimide (NHS)- or aldehyde-based chemistry or other chemistry, as is known in the art. Such CNP variants can be generated using, e.g., wtCNP-22 (2.2 kDa), CNP-17 retaining only the cyclized region (residues 6-22) of wtCNP22, CNP variants having an amino acid extension at the N-terminus and/or C-terminus of CNP22, or variants with amino acid substitutions, additions and/or deletions, for example, GANRR-CNP22(K4R) (SEQ ID NO: 36), GANPR-CNP22(K4R) (SEQ ID NO: 37), R-CNP22 (SEQ ID NO: 40), R-CNP22(K4R) (SEQ ID NO: 41), ER-CNP22 (SEQ ID NO: 38) and ER-CNP22(K4R) (SEQ ID NO: 39). In an embodiment, the PEG-CNP variants having a total mass characterized by the ranges described generally herein, e.g., from about 2.6 kDa or 2.8 kDa to about 6 or 7 kDa, contain a monodispersed, linear PEG (or PEO) group conjugated at the N-terminus and/or C-terminus via NHS- or aldehyde-based chemistry, or a two-arm or three-arm branched PEG group conjugated at the N-terminus and/or C-terminus via NHS-based chemistry. The disclosure further contemplates negatively charged PEG-CNP variants designed for reduced renal clearance, including but not limited to carboxylated, sulfated and phosphorylated compounds.

In a related embodiment, the disclosure contemplates PEG-CNP conjugates comprising NHS- or aldehyde-based PEG of the formula $(CH_2CH_2O)_n$, wherein n is an integer from 12 to 50, and the PEG polymer is up to about 2.5 kDa in molecular weight. In a specific embodiment, n is 12 or 24. In an embodiment, the terminal hydroxyl group of the PEG polymer is capped with a non-reactive group. In a particular embodiment, the capping group is an alkyl group, e.g., a lower alkyl group such as methyl.

In an additional embodiment, the PEG polymers or derivatives thereof have a polymer number-average molecular weight in the range from about 0.4 kDa to about 2.5 kDa or from about 0.6 kDa to about 1.5 kDa.

In a further embodiment, the wtCNP or CNP variant peptide is conjugated to a moiety including, e.g., bisphosphonates, carbohydrates, hydrophobic acids (including fatty acids) or amino acid sequences. Such amino acid sequences include for example polyAsp or polyGlu useful in bone/cartilage targeting, or can be derived from bone proteins with elucidated bone-targeting domains or derivatives thereof, such as for example fusion proteins or peptide sequences of osteopontin, osteocalcin, sialoprotein, etc. In embodiments described herein where CNP22 or a variant thereof is attached to a bone- or cartilage-targeting moiety, such a moiety is designed to promote getting the modified CNP peptide to

chondrocytes of bone growth plates, where the peptide can bind and activate NPR-B on the chondrocytes.

In another embodiment, the disclosure provides CNP variants with a peptide bond that is less susceptible to cleavage by peptidases including NEP. The disclosure encompasses a CNP variant comprising at least one modified residue at a site of endopeptidase cleavage. In one embodiment, the Cys6-Phe7 peptide bond (—C(═O)—NH—) at an NEP cleavage site in CNP can be replaced with anyone of the following peptide-bond isosteres:

—CH_2—NH—,

—C(═O)—N(R)—, where the amide group is alkylated with any of the following R groups: methyl, ethyl, n-propyl, isopropyl, cyclopropyl, n-butyl, isobutyl, sec-butyl or tert-butyl,

—C(═O)—NH—CH_2—,

—CH_2—S—,

—CH_2—$S(O)_n$—, where n is 1 or 2,

—CH═CH—,

—C(═O)—CH_2—,

—CH(CN)—NH—,

—CH(OH)—CH_2—,

—O—C(═O)—NH—, and

—NHC(═O)NH—.

In another embodiment, Phe7 is substituted with its enantiomer D-Phe. In yet another embodiment, D-enantiomers are introduced to one or more, up to all 22, positions within wtCNP-22. In a further embodiment, a beta amino acid such as 3-amino-2-phenylpropionic acid is substituted for Phe7, which increases the length of the backbone while reducing the length of the side chain. In yet another embodiment, the disclosure contemplates Cys analogs at the Cys6 position including but not limited to homocysteine, penicillamine, 2-mercaptopropionic acid, and 3-mercaptopropionic acid.

Even in the presence of a NEP-resistant bond between Cys6 and Phe7, other peptide bonds can be hydrolyzed by NEP, including Gly8-Leu9, Lys10-Leu11, Arg13-Ile14, Ser16-Met17 and Gly19-Leu20. Accordingly, the disclosure encompasses CNP analogs containing peptide bond isosteres at multiple locations in the backbone of the CNP analogs. In one embodiment, CNP analogs or variants comprise modifications at more than one peptidase cleavage site. In a further embodiment, such variant comprises a CNP with substitutions at amino acid residues important in binding to the NEP active site, thereby increasing resistance to NEP degradation. One or more NEP-binding residues, including but not limited to Gly8, Gly15, Ser18, Gly19 and/or Gly21, are replaced with larger-size natural or unnatural amino acid residues to reduce affinity to the NEP active site. In yet another embodiment, one or more hydrophobic residues essential in NEP recognition, including but not limited to Phe7, Leu9, Leu11, Ile14, Met17 and Leu20, are substituted with natural or unnatural amino acids and/or peptidomimetics that decrease NEP binding. In yet another embodiment, one to five of the first five amino acids of CNP can be deleted or substituted with any other natural amino acids or unnatural amino acids or peptidomimetics, or one or more natural or unnatural amino acids or peptidomimetics can be added to any one or to all of the first five positions of CNP.

In a further embodiment, the CNP variants have a total mass characterized by the ranges described generally herein, e.g., from about 2.6 kDa or 2.8 kDa to about 6 or 7 kDa for increased resistance to NEP, and are represented by the formula:

(x)-Gly_1-Leu_2-Ser_3-$(a)_4$-Gly_5-$(b)_6$-$(c)_7$-$(d)_8$-$(e)_9$-$(f)_{10}$-$(g)_{11}$-Asp_{12}-Arg_{13}-$(h)_{14}$-Gly_{15}-Ser_{16}-$(i)_{17}$-Ser_{18}-Gly_{19}-$(j)_{20}$-Gly_{21}-Cys_{22}-(z) (SEQ ID NO: 46), wherein:

(x) may be absent or may be selected from the group consisting of synthetic bone-targeting compounds such as, e.g., bisphosphonates; amino acid sequences useful in bone or cartilage targeting such as, e.g., polyAsp and polyGlu; amino acid sequences derived from bone proteins with elucidated bone-targeting domains, such as, e.g., fusion proteins or peptide sequences of osteopontin, osteocalcin, sialoprotein, etc.; polymeric or non-polymeric molecules that reduce renal clearance such as, e.g., charged PEG molecules; and extensions comprising, e.g., polymers (e.g., PEGs), carbohydrates, hydrophobic acids (including fatty acids), and/or amino acids, and wherein such amino acid extensions can contain, e.g., from 1 to 31, or 1 to 35, or 5 to 35, or 10 to 35, or 15 to 35 amino acid residues, and can be derived from NPPC, ANP, BNP, other non-CNP (poly)peptides such as, e.g., serum albumin or IgG, or variants of the aforementioned polypeptides having substitutions, additions and/or deletions, or combinations thereof;

(z) may be absent or may be selected from the group consisting of amino acid sequences useful in bone or cartilage targeting such as for example polyAsp and polyGlu, amino acid sequences from bone-targeting domains of bone proteins such as, e.g., osteopontin, osteocalcin and sialoprotein, and amino acid sequences derived from non-CNP (poly)peptides such as, e.g., ANP or BNP;

(a) may be the wild type Lys at that position or may be replaced with a conservative amino acid substitution or a natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amine on a side chain, including but not limited to Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu, wherein in one embodiment (a) is Arg;

(b) is selected from the group consisting of Cys and peptide-bond isosteres between Cys6 and Phe7 such as, e.g., Cys-CH_2—NH;

(c) is selected from the group consisting of L-Phe; D-Phe; 3-amino-2-phenylpropionic acid; N-alkylated derivatives of Phe, wherein the N-alkyl group is methyl, ethyl, n-propyl, isopropyl, cyclopropyl, n-butyl, isobutyl, sec-butyl or tert-butyl; and Phe analogs, wherein one or more ortho-, meta-, and/or para-positions of the benzene ring of the Phe analog are substituted with one or more substituents selected from the group consisting of halogen, hydroxyl, cyano, straight or branched C_{1-6} alkyl, straight or branched C_{1-6} alkoxy, straight or branched halo-C_{1-6} alkyl, C_{3-10} cycloalkyl, heterocyclyl, C_{6-14} aryl and heteroaryl (examples include, but are not limited to, tyrosine, 3-chlorophenylalanine, 2,3-chloro-phenylalanine, 3-chloro-5-fluoro-phenylalanine, 2-chloro-6-fluoro-3-methyl-phenylalanine), or wherein the benzene ring of the Phe analog can be replaced with another aryl group (non-limiting examples include 1- and 2-naphthylalanine) or with a heteroaryl group (non-limiting examples include pyridylalanine, thienylalanine and furylalanine);

(d) is selected from the group consisting of Gly, tert-butyl-Gly (tBu-Gly), Thr, Ser, Val and Asn;

(e) is selected from the group consisting of Leu, Ser, Thr and peptide-bond isosteres such as, e.g., N-Me-Leu;

(f) may be the wild type Lys at that position or may be replaced with a conservative amino acid substitution or a natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amine on a side chain, including but not limited to Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu, wherein in one embodiment (f) is not Arg;

(g) is selected from the group consisting of Leu and peptide-bond isosteres such as, e.g., N-Me-Leu;

(h) is selected from the group consisting of Ile, tBu-Gly, and peptide-bond isosteres such as, e.g., N-Me-Ile;

(i) is selected from the group consisting of Met, Val, Asn, beta-Cl-Ala, 2-aminobutyric acid (Abu) and 2-amino-isobutyric acid (Aib); and

(j) is selected from the group consisting of Leu, norleucine (Nle), homoleucine (Hleu), Val, tert-butyl-Ala (tBu-Ala), Ser, Thr, Arg, and peptide-bond isosteres such as, e.g., N-Me-Leu.

In one embodiment, the CNP variants comprise a modification at one or more of positions 6, 7, 8, 9, 10, 11, 13, 14, 16, 17, 19 and/or 20, and may optionally have modifications at any of the other positions disclosed herein.

In embodiments described herein where CNP22 or variants thereof can be attached to a hydrophobic acid, the CNP peptides can be attached to one or hydrophobic acids. Non-limiting examples of hydrophobic acids include straight-chain or branched, saturated or unsaturated C_5-C_{12} carboxylic acids (e.g., pentanoic acid, heptanoic acid, etc.) and natural fatty acids. The hydrophobic acids can be attached to the N-terminus, the C-terminus, and/or the side chain of one or more amino acid residues. In one embodiment, the hydrophobic acids are conjugated to the N-terminus. In an embodiment, conjugation of CNP22 or a variant thereof to a hydrophobic acid is designed, inter alia, to promote non-specific interaction between the modified CNP peptide and serum albumin, thereby increasing the size of the CNP peptide and protecting it from cleavage by proteases such as, e.g., NEP. The interaction between the hydrophobic acid-conjugated CNP peptide and albumin is designed to be not too strong, so that the modified CNP peptide can diffuse through cartilage, get to chondrocytes of bone growth plates, and bind and activate NPR-B.

In a further embodiment, the disclosure provides CNP variants that in vitro or in vivo stimulate the production of at least about 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140% or 150% of the cGMP level produced under the same concentration of wtCNP22 (e.g., 1 uM), comprise at least one modified amino acid at position $(b)_6$, $(c)_7$ and/or $(d)_8$, and are represented by the general formula:

(x)-Gly_1-Leu_2-Ser_3-$(a)_4$-Gly_5-$(b)_6$-$(c)_7$-$(d)_8$-$(e)_9$-$(f)_{10}$-$(g)_{11}$-Asp_{12}-Arg_{13}-$(h)_{14}$-Gly_{15}-Ser_{16}-$(i)_{17}$-Ser_{18}-Gly_{19}-$(j)_{20}$-Gly_{21}-Cys_{22}-(z) (SEQ ID NO: 47), wherein:

(x) may be absent or may be a peptide sequence containing one to five amino acids which is derived from a natriuretic polypeptide (e.g. NPPC, CNP, ANP or BNP) or a non-natriuretic polypeptide as described herein (e.g., HSA, IgG, a bone-targeting protein, etc.);

(z) may be absent or may be selected from the group consisting of synthetic bone-targeting compounds such as, e.g., bisphosphonates; amino acid sequences useful in bone/cartilage targeting such as, e.g., polyAsp and polyGlu; bone proteins with bone-targeting domains and derivatives thereof, such as fusion proteins or peptides sequences of osteopontin, osteocalcin, and sialoprotein; molecules that reduce renal clearance, such as, e.g., charged PEGs; and molecules that increase resistance of CNP to NEP-mediated degradation, as described herein;

(a) may be the wild type Lys at that position or may be replaced with a conservative amino acid substitution or a natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amine on a side chain, including but not limited to Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu, wherein in one embodiment (a) is Arg;

(b) may be Cys or descarboxy cysteine, or the $(b)_6$-$(c)_7$ peptide bond (—C(═O)—NH—) may be replaced with any one of the following peptide bond isosteres:

—CH_2—NH—,
—C(═O)—N(R)—, where the amide group is alkylated with any of the following R groups: methyl, ethyl, n-propyl, isopropyl, cyclopropyl, n-butyl, isobutyl, sec-butyl or tert-butyl,
—C(═O)—NH—CH_2—,
—CH_2—S—,
—CH_2—$S(O)_n$—, where n is 1 or 2,
—CH_2—CH_2—,
—CH═CH—,
—C(═O)—CH_2—,
—CH(CN)—NH—,
—CH(OH)—CH_2—,
—O—C(═O)—NH—, or
—NHC(═O)NH—;

(c) is selected from the group consisting of L-Phe; D-Phe; 3-amino-2-phenylpropionic acid; peptide bond isosteres of Phe such as N-alkylated derivatives of Phe wherein the N-alkyl group is selected from the group consisting of methyl, ethyl, n-propyl, isopropyl, cyclopropyl, n-butyl, isobutyl, sec-butyl and tert-butyl; and Phe analogs, wherein one or more ortho-, meta-, and/or para-positions of the benzene ring of the Phe analog are substituted with one or more substituents selected from the group consisting of halogen, hydroxyl, cyano, straight or branched C_{1-6} alkyl, straight or branched C_{1-6} alkoxy, straight or branched halo-C_{1-6} alkyl, C_{3-10} cycloalkyl, C_{6-14} aryl, heterocyclyl and heteroaryl (examples include, but are not limited to, tyrosine, 3-chlorophenylalanine, 2,3-chloro-phenylalanine, 3-chloro-5-fluoro-phenylalanine, 2-chloro-6-fluoro-3-methyl-phenylalanine), or wherein the benzene ring of the Phe analog can be replaced with another aryl group (non-limiting examples include 1- and 2-naphthylalanine) or with a heteroaryl group (non-limiting examples include pyridylalanine, thienylalanine and furylalanine);

(d) is selected from the group consisting of Gly, tert-butyl-Gly (tBu-Gly), Val, Ser, Thr and Asn;

(e) is selected from the group consisting of Leu, Ser, Thr, and peptide-bond isosteres such as, e.g., N-Me-Leu;

(f) is any natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amino group on a side chain, including but not limited to Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu, wherein in one embodiment (f) is not Arg;

(g) is selected from the group consisting of Leu and peptide-bond isosteres such as, e.g., N-Me-Leu;

(h) is selected from the group consisting of Ile, tBu-Gly and peptide-bond isosteres such as, e.g., N-Me-Ile;

(i) is selected from the group consisting of Met, Val, Asn, beta-Cl-Ala, 2-aminobutyric acid (Abu) and 2-amino-isobutyric acid (Aib); and

(j) is selected from the group consisting of Leu, norleucine (Nle), homoleucine (Hleu), Val, tert-butyl-Ala (tBu-Ala), Ser, Thr, Arg, and peptide-bond isosteres such as, e.g., N-Me-Leu.

In a further embodiment, the disclosure encompasses CNP variants that in vitro or in vivo stimulate the production of at least about 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140% or 150% of the cGMP level produced under the same concentration of wtCNP22 (e.g., 1 uM), have a total mass characterized by the ranges described generally herein, e.g., from about 2.6 kDa or 2.8 kDa to about 6 or 7 kDa for increased resistance to NEP degradation, and are represented by the general formula:

(x)-(y)-Cys_6-Phe_7-Gly_8-Leu_9-$(h)_{10}$-Leu_{11}-Asp_{12}-Arg_{13}-Ile_{14}-Gly_{15}-Ser_{16}-Met_{17}-Ser_{18}-Gly_{19}-Leu_{20}-Gly_{21}-Cys_{22}-(z) (SEQ ID NO: 48), wherein:

(x) is a synthetic or natural polymeric group, or a combination thereof, wherein a non-limiting example of a synthetic

polymeric group is polyethylene glycol (PEG), and a non-limiting example of a natural polymeric group is an amino acid sequence containing from 1 to 35 amino acids and derived from NPPC or variants thereof with substitutions and/or deletions, ANP, BNP, or other non-CNP (poly)peptides such as, e.g., serum albumin, IgG, histidine-rich glycoproteins, fibronectin, fibrinogen, zinc finger-containing polypeptides, osteocrin or FGF2;

(y) may be absent or may be one or more amino acids from Gly_1-Leu_2-Ser_3-Lys_4-Gly_5 (corresponding to positions 1 to 5 of CNP22) (SEQ ID NO: 1) and/or substitutions at one or more of those positions using natural or unnatural amino acids (e.g., K4R substitution);

(h) may be the wild-type Lys at that position or may be replaced with a conservative amino acid substitution or a natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amine on a side chain, including but not limited to Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu, wherein in one embodiment (h) is not Arg; and

(z) may be absent or may be a synthetic or natural polymeric group, or a combination thereof, wherein a non-limiting example of a synthetic polymeric group is PEG, and a non-limiting example of a natural polymeric group is an amino acid sequence derived from a natriuretic polypeptide (e.g., NPPC, CNP, ANP or BNP) or non-natriuretic polypeptide (e.g., serum albumin or IgG).

In an embodiment, (x), (y) and (z) together contain from about 10 to about 40, or from about 15 to about 35 amino acids. In another embodiment, (x) is an amino acid sequence comprising from 1 to 40 amino acids, or from 1 to 20 amino acids.

Further contemplated are CNP variants that in vitro or in vivo stimulate the production of at least about 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140% or 150% of the cGMP level produced under the same concentration of wtCNP22 (e.g., 1 uM), and comprise the sequence:

(y)-Cys_6-Phe_7-Gly_8-Leu_9-Lys_{10}-Leu_{11}-Asp_{12}-Arg_{13}-Ile_{14}-Gly_{15}-Ser_{16}-Met_{17}-Ser_{18}-Gly_{19}-Leu_{20}-Gly_{21}-Cys_{22} (SEQ ID NO: 138), wherein:

(y) comprises one or more amino acids selected from Gly_1-Leu_2-Ser_3-Lys_4-Gly_5 (SEQ ID NO: 1) and/or substitutions at one or more of those positions using natural or unnatural amino acids (e.g., K4R substitution), and further comprises a hydrophilic or water soluble polymer of molecular weight from about 0.6 kDa to about 5 kDa. In an embodiment, the hydrophilic or water-soluble polymer is conjugated to the N-terminus of such amino acid-extended CNP variant. In a particular embodiment, the hydrophilic or water-soluble polymer is PEG (or PEO).

In yet another embodiment, the disclosure provides CNP variants that in vitro or in vivo stimulate the production of at least about 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140% or 150% of the cGMP level produced under the same concentration of wtCNP22 (e.g. 1 uM), wherein the CNP variants comprise an N-terminal and/or C-terminal peptide extension containing from 1 to 15 amino acids, and are conjugated to a hydrophilic or water soluble polymer. In an embodiment, the peptide extension contains from 5 to 10 amino acids. In a specific embodiment, the peptide extension contains 5 amino acids. In another specific embodiment, the hydrophilic or water-soluble polymer is PEG (or PEO).

In a still further embodiment, the CNP variants of the disclosure in vitro or in vivo stimulate the production of at least about 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140% or 150% of the cGMP level produced under the same concentration of wtCNP22 (e.g. 1 uM), and comprise at least a 15 amino acid fragment derived from natriuretic peptide precursor C(NPPC), wherein the fragment is at least 70% homologous to a sequence from wild type NPPC containing the same number of amino acid residues.

In still another embodiment, the CNP variants have a total mass characterized by the ranges described generally herein, e.g., from about 2.6 kDa or 2.8 kDa to about 6 or 7 kDa for increased NEP resistance, and are represented by the formula: (x)-$(b)_6$-$(c)_7$-$(d)_8$-$(e)_9$-$(f)_{10}$-$(g)_{11}$-Asp_{12}-Arg_{13}-$(h)_{14}$-Gly_{15}-Ser_{16}-$(i)_{17}$-Ser_{18}-Gly_{19}-$(j)_{20}$-Gly_{21}-Cys_{22}-(z) (SEQ ID NO: 49), wherein:

(x) may be absent (i.e., the N-terminus ends with an —NH_2 group) or may be selected from the group consisting of a sequence of 1, 2, 3, 4 or 5 amino acids from the peptide Gly_1-Leu_2-Ser_3-Lys_4-Gly_5 (SEQ ID NO: 1); amino acid sequences useful in bone/cartilage targeting such as for example polyAsp or polyGlu; bone-targeting domains from bone proteins such as for example osteopontin, osteocalcin or sialoprotein; molecules that reduce renal clearance such as hydrophilic or water-soluble polymers, including but not limited to charged PEG molecules; and moieties comprising PEG, carbohydrates, hydrophobic acids, amino acids, or combinations thereof, wherein such moieties can be amino acid extensions including but not limited to amino acid sequences derived from NPPC or non-CNP (poly)peptides such as, e.g., BNP, ANP, serum albumin or IgG;

(z) may be absent or may be selected from the group consisting of amino acid sequences useful in bone/cartilage targeting such as for example polyAsp or polyGlu; amino acid sequences derived from bone-targeting proteins, such as for example osteopontin, osteocalcin or sialoprotein; and amino acid sequences derived from NPPC or non-CNP (poly) peptides, as described herein;

(b) is selected from the group consisting of Cys and peptide bond isosteres between Cys6 and Phe7 such as, e.g., Cys-CH_2—NH;

(c) is selected from the group consisting of L-Phe; D-Phe; 3-amino-2-phenylpropionic acid; N-alkylated derivatives of Phe, wherein the N-alkyl group is methyl, ethyl, n-propyl, isopropyl, cyclopropyl, n-butyl, isobutyl, sec-butyl or tert-butyl; and Phe analogs, wherein one or more ortho-, meta-, and/or para-positions of the benzene ring of the Phe analog are substituted with one or more substituents selected from the group consisting of halogen, hydroxyl, cyano, straight or branched C_{1-6} alkyl, straight or branched C_{1-6} alkoxy, straight or branched halo-C_{1-6} alkyl, C_{3-10} cycloalkyl, C_{6-14} aryl, heterocyclyl and heteroaryl (examples include, but are not limited to, tyrosine, 3-chlorophenylalanine, 2,3-chloro-phenylalanine, 3-chloro-5-fluoro-phenylalanine, 2-chloro-6-fluoro-3-methyl-phenylalanine), or wherein the benzene ring of the Phe analog can be replaced with another aryl group (non-limiting examples include 1- and 2-naphthylalanine) or with a heteroaryl group (non-limiting examples include pyridylalanine, thienylalanine and furylalanine);

(d) is selected from the group consisting of Gly, tert-butyl-Gly (tBu-Gly), Val, Ser, Thr and Asn;

(e) is selected from the group consisting of Leu, Ser, Thr, and peptide-bond isosteres such as, e.g., N-Me-Leu;

(f) may be the wild type Lys at that position or may be replaced with a conservative amino acid substitution or a natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amine on a side chain, including but not limited to Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu, wherein in one embodiment (f) is not Arg;

(g) is selected from the group consisting of Leu and peptide-bond isosteres such as, e.g., N-Me-Leu;

(h) is selected from the group consisting of Ile, tBu-Gly and peptide-bond isosteres such as, e.g., N-Me-Ile;

(i) is selected from the group consisting of Met, Val, Asn, beta-Cl-Ala, 2-aminobutyric acid (Abu) and 2-amino-isobutyric acid (Aib); and

(j) is selected from the group consisting of Leu, norleucine (Nle), homoleucine (Hleu), Val, tert-butyl-Ala (tBu-Ala), Ser, Thr, Arg, and peptide-bond isosteres such as, e.g., N-Me-Leu.

In a further embodiment, the CNP variants have a total mass characterized by the ranges described generally herein, e.g., from about 2.6 kDa or 2.8 kDa to about 6 or 7 kDa for increased NEP resistance, and are represented by the formula: (x)-Gly_1-Leu_2-Ser_3-$(a)_4$-Gly_5-$(b)_6$-$(c)_7$-$(d)_8$-$(e)_9$-$(f)_{10}$-$(g)_{11}$-Asp_{12}-Arg_{13}-$(h)_{14}$-$(i)_{15}$-Ser_{16}-$(j)_{17}$-Ser_{18}-Gly_{19}-$(k)_{20}$-Gly_{21}-Cys_{22}-(z) (SEQ ID NO: 50), wherein:

(x) and (z) independently may be absent or may be selected from the group consisting of synthetic bone-targeting compounds such as, e.g., bisphosphonates; amino acid sequences useful in bone/cartilage targeting such as for example polyAsp or polyGlu; amino acid sequences derived from bone-targeting domains of bone proteins and derivatives thereof, such as for example fusion proteins or peptide sequences of osteopontin, osteocalcin, sialoprotein, etc.; moieties that reduce renal clearance, including but not limited to hydrophilic or water-soluble polymers such as, e.g., charged PEG molecules; and moieties comprising, e.g., hydrophilic polymers (e.g., PEG), carbohydrates, hydrophobic acids, and/or amino acids;

(a) may be the wild type Lys at that position or may be replaced with a conservative amino acid substitution or a natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amine on a side chain, including but not limited to Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu, wherein in one embodiment (a) is Arg;

(b) is selected from the group consisting of Cys and peptide bond isosteres between Cys6 and Phe7 such as, e.g., Cys-CH_2—NH;

(c) is selected from the group consisting of L-Phe; D-Phe; 3-amino-2-phenylpropionic acid; N-alkylated derivatives of Phe, wherein the N-alkyl group is methyl, ethyl, n-propyl, isopropyl, cyclopropyl, n-butyl, isobutyl, sec-butyl or tert-butyl; and Phe analogs, wherein one or more ortho-, meta-, and/or para-positions of the benzene ring of the Phe analog are substituted with one or more substituents selected from the group consisting of halogen, hydroxyl, cyano, straight or branched C_{1-6} alkyl, straight or branched C_{1-6} alkoxy, straight or branched halo-C_{1-6} alkyl, C_{3-10} cycloalkyl, C_{6-14} aryl, heterocyclyl and heteroaryl (examples include, but are not limited to, tyrosine, 3-chlorophenylalanine, 2,3-chloro-phenylalanine, 3-chloro-5-fluoro-phenylalanine, 2-chloro-6-fluoro-3-methyl-phenylalanine), or wherein the benzene ring of the Phe analog can be replaced with another aryl group (non-limiting examples include 1- and 2-naphthylalanine) or with a heteroaryl group (non-limiting examples include pyridylalanine, thienylalanine and furylalanine);

(d) is selected from the group consisting of Gly, tert-butyl-Gly, Thr, Ser, Val and Asn;

(e) is selected from the group consisting of Leu, Ser, Thr, and peptide bond isosteres such as, e.g., N-Me-Leu;

(f) may be the wild type Lys at that position or may be replaced with a conservative amino acid substitution or a natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amine on a side chain, including but not limited to Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu, wherein in one embodiment (f) is not Arg;

(g) is selected from the group consisting of Leu, Asn, and peptide bond isosteres such as, e.g., N-Me-Leu;

(h) is selected from the group consisting of Ile, tert-butyl-Gly (tBu-Gly), Asn, and peptide bond isosteres such as, e.g., N-Me-Ile;

(i) is selected from the group consisting of Gly, Arg, Ser and Asn;

(j) is selected from the group consisting of Met, Val, Asn, beta-Cl-Ala, 2-aminobutyric acid (Abu) and 2-amino-isobutyric acid (Aib); and

(k) is selected from the group consisting of Leu, norleucine (Nle), homoleucine (Hleu), Val, tert-butyl-Ala (tBu-Ala), Arg, Thr, Ser, and peptide bond isosteres such as, e.g., N-Me-Leu.

In a further embodiment, the CNP variants can have amino acid substitution(s) at one or more of any of positions 1 to 22 of CNP22. In one embodiment, Gly1 is substituted with Arg or Glu. In another embodiment, Lys4 is replaced with Arg. In still another embodiment, Gly5 is substituted with Arg, Gln or Ser. In yet another embodiment, Gly15 is substituted with Ser, Asn, Arg or Cit. In a further embodiment, Gly19 is substituted with Ser, Arg or Asn. In yet another embodiment, Gly21 is substituted with Ser, Thr, or Arg.

In one embodiment, the CNP variant is selected from the group consisting of GLSKGC(CH_2NH)FGLKLDRIGSMSGLGC (formed using descarboxy-Cys) (SEQ ID NO: 56), GLSKGC-(N-Me-Phe)-GLKLDRIGSMSGLGC (SEQ ID NO: 57), GLSKGC-(D-Phe)-GLKLDRIGSMSGLGC(SEQ ID NO:136), GLSKGCF-(tBuG)-LKLDRIGSMSGLGC (SEQ ID NO: 58), GLSKGC-(3-Cl-Phe)-GLKLDRIGSMSGLGC (SEQ ID NO:137), and GLSKGC-[NHCH_2CH(Ph)CO]-GLKLDRIGSMSGLGC (formed using 3-amino-2-phenylpropionic acid) (SEQ ID NO: 59). In a further embodiment, a disulfide bond exists between Cys6, descarboxy-Cys or another sulfhydryl-containing cysteine analog at the Cys6 position, and Cys22 of any CNP variant described herein.

In another embodiment, the CNP variants contain an amino acid extension at the N-terminus and/or C-terminus of CNP22 or CNP17, including but not limited to:

(SEQ ID NO: 4)
DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-53);

(SEQ ID NO: 60)
QEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-37, Analog BL);

(SEQ ID NO: 61)
AAWARLLQEHPNAGLSKGCFGLKLDRIGSMSGLGC (Analog CA);

(SEQ ID NO: 62)
AAWARLLQEHPNARGLSKGCFGLKLDRIGSMSGLGC (Analog CB);

(SEQ ID NO: 63)
DLRVDTKSRAAWARGLSKGCFGLKLDRIGSMSGLGC (Analog CC);

(SEQ ID NO: 40)
RGLSKGCFGLKLDRIGSMSGLGC;

(SEQ ID NO: 38)
ERGLSKGCFGLKLDRIGSMSGLGC;

(SEQ ID NO: 64)
GANQQGLSKGCFGLKLDRIGSMSGLGC;

(SEQ ID NO: 65)
GANRRGLSKGCFGLKLDRIGSMSGLGC;

-continued

(SEQ ID NO: 66)
GANPRGLSKGCFGLKLDRIGSMSGLGC;

(SEQ ID NO: 67)
GANSSGLSKGCFGLKLDRIGSMSGLGC;

(SEQ ID NO: 144)
GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC
(sometimes designated "CNP27-HSA" or "HSA-CNP27"
in the Examples and figures);

(SEQ ID NO: 68)
SPKMVQGSG-CNP17-KVLRRH (Analog CD) (CNP17 having
N-terminal and C-terminal tails derived from BNP).

In a further embodiment, the CNP variants have a K4R substitution at position 4 of CNP22. Non-limiting examples of CNP(K4R) variants include:

((SEQ ID NO: 36)
GANRRGLSRGCFGLKLDRIGSMSGLGC (Analog AY);

(SEQ ID NO: 37)
GANPRGLSRGCFGLKLDRIGSMSGLGC (Analog CI);

(SEQ ID NO: 41)
RGLSRGCFGLKLDRIGSMSGLGC (Analog AZ);

(SEQ ID NO: 39)
ERGLSRGCFGLKLDRIGSMSGLGC (Analog BA);

(SEQ ID NO: 69)
GANQQGLSRGCFGLKLDRIGSMSGLGC (Analog CH);
and

(SEQ ID NO: 70)
GANSSGLSRGCFGLKLDRIGSMSGLGC (Analog CG).

In one embodiment, CNP variants having a PEG (or PEO) moiety and an amino acid extension at the N-terminus contain arginine at the position immediately preceding the position corresponding to Gly1 of CNP22. Such PEGylated CNP variants are designed for increased resistance to NEP degradation, reduced binding to serum albumin, and enhanced CNP functional activity (e.g., activation of cGMP signaling). Non-limiting examples of PEGylated CNP variants include PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36), PEO12-GANRR-CNP22(K4R) (SEQ ID NO: 36), PEO24-GANRR-CNP22(SEQ ID NO: 65), PEO12-GANRR-CNP22(SEQ ID NO: 65), PEO24-GANPR-CNP22(K4R) (SEQ ID NO: 37), PEO12-GANPR-CNP22(K4R) (SEQ ID NO: 37), PEO24-GANPR-CNP22(SEQ ID NO: 37), PEO12-GANPR-CNP22 (SEQ ID NO: 66), PEO24-GANQQ-CNP22(SEQ ID NO: 64), PEO12-GANQQ-CNP22(SEQ ID NO: 64), PEO24-ER-CNP22(K4R) (SEQ ID NO: 39), PEO12-ER-CNP22(K4R) (SEQ ID NO: 39), PEO24-ER-CNP22(SEQ ID NO: 38), PEO12-ER-CNP22(SEQ ID NO: 38), PEO24-R-CNP22 (K4R) (SEQ ID NO: 41), PEO12-R-CNP22(K4R) (SEQ ID NO: 41), PEO24-R-CNP22(SEQ ID NO: 40), and PEO12-R-CNP22(SEQ ID NO: 40), wherein PEO24 is a monodispersed 1.2 kDa PEG polymer and PEO12 is a monodispersed 0.6 kDa PEG polymer. In an embodiment, the PEG (or PEO) polymer is attached to the N-terminus of the CNP variants.

Additional CNP variants include, but are not limited to, derivatives of CNP37 having mutation(s) at the furin cleavage site (underlined), designed to improve in vivo resistance to the furin protease, and/or having glycine (underlined) preceding glutamine, designed to prevent pyroglutamine formation, including but not limited to:

(SEQ ID NO: 71)
GQEHPNARKYKGANPKGLSKGCFGLKLDRIGSMSGLGC (An. CS);

(SEQ ID NO: 72)
GQEHPNARKYKGANQKGLSKGCFGLKLDRIGSMSGLGC (An. CT);

(SEQ ID NO: 73)
GQEHPNARKYKGANQQGLSKGCFGLKLDRIGSMSGLGC (An. CU);

(SEQ ID NO: 74)
GQEHPNARKYKGANKPGLSKGCFGLKLDRIGSMSGLGC (An. CW);

(SEQ ID NO: 75)
GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC
(Gly-CNP37, An. DB);

(SEQ ID NO: 145)
PGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC
(Pro-Gly-CNP37).

In another embodiment, the CNP variants are chimera comprising CNP22 and an N-terminal peptide fragment, including but not limited to:

(SEQ ID NO: 76)
GHHSHEQHPHGANQQGLSKGCFGLKLDRIGSMSGLGC (Analog CQ)
(histidine-rich glycoprotein (HRGP) fragment-
CNP22 chimera);

(SEQ ID NO: 77)
GAHHPHEHDTHGANQQGLSKGCFGLKLDRIGSMSGLGC (Analog CR)
(HRGP fragment-CNP22 chimera);

(SEQ ID NO: 78)
GHHSHEQHPHGANPRGLSKGCFGLKLDRIGSMSGLGC (Analog CX)
(HRGP fragment-CNP22 chimera);

(SEQ ID NO: 79)
GQPREPQVYTLPPSGLSKGCFGLKLDRIGSMSGLGC (Analog CF)
($IgG_1(F_c)$ fragment-CNP22 chimera);

(SEQ ID NO: 80)
GQHKDDNPNLPRGANPRGLSKGCFGLKLDRIGSMSGLGC (Analog
CY) (human serum albumin (HSA) fragment-CNP22
chimera);

(SEQ ID NO: 81)
GERAFKAWAVARLSQGLSKGCFGLKLDRIGSMSGLGC (Analog CE)
(HSA fragment-CNP22 chimera);

(SEQ ID NO: 82)
FGIPMDRIGRNPRGLSKGCFGLKLDRIGSMSGLGC (Analog CZ)
(osteocrin "NPR C inhibitor" fragment-CNP22
chimera);
and

(SEQ ID NO: 83)
GKRTGQYKLGSKTGPGPKGLSKGCFGLKLDRIGSMSGLGC
(Analog DA) (FGF2 "heparin-binding domain"
fragment-CNP22 chimera).

In a further embodiment, the CNP variants are chimera comprising an N-terminal peptide fragment and CNP22 in which arginine is substituted for Lys4 of CNP22 ("CNP22 (K4R)"), including but not limited to:

(SEQ ID NO: 84)
GQPREPQVYTGANQQGLSRGCFGLKLDRIGSMSGLGC (Analog CK)
($IgG_1(F_c)$ fragment-CNP22(K4R) chimera);

(SEQ ID NO: 85
GVPQVSTSTGANQQGLSRGCFGLKLDRIGSMSGLGC (Analog CL)
(HSA fragment-CNP22(K4R) chimera)

-continued

(SEQ ID NO: 86)
GQPSSSSQSTGANQQGLSRGCFGLKLDRIGSMSGLGC
(Analog CM) (fibronectin fragment-CNP22(K4R)
chimera);

(SEQ ID NO: 87)
GQTHSSGTQSGANQQGLSRGCFGLKLDRIGSMSGLGC
(Analog CN) (fibrinogen fragment-CNP22(K4R)
chimera);

(SEQ ID NO: 88)
GSTGQWHSESGANQQGLSRGCFGLKLDRIGSMSGLGC
(Analog CO) (fibrinogen fragment-CNP22(K4R)
chimera);
and

(SEQ ID NO: 89)
GSSSSSSSSSGANQQGLSRGCFGLKLDRIGSMSGLGC
(Analog CP) (zinc finger fragment-CNP22(K4R)
chimera).

In yet another embodiment, the CNP variants are chimera, or fusion proteins, comprising a CNP peptide or variant, and a cleavable peptide or protein, or peptide tag. Exemplary cleavable proteins or peptides include, but are not limited to, histidine (e.g., hexa-His) tags; TAF12: human transcription factor TAF12; KSI: ketosteroid isomerase; MBP: maltose-binding protein; β-Gal: β-galactosidase; GST: glutathione-S-transferase; Trx: thioredoxin; CBD: chitin binding domain; BMPM: BMP-2 mutation, SUMO, CAT, TrpE, staphylococcal protein A, streptococcal proteins, starch-binding protein, cellulose-binding domain of endoglucanase A, cellulose-binding domain of exoglucanase Cex, biotin-binding domain, recA, Flag, c-Myc, poly(His), poly(Arg), poly(Asp), poly (Gln), poly(Phe), poly(Cys), green fluorescent protein, red fluorescent protein, yellow fluorescent protein, cyan fluorescent protein, biotin, avidin, streptavidin, antibody epitopes, and fragments thereof.

In yet another embodiment, the CNP variant may be a monomer or a dimer. In a related embodiment the monomers of dimeric CNP variants can be attached N-terminus to N-terminus via a linker or no linker, N-terminus to C-terminus via a linker or no linker, or C-terminus to C-terminus via a linker or no linker.

Chimera comprising an IgG fragment and CNP22 or a variant thereof are designed for, inter alia, increased resistance to NEP degradation and reduced binding to serum albumin. CNP chimera comprising a surface fragment of HSA are designed for, inter alia, reduced immunogenicity and reduced binding to serum albumin. HRGP-CNP22 and HRGP-CNP22(K4R) chimera containing a cationic, histidine-rich, non-lysine, non-arginine sequence at the N-terminus are designed for, inter alia, increased stability to proteases. Chimera containing an osteocrin fragment are designed to release, upon protease (e.g., furin) cleavage, the osteocrin fragment at bone growth plates, where the fragment would inhibit the clearance receptor NPR-C. With respect to chimera comprising an FGF2 heparin-binding fragment, heparin binding to the fragment is designed to protect the chimera from degradation, thereby providing a longer serum half-life. Chimera containing a fibronectin, fibrinogen, or zinc-finger fragment are designed for reduced binding to serum albumin, among other advantageous features.

Not intending to be bound by theory, a CNP variant of molecular weight from about 2.6 kDa or 2.8 kDa to about 6 or 7 kDa which has increased resistance to NEP degradation and has similar or improved functionality (e.g., binding to NPR-B and stimulation of cGMP signaling) as compared to wtCNP22, may be more effective if it does not bind tightly to plasma proteins such as serum albumin. A CNP variant that does not bind tightly to plasma proteins (e.g., serum albumin) may be more effective in diffusing through cartilage, getting to chondrocytes of bone growth plates, and binding to and activating NPR-B for cGMP signaling. In one embodiment, CNP variants designed for reduced binding to plasma proteins (e.g., serum albumin) are chimeras comprising CNP22 or a variant thereof and a peptide fragment from IgG. In another embodiment, CNP variants designed for reduced binding to plasma proteins are chimeras comprising CNP22 or CNP22(K4R) and a fragment from a polypeptide (e.g., IgG, HSA, fibronectin, fibrinogen, a zinc finger-containing polypeptide, etc.). In yet another embodiment, CNP variants designed for reduced binding to plasma proteins comprise CNP22 or a variant thereof conjugated to a hydrophilic or water-soluble polymer. In one embodiment, the hydrophilic or water-soluble polymer is PEG (or PEO). In another embodiment, the hydrophilic or water-soluble polymer (e.g., PEG) is functionalized with one or more functional groups that impart a negative charge to the polymer under physiological conditions, such as, e.g, carboxyl, sulfate or phosphate groups, or a combination thereof.

In any of the embodiments disclosed herein, the CNP variants may have substantially the same or better biological activity than wild-type CNP22. For example, the CNP variants may retain at least 50%, 60%, 70%, 80%, 90%, 95% or more of the activity of wild-type CNP22, or may have greater activity than CNP22, e.g., with respect to interaction with NPR-B (GC-B) to stimulate the generation of cGMP. Alternatively, or in addition, the CNP variants may retain at least 50%, 60%, 70%, 80%, 90%, 95% or more of the activity of wild-type CNP22, or may have greater activity than CNP22, with respect to regulating endochondral bone growth and chondrocyte activity, including but not limited to chondrocyte proliferation, chondrocyte differentiation, inhibition of the mitogen activated protein (MAP) kinase/MEK (Raf-1) kinase signaling pathway, and promoting endochondral ossification. In any of the embodiments described herein, the CNP variants may comprise an amino acid sequence that is at least 40%, 50%, 60%, 70%, 80%, 90%, 95% or more identical or homologous to amino acids 6-22 or 1-22 of wild-type CNP22.

In a further embodiment, the disclosure provides variants of CNP22 having less affinity to the NPR-C clearance receptor while retaining the ability to bind and activate NPR-B. The present disclosure encompasses variants that were, or can be, generated from a homology-based structural model of the NPR-B/CNP complex as described in the Detailed Description. In another embodiment, the CNP variants have substitution(s) at one or more Gly sites at positions 1, 5, 8, 15, 19 and 21, to reduce conformational flexibility, which may increase their specificity for binding to NPR-B over NPR-C. Variants of CNP having potentially reduced affinity to the NPR-C include but are not limited to those having one or more of the following substitutions: G1R, G1E, G5R, G5Q, G5S, F7Y, G8T, G8S, G8V, G8N, L9S, L9T, K10Cit, K10Q, K10S, I14N, G15R, G15S, G15N, G15Cit, S16Q, M17V, M17N, G19S, G19R, G19N, L20V, L20R, L20T, L20S, G21S, G21T and G21R.

In yet another embodiment, the CNP variants have a modification and/or substitution at one or more of positions 5, 7, 8, 9, 10, 14, 15, 16, 17, 19, 20 and 21, and may optionally have modifications and/or substitutions at any of the other positions disclosed herein. In a further embodiment, the CNP variants can optionally have conjugation(s) or extension(s), e.g., at the N- and/or C-terminus to facilitate bone/cartilage targeting, reduce renal clearance, and/or increase resistance to NEP degradation. Such conjugation(s) or extension(s) can comprise molecules or sequences formed or derived from,

e.g., polyAsp, polyGlu, bone- or cartilage-targeting peptides, osteopontin, osteocalcin, sialoprotein, PEGs, carbohydrates, hydrophobic acids, NPPC or non-CNP (poly)peptides, or combinations thereof.

In still another embodiment, the CNP variants are prepared by standard solid-phase peptide synthesis methods with natural or unnatural amino acid(s) or peptidomimetic(s) being substituted and/or added where appropriate. In another embodiment, the CNP variants are produced by recombinant synthesis processes, e.g., via fusion proteins containing a tag or carrier protein, wherein use of the tag or carrier protein facilitates, e.g., detection, isolation and/or purification of the fusion protein, and selective chemical or proteolytic cleavage of the tag or carrier protein from the fusion protein provides the target CNP variant. In a further embodiment, PEGylation of the CNP variants occurs following, or part of, chemical or biological synthesis with the conjugation reaction being performed by NHS- or aldehyde-based chemistry or other chemistry known in the art. In another embodiment, the CNP variants comprise a disulfide bond. In a related embodiment, the disulfide bond forms a cyclic peptide. In a particular embodiment, the disulfide bond is formed between cysteine residues at positions corresponding to positions 6 and 22 of CNP22.

It is further contemplated that the CNP variants can be conjugated to a hydrophobic polymeric or non-polymeric moiety, such as, e.g., heptanoic acid, pentanoic acid, or fatty acids. The hydrophobic moiety can be conjugated to the side chain of an amino acid residue, including but not limited to a lysine, a serine, a cysteine or a threonine, or can be attached to the N-terminus and/or C-terminus of the CNP variant.

In an embodiment, the CNP variants as described herein have a pI in the range from about 8 to about 10.5 or from about 8.5 to about 10.

In a further embodiment, the disclosure provides a pharmaceutical composition comprising a CNP variant, optionally another biologically active agent, and optionally a pharmaceutically acceptable excipient, carrier or diluent. In some embodiments, the compositions are sterile pharmaceutical compositions suitable for parenteral injection. In some embodiments, the compositions comprise substantially pure CNP variant, e.g. at least about 90% or 95% pure. In some embodiments, the compositions contain less than about 5%, 4%, 3%, 2%, 1% or 0.5% contaminants, such as other human proteins, porcine proteins, or CNP53 or fragments thereof (other than the desired CNP variant). In certain embodiments, the sterile composition is administered to a subject for treating or preventing any of the CNP-responsive conditions or disorders disclosed herein.

CNP variants of the disclosure advantageously retain CNP activity and exhibit increased serum half-life. Retention of CNP activity can be shown, for example, as retention of desired in vivo biological effect, or retention of at least about 50%, 60%, 70%, 80%, 90%, 95% or at least about 100% of the cGMP stimulating activity of CNP22, under the same concentration (e.g., 1 uM of CNP peptide or greater than the ED80). In some embodiments, CNP variants exhibit at least about 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, 15-fold, 20-fold, 25-fold, 30-fold, 35-fold or 40-fold increase in serum half-life compared to CNP22.

In a related embodiment, the CNP variants described herein have increased NEP resistance and exhibit increased half-life compared to wild-type CNP22. In one embodiment, the half-life of the CNP variants is increased by about 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or about 100% compared to wild-type CNP22.

In certain embodiments, the CNP variants described herein increase cGMP production in vitro, increase cGMP production in vivo, increase in vivo the level of one or more biomarkers associated with cartilage or bone formation or growth, increase resistance to NEP cleavage in vitro, increase plasma or serum half-life in vivo, increase bioavailability in vivo, or increase the length of particular bones in vivo, or effect combinations of such increases, by about 1.5-fold, about 2-fold, about 2.5-fold, about 3-fold, about 3.5-fold, about 4-fold, about 4.5-fold, or about 5-fold or more compared to wild-type CNP22.

In yet another embodiment, the disclosure provides methods of treating conditions or disorders responsive to CNP, comprising administering a therapeutically effective amount of a CNP variant or a composition comprising the same to a subject in need thereof. In one embodiment, disorders responsive to CNP are disorders of bone growth, including but not limited to skeletal dysplasias and inherited skeletal malformations such as disorders associated with fibroblast growth factor receptor 3 (FGFR-3) mutations. In a specific embodiment, the disorder associated with FGFR-3 mutation(s) is achondroplasia. In another embodiment, the disorders responsive to CNP are disorders associated with vascular smooth muscle cells and tissues. In a further embodiment, the CNP variants are useful for increasing the size of the growth plate of a bone (e.g., a limb bone). In another embodiment, the CNP variants are useful for elongating a bone or increasing long bone growth. In still another embodiment, the CNP variants are useful for enhancing matrix production, proliferation and differentiation of chondrocytes.

In certain embodiments, the CNP variants described herein are administered at a dose in the range from about 5 or 10 nmol/kg to about 300 nmol/kg, or from about 20 nmol/kg to about 200 nmol/kg. In some embodiments, the CNP variants are administered at a dose of about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 125, 150, 175, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 350, 400, 450, 500, 750, 1000, 1250, 1500, 1750 or 2000 nmol/kg or other dose deemed appropriate by the treating physician. In other embodiments, the CNP variants are administered at a dose of about 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750 or 800 ug/kg, or about 1 mg/kg, 1.25 mg/kg, 1.5 mg/kg, 2 mg/kg or other dose deemed appropriate by the treating physician. The doses of CNP variants described herein can be administered according to the dosing frequency/frequency of administration described herein, including without limitation daily, 2 or 3 times per week, weekly, every 2 weeks, every 3 weeks, monthly, etc.

In another embodiment, the CNP variants are administered in a single treatment or in multiple doses. The multiple doses may be administered daily, or in multiple doses over the course of treatment. In certain embodiments, it is contemplated that the CNP variant is administered, in a single dose or in multiple doses, daily, every other day, every 3 days, 2 times per week, 3 times per week, weekly, bi-weekly, every 3 weeks, monthly, every 6 weeks, every 2 months, every 3 months or as deemed appropriate by a treating physician.

In certain embodiments, administration of the CNP variant is adjusted to allow for periods of growth (e.g., chondrogenesis), followed by a recovery period (e.g., osteogenesis). For example, the CNP variant may be administered subcutaneously, intravenously, or by another mode daily or multiple times per week for a period of time, followed by a period of no treatment, then the cycle is repeated. In some embodiments, the initial period of treatment (e.g., administration of the CNP

variant daily or multiple times per week) is for 3 days, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks or 12 weeks. In a related embodiment, the period of no treatment lasts for 3 days, 1 week, 2 weeks, 3 weeks or 4 weeks. In certain embodiments, the dosing regimen of the CNP variant is daily for 3 days followed by 3 days off; or daily or multiple times per week for 1 week followed by 3 days or 1 week off; or daily or multiple times per week for 2 weeks followed by 1 or 2 weeks off; or daily or multiple times per week for 3 weeks followed by 1, 2 or 3 weeks off; or daily or multiple times per week for 4, 5, 6, 7, 8, 9, 10, 11 or 12 weeks followed by 1, 2, 3 or 4 weeks off.

In additional embodiments, the disclosure provides a method of treating a CNP-responsive condition or disorder, comprising administering a CNP peptide or variant to a subject, and monitoring the level of at least one bone- or cartilage-associated biomarker in the subject (e.g., in a biological sample from the subject), wherein an increase or decrease in the level of the bone- or cartilage-associated biomarker indicates a therapeutic effect of the CNP peptide or variant on the subject. In some embodiments, when the level of a biomarker increases in association with bone or cartilage formation or growth, an increase in the level of that biomarker indicates a therapeutic effect of the CNP peptide or variant on the subject. In other embodiments, when the level of a biomarker decreases in association with bone or cartilage formation or growth, a decrease in the level of that biomarker indicates a therapeutic effect of the CNP peptide or variant on the subject.

In further embodiments, the therapeutic method further comprises adjusting the amount (or dose) or frequency of administration of the CNP peptide or variant, wherein:

(i) the amount (or dose) or frequency of administration of the CNP peptide or variant is increased if the level of the at least one bone- or cartilage-associated biomarker is below a target level, where the level of the biomarker increases in association with bone or cartilage formation or growth; or

(ii) the amount (or dose) or frequency of administration of the CNP peptide or variant is decreased if the level of the at least one bone- or cartilage-associated biomarker is above a target level, where the level of the biomarker increases in association with bone or cartilage formation or growth; or

(iii) the amount (or dose) or frequency of administration of the CNP peptide or variant is increased if the level of the at least one bone- or cartilage-associated biomarker is above a target level, where the level of the biomarker decreases in association with bone or cartilage formation or growth; or

(iv) the amount (or dose) or frequency of administration of the CNP peptide or variant is decreased if the level of the at least one bone- or cartilage-associated biomarker is below a target level, where the level of the biomarker decreases in association with bone or cartilage formation or growth.

It is contemplated that the target level of a biomarker refers to the level or range of levels of the biomarker that is associated with therapeutic effect in the subject and/or beneficial effect in alleviating or ameliorating symptoms of the disorder or condition. In certain embodiments, a level of a biomarker above or below a target level may be deleterious to the subject.

In other embodiments, the disclosure contemplates a method for assessing the effect of administration of a CNP peptide or variant on bone or cartilage formation or growth. In one embodiment, the method provides for assaying or measuring the level of at least one bone- or cartilage-associated biomarker in a subject that has been administered a CNP peptide or variant in order to assess the effect of the CNP peptide or variant on bone and cartilage formation and growth in vivo. In a related embodiment, an increase in the level of the at least one bone- or cartilage-associated biomarker may indicate that administration of a CNP peptide or variant has a positive effect on bone or cartilage formation or growth and is a useful treatment for skeletal dysplasias and other bone- or cartilage-related diseases or disorders associated with decreased CNP activity. Exemplary bone- or cartilage-associated biomarkers include, but are not limited to, CNP (e.g, endogenous level of CNP-22 or CNP-53), cGMP, osteocalcin, proliferating cell nuclear antigen (PCNA), propeptides of type I procollagen (PINP) and fragments thereof, collagen type I and fragments thereof, propeptides of collagen type II and fragments thereof, collagen type II and fragments thereof, aggrecan chondroitin sulfate, and alkaline phosphatase.

In further embodiments, the disclosure contemplates a method for assessing the effect of a CNP peptide or variant on the level of at least one bone- or cartilage-associated biomarker in a subject, comprising assaying or measuring the level of the bone- or cartilage-associated biomarker in a biological sample from a subject that has been administered a CNP peptide or variant. In some embodiments, the method further comprises administering the CNP peptide or variant to the subject before assaying or measuring the level of the bone- or cartilage-associated biomarker.

In certain embodiments, the at least one bone- or cartilage-associated biomarker is selected from the group consisting of CNP (e.g, endogenous level of CNP-22 or CNP-53), cGMP, propeptides of collagen type II and fragments thereof, collagen type II and fragments thereof, osteocalcin, proliferating cell nuclear antigen (PCNA), propeptides of type I procollagen (PINP) and fragments thereof, collagen type I and fragments thereof, aggrecan chondroitin sulfate, and alkaline phosphatase.

In some embodiments of methods (e.g., therapeutic, diagnostic and assay methods) relating to bone- or cartilage-associated biomarkers, the CNP peptide or variant is CNP-22, CNP-53, or any of the CNP peptides and variants described herein. In certain embodiments of such methods, the CNP peptide or variant is not CNP-22 or CNP-53.

In other embodiments, the disclosure provides a method for recombinant production of a CNP variant, comprising culturing in a medium a host cell comprising a polynucleotide encoding a CNP variant peptide linked to a polynucleotide encoding a cleavable peptide or protein, under conditions that result in expression of a fusion polypeptide encoded by the polynucleotides. In a related embodiment, the host cell is transformed with an expression vector comprising a polynucleotide encoding a CNP variant peptide linked to a polynucleotide encoding a cleavable peptide or protein.

In one embodiment, the vector is a plasmid. In still another embodiment, the plasmid is selected from the group consisting of pET-21a, pJexpress, pET-31b, pET-15b, pET-32a, pET-41a, pMAL, pQE-30, pET-SUMO, pET-22b, and pTYB11.

In certain embodiments, the cleavable peptide or protein comprises a polypeptide that is selected from the group consisting of a histidine tag, human transcription factor TAF12, ketosteroid isomerase, maltose-binding protein, β-galactosidase, glutathione-S-transferase, thioredoxin, chitin binding domain, and BMP-2 mutation, or fragments thereof.

In a related embodiment, the cleavable peptide or protein is cleaved by a cleaving agent. In some embodiments, the cleaving agent is selected from the group consisting of formic acid, cyanogen bromide (CNBr), hydroxylamine, protein self cleavage, Factor Xa, enterokinase, ProTEV, and SUMO protease. Additional exemplary cleaving agents include, but are not limited to, palladium, clostripain, thrombin, chymotrypsin, trypsin, trypsin-like proteases, carboxypeptidase,

enteropeptidase, Kex 2 protease, Omp T protease, subtilisin, V8 protease, HIV protease, rhinovirus protease, furilisin protease, IgA proteases, human Pace protease, collagenase, Nia protease, poliovirus 2Apro protease, poliovirus 3C protease, genenase, furin, elastase, Proteinase K, pepsin, rennin (chymosin), microbial aspartic proteases, papain, calpain, chymopapain, ficin (ficain), bromelain (bromelase), cathespisin B, caspases, thermolysin, Endoprotease Arg-C, Endoprotease Glu-C, Endoprotease Lys-C, kallikrein, and plasmin.

In certain embodiments, the fusion polypeptide is expressed as a soluble protein or as an inclusion body. In a related embodiment, the disclosure contemplates isolating the expressed fusion polypeptide from the host cell or culture medium. In a further embodiment, the isolated fusion polypeptide is contacted with a cleaving agent as described herein.

In one embodiment, the disclosure provides a bacterial host cell comprising an expression vector, said vector comprising a polynucleotide encoding a CNP variant peptide linked to a polynucleotide encoding a cleavable peptide or protein. In some embodiments, the cleavable peptide or protein is selected from the group consisting of a histidine tag, human transcription factor TAF12, ketosteroid isomerase, maltose-binding protein, β-galactosidase, glutathione-S-transferase, thioredoxin, chitin binding domain, and BMP-2 mutation, or fragments thereof.

In another embodiment, the host cell is a bacteria, such as *E. coli*. In a related embodiment, the *E. coli* cell is selected from the group consisting of BL21(DE3), BL21(DE3)pLysS, BL21(DE3)pGro7, ArcticExpress(DE3), C41 [also called C41(DE3)], C43 [also called C43(DE3)], Origami B(DE3), Origami B(DE3)pLysS, KRX, and Tuner(DE3). In still a further embodiment, the host cell comprises a vector as described above. In some embodiments, the host cell is transformed with the vector prior to cell culture.

In certain embodiments, it is contemplated that the host cell is cultured in a medium under conditions suitable for expression of a fusion polypeptide encoded by the polynucleotides. In one embodiment, the fusion polypeptide is expressed as a soluble protein or as inclusion body. In a related embodiment, the expressed fusion polypeptide is isolated from the host cell or culture medium. In still another embodiment, the isolated fusion polypeptide is contacted with a cleaving agent as described herein.

BRIEF DESCRIPTION OF THE FIGURES

FIG. **1** shows expression of CNP fusion proteins in *E. coli* (FIG. **1**A: Coomassie blue stain, FIG. **1**B: Western blot). M: protein marker; T: total cell lysates; S: soluble supernatants; P: 2 ug CNP22; KSI: KSI-CNP(M/N) fusion protein expression (insoluble); N: Un-induced KSI-CNP fusion protein total lysates; KSI': KSI-P-CNP fusion protein expression (insoluble); Trx: Trx-P-CNP fusion protein expression (soluble); MBP: MBP-P-CNP fusion protein expression (soluble); TAF: TAF-P-CNP fusion protein expression (insoluble) (BL21); TAF': TAF-P-CNP fusion protein expression (insoluble) BL21(DE3), where CNP is Gly-CNP37.

FIG. **2** shows formic acid cleavage of TAF-CNP inclusion bodies. M: protein marker; 1: Gly-CNP37 positive control; 2: uncleaved TAF-CNP inclusion bodies; 3: 2% formic acid cleaved TAF-CNP inclusion bodies, where CNP is Gly-CNP37.

FIGS. **3**A-E depict expression of CNP fusion proteins in *E. coli*. M: protein marker; Tu: total un-induced cell lysates; Su: un-induced soluble supernatants; T: total induced cell lysates; S: soluble supernatants; C1: CNP22; C: Gly-wtCNP37 ("CNP38"); P: insoluble pellets. A: KSI: KSI-CNP38(M/N) fusion protein expression (Insoluble); KSI': KSI-Pro-CNP38 (Pro-Gly-wtCNP37 is designated "Pro-CNP38") fusion protein expression (Insoluble); Trx: Trx-Pro-CNP38 fusion expression (Soluble); MBP: MBP-Pro-CNP38 fusion protein expression (Soluble); TAF: TAF-Pro-CNP38 fusion protein expression (Insoluble) from BL21 cell; TAF': TAF-Pro-CNP38 fusion protein expression (Insoluble) from BL21 (DE3) cell. B: TAF-Pro-CNP37 and BMP-Pro-CNP37 fusion protein expression. C: BMP-Pro-CNP38 fusion protein and BMP protein expression. D: TAF-Pro-HSA-CNP ("Pro-GH-KSEVAHRFK-wtCNP27 (SEQ ID NO: 188) is designated "Pro-HSA-CNP") fusion protein expression. E: TAF-Pro-CNP38 fusion protein and TAF protein expression.

FIGS. **4**A-C depict formic acid cleavage of TAF-Pro-CNP38 inclusion bodies. A: 50% formic acid cleavage of TAF-Pro-CNP38 inclusion bodies. M: protein marker; U: un-cleaved TAF-Pro-CNP38 inclusion bodies; 25° C., 37° C., 42° C., 55° C.: TAF-Pro-CNP38 inclusion bodies were cleaved in 50% formic acid at 25° C., 37° C., 42° C. or 55° C. for 24 hours. 37° C.-S and 55° C.-S: soluble supernatants from the cleavage reactions at 37° C. and 55° C. neutralized with 10N NaOH and centrifuged at 14,000 rpm for 15 minutes. B: 10% and 2% formic acid cleavage of TAF-Pro-CNP38 inclusion bodies. M: protein marker; U: un-cleaved TAF-Pro-CNP38 inclusion bodies; C: formic acid cleaved TAF-Pro-CNP38; S: soluble supernatant after centrifuged at 14,000 rpm for 5 minutes without neutralization; P: insoluble pellet after centrifuged at 14,000 rpm for 5 minutes without neutralization. C: LC/MS analysis of 2% and 10% formic acid cleaved products from TAF-Pro-CNP38 inclusion bodies.

FIGS. **5**A-C depict formic acid cleavage of TAF-Pro-CNP38 inclusion bodies at different temperature and time of formic acid cleavage. M: protein marker; C: Gly-wtCNP37 ("CNP38") positive control; U: un-cleaved TAF-Pro-CNP38 inclusion bodies. A: 2% formic acid cleaved TAF-Pro-CNP38 at 42° C., 55° C. or 70° C. for 6, 24 or 48 hours. B: 2% formic acid cleaved TAF-Pro-CNP38 at 55° C., 60° C., 65° C. or 70° C. for 17 or 24 hours. C: LC/MS analysis of 2% formic acid cleaved products from TAF-Pro-CNP38 inclusion bodies.

FIG. **6** is an SDS-PAGE (Coomassie blue stain) for the expression of a TAF-CNP34 fusion protein. M: protein marker; C: control [Gly-CNP37 ("CNP38")]; T: total cell lysates; S: supernatant; TI: total cell lysates induced; SI: supernatant induced.

FIG. **7** is an SDS-PAGE (Coomassie blue stain) for the expression of fusion proteins TAF-NL-(C/A & 6D/6E)-Pro-CNP38, TAF(C/A & 10D/10E)-Pro-CNP38, and TAF-Pro-CNP53, where "Pro-CNP38" denotes Pro-Gly-CNP37. M: protein marker; C: control [Gly-CNP37 ("CNP38")]; T: total cell lysates; TI: total cell lysates induced; S: supernatant; SI: supernatant induced.

FIG. **8** is an SDS-PAGE (Coomassie blue stain) of the products of formic acid cleavage of the fusion proteins TAF-CNP34 and TAF-Pro-CNP53. M: protein marker; P: positive control [Gly-CNP37 ("CNP38")]; U: uncleaved; C: cleaved; CS: cleaved supernatant; CP: cleaved pellet.

FIG. **9** is an LC/MS chromatogram showing the peak for CNP-34 after formic acid cleavage of TAF-CNP34.

FIG. **10** is an LC/MS chromatogram showing the peak for Pro-CNP53 after formic acid cleavage of TAF-Pro-CNP53.

FIG. **11** is an SDS-PAGE (Coomassie blue stain) for the expression of fusion proteins TAF(C/A & 4D/4E)-Pro-CNP38 and TAF(4D/4E)-Pro-CNP38, where "Pro-CNP38" denotes Pro-Gly-CNP37. M: protein marker; C: control [Gly-

CNP37 ("CNP38")]; T: total cell lysates; TI: total cell lysates induced; S: supernatant; SI: supernatant induced.

FIG. **12** is an SDS-PAGE (Coomassie blue stain) of the products of formic acid cleavage of the fusion proteins TAF (4D/4E)-Pro-CNP38 and TAF(C/A & 4D/4E)-Pro-CNP38, where "Pro-CNP38" denotes Pro-Gly-CNP37. M: protein marker; P: positive control [Gly-CNP37 ("CNP38")]; U: uncleaved; C: cleaved; CS: cleaved supernatant; CP: cleaved pellet.

FIG. **13** is an SDS-PAGE (Coomassie blue stain) of the products of formic acid cleavage of the fusion proteins TAF-NL-(C/A & 6D/6E)-Pro-CNP38 and TAF(C/A & 10D/10E)-Pro-CNP38, where "Pro-CNP38" denotes Pro-Gly-CNP37. M: protein marker; P: positive control [Gly-CNP37 ("CNP38")]; U: uncleaved; C: cleaved; CS: cleaved supernatant; CP: cleaved pellet.

FIG. **14** is a Western blot, using an anti-CNP antibody, of a TAF-Pro-CNP38 fusion protein produced in a 10 L fermentation of BL21(DE3) cells, where the cells were induced at OD_{600}=64 and hour 17 and "Pro-CNP38" denotes Pro-Gly-CNP37.

FIG. **15** is an SDS-PAGE (Coomassie blue stain) of eluate fractions from SP-Sepharose cation-exchange column chromatography of a cruder Pro-Gly-CNP37 ("Pro-CNP38") product. A: TAF-Pro-CNP38 inclusion body (IB) in water; B: IB in formate; C: IB in formate, neutralized; D: neutralized pellet; E: neutralized supernatant; F: TMAE Hi-CAP load; G: TMAE Hi-CAP flow through/SP-Sepharose load; H: SP-Sepharose flow through; SP-Sepharose eluate fractions 1-47: 10 uL/lane.

FIG. **16** shows the degree of resistance of N-terminal PEGylated CNP22 conjugates to neutral endopeptidase (NEP) in vitro.

FIG. **17** depicts the degree of NEP resistance of CNP variants having an amino acid extension at the N-terminus ["CNP27" is GANRR-CNP22(K4R) (SEQ ID NO: 36)].

FIG. **18** illustrates the degree of NEP resistance of N-terminal PEGylated CNP17 and GANRR-CNP22(K4R) ("CNP27") (SEQ ID NO: 36).

FIG. **19** illustrates the degree of NEP resistance of wtCNP22 and CNP variants Gly-CNP37, GHKSEVAHRFK-wtCNP27 ("CNP27-HSA" in the figures) (SEQ ID NO: 144) and PEO12-GANRR-CNP22(K4R) ("CNP27-PEO12" in the figures) (SEQ ID NO: 36).

FIG. **20** shows the ability of CNP variants having an N-terminal amino acid extension to stimulate cGMP production in NIH3T3 cells in vitro. The results are relative to the level of cGMP produced in the presence of 1 uM CNP22. "CNP27" is GANRR-CNP22(K4R) (SEQ ID NO: 36)

FIG. **21** displays the ability of N-terminal PEGylated CNP17 and GANRR-CNP22(K4R) ("CNP27") (SEQ ID NO: 36) to stimulate cGMP production in NIH3T3 cells.

FIG. **22** illustrates the effects of N-terminal PEGylation of CNP22 on cGMP production.

FIG. **23** illustrates the cGMP production induced by wtCNP22 and CNP variants Gly-CNP37, GHKSEVAHRFK-wtCNP27 ("CNP27-HSA") (SEQ ID NO: 144), wtCNP29 and PEO12-GANRR-CNP22(K4R) ("CNP27-PEO12") (SEQ ID NO: 36) in NIH3T3 cells.

FIGS. **24**A and B show that CNP-22 and Pro-Gly-CNP37 ("Pro-CNP38") stimulated cGMP production through NPR-B with similar dose-response curves, and to a much greater extent than through NPR-A, and exhibited a similar profile for NPR-B vs. NPR-C selectivity in in vitro signaling competition assays.

FIG. **25** demonstrates that exposure of rat chondrosarcoma cells to CNP22 1 hour once daily or 2 hours once daily has substantially similar effectiveness in reversing FGF2-induced arrest of chondrocyte growth as continuous exposure to CNP22.

FIGS. **26**A and B show results from a dose response study of CNP22 effects on FGF2-arrested rat chondrosarcoma (RCS) cells.

FIGS. **27**A-D show that addition of CNP22 to RCS cells arrested by FGF2 increases matrix synthesis and partly inhibits FGF2, as assessed by ^{35}S-sulfate and ^{3}H-Pro incorporation into or decrease from matrix. Panels A and C, synthesis; B and D, degradation; A and B, ^{35}S measurement; C and D, ^{3}H measurement. Statistically significant differences are highlighted (ANOVA; *p<0.05, **p<0.01).

FIGS. **28**A-C show the levels of aggrecan and fibronectin production (mRNA, panels A and C, and protein, panel B) in RCS cells cultured with FGF2 and CNP22.

FIG. **29** shows the efficacy of CNP37 and PEO24-GANRR-CNP22(K4R) ("CNP27-PEO24" in the figures) (SEQ ID NO: 36) in stimulating longitudinal growth of wild-type femur in an ex vivo mouse organ model.

FIG. **30** shows longitudinal bone growth of 2-3 day-old wild-type mouse tibias treated with CNP22, CNP37 or PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) every two days. Results are normalized to measurements prior to treatment (day 0). Data is represented as means±SEM (n=8).

FIG. **31** shows longitudinal bone growth of 2-3 day old achondroplastic FGFR3^{ach} mouse tibias treated with CNP22, CNP37 or PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) every two days. Results are normalized to measurements prior to treatment (day 0). Data is represented as means±SEM (n=7-8).

FIG. **32** shows longitudinal bone growth of 2-3 day-old wild-type mouse femurs treated with CNP22, CNP37 or PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) every two days. Results are normalized to measurements prior to treatment (day 0). Data is represented as means±SEM (n=8).

FIG. **33** shows longitudinal bone growth of 2-3 day-old FGFR3^{ach} mouse femurs treated with CNP22, CNP37 or PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) every two days. Results are normalized to measurements prior to treatment (day 0). Data is represented as means±SEM (n=3-7).

FIGS. **34**A-I depict CNP37 biodistribution in FGFR3^{ach} mouse femurs treated ex vivo every two days. Panels A-C illustrate distribution in distal femurs, panels D-F illustrate distribution in articular chondrocytes and panels G-I illustrate distribution in hypertrophic chondrocytes.

FIGS. **35**A-C depict the cellularity of proliferating columns in growth plates after treatment of wild-type and FGFR3^{ach} mouse femurs with CNP22 or CNP37 every two days for 8 days. (A) no treatment, (B) cell numbers per column after treatment, (C) morphological studies after treatment. The panels in FIGS. **35**C(i) to C(vi). correspond to the sample order set out in FIG. **35**B. Data is represented as means±SEM (n=4-8).

FIGS. **36**A-C depict chondrocyte hypertrophy after ex vivo treatment of wild-type and FGFR3^{ach} mouse femurs with CNP22 or CNP37 every two days for 8 days. (A) no treatment, (B) cell size after treatment, (C) morphological studies after treatment. The panels in FIGS. **36**C(i) to C(vi) correspond to the sample order set out in FIG. **36**B. Data is represented as means±SEM (n=4-9).

FIGS. **37**A-I depict the biodistribution of CNP37 in FGFR3^{ach} mouse tibias treated in vivo. Panels A-C show distribution in distal femurs, panels D-F illustrate distribution in articular chondrocytes and panels G-I illustrate distribution in hypertrophic chondrocytes.

FIGS. **38**A-C illustrate the in vivo effects of CNP37 on FGFR3^{ach} mouse tibia growth plate: (A) total growth plate thickness, (B) proliferating zone thickness, and (C) hypertrophic zone thickness. Data is represented as means±SEM (n=7-15).

FIG. **39** shows cGMP levels in conditioned media from wild-type mouse femurs treated ex vivo with CNP22, CNP37 or PEO24-GANRR-CNP22(K4R) ("CNP27-PEO24") (SEQ ID NO: 36) ($p<0.01$).

FIG. **40** shows cGMP levels in conditioned media from FGFR3^{ach} mouse femurs treated ex vivo with CNP22, CNP37 or PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) ($p<0.01$).

FIG. **41** depicts cGMP levels in conditioned media from wild-type mouse tibias treated ex vivo with CNP22, CNP37 or PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) ($p<0.01$).

FIG. **42** shows cGMP levels in conditioned media from FGFR3^{ach} mouse tibias treated ex vivo with CNP22, CNP37 or PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) ($p<0.01$).

FIG. **43** demonstrates that ex vivo exposure of wild-type and FGFR3^{ach} mouse femurs to CNP22, CNP37 or PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) increased the levels of cleaved collagen type II in the conditioned media ($p<0.05$).

FIG. **44** depicts the hypertrophic region of femoral bones isolated from wild-type mice and FGFR3^{Y367C} mice (a mouse model of severe achondroplasia) and treated ex vivo with vehicle or 1 uM Pro-Gly-CNP37 ("ProCNP38") for 6 days, demonstrating that Pro-Gly-CNP37 treatment resulted in increase in bone growth and expansion in the growth plate.

FIG. **45** shows that CNP37 and PEO24-GANRR-CNP22 (K4R) (SEQ ID NO: 36) intravenously (i.v.) administered to rats have a much longer half-life and a much greater bioavailability in the plasma than CNP22.

FIG. **46** illustrates that PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) subcutaneously (s.c.) administered to rats also has a much longer half-life and a much greater bioavailability in the plasma than CNP22.

FIG. **47** demonstrates that i.v. administered CNP37 and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) stimulate a much greater level of cGMP production in rats than CNP22.

FIG. **48** shows that s.c. administered PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) and, to a lesser extent, CNP37 are substantially more effective in stimulating cGMP production in rats than CNP22.

FIG. **49** shows body weight measurements of wild-type mice treated with Gly-CNP37 or PEO12-GANRR-CNP22 (K4R) (SEQ ID NO: 36).

FIG. **50** shows tail length measurements of wild-type mice treated with Gly-CNP37 or PEO12-GANRR-CNP22(K4R) (SEQ ID NO: 36).

FIG. **51** illustrates the effect of treatment of FGFR3^{ach} mice with CNP22, CNP37 or PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) on body length ($p=0.02$, 1-tailed t-test, unequal variance).

FIG. **52** shows the effect of CNP22, CNP37 and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) on tail length in FGFR3^{ach} mice.

FIGS. **53**A and B show the effect of CNP22, CNP37 and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) on the length of distal long bones (A, ulna; B, tibia) in FGFR3^{ach} mice ($p<0.01$, one-tailed t-test, unequal variance).

FIGS. **54**A and B show the effect of CNP22, CNP37 and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) on the length of proximal bones (A, humerus; B, femur) in FGFR3^{ach} mice ($p<0.01$, one-tailed t-test, unequal variance).

FIG. **55** illustrates that CNP37 administration corrects rhizomelia (disproportion of the length of the proximal limbs) as assessed by the femur:tibia ratio observed in FGFR3^{ach} mice ($p<0.01$, one-tailed t-test, unequal variance).

FIG. **56** shows the effect of CNP22, CNP37 and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) on head length in FGFR3^{ach} mice ($p<0.01$ one-tailed t-test, unequal variance).

FIG. **57** shows that treatment of FGFR3^{ach} mice with CNP37 increases the size of the external auditory meatus (EAM) ($P=0.03$, one-tailed t-test, unequal variance).

FIG. **58** shows the effect of CNP22, CNP37 and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) on spinal length in achondroplasic mice, expressed as extension of vertebral bodies (e.g., lumbar vertebra 5).

FIG. **59** shows that treatment of FGFR3^{ach} mice with CNP37 or PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) results in increased cGMP plasma levels 15-min post-dose.

FIG. **60** illustrates the serum levels of cleaved collagen type II in FGFR3^{ach} mice treated 5 weeks with CNP22, CNP37 or PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36).

FIG. **61** shows the serum levels of osteocalcin in FGFR3^{ach} mice treated 5 weeks with CNP22, CNP37 or PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36).

FIG. **62** shows cGMP plasma levels 15-min post-dose from wild-type mice treated with Gly-CNP37 or PEO12-GANRR-CNP22(K4R) (SEQ ID NO: 36) ($p<0.05$).

FIG. **63** shows the serum levels of cleaved collagen type II in wild-type mice treated 5 weeks with Gly-CNP37 or PEO12-GANRR-CNP22(K4R) (SEQ ID NO: 36).

FIGS. **64-66** display the levels of cGMP, cleaved collagen type II and alkaline phosphatase after administration of vehicle or 20 nmol/kg or 70 nmol/kg Pro-Gly-CNP37 ("Pro-CNP38") to wild-type mice.

FIGS. **67** and **68** depict cleaved collagen type II and total alkaline phosphatase levels after administration of vehicle or Pro-Gly-CNP37 ("Pro-CNP38") under different dosing regimens.

FIG. **69** illustrates the relative increase in body length of Pro-Gly-CNP37 ("Pro-CNP38") and vehicle treated animals in two separate studies (S1 and S2) at Day 37 vs Day 1.

FIGS. **70**A and B show changes in bone mineral density (A) and bone mineral content (B) after administration of Pro-Gly-CNP37 ("Pro-CNP38").

FIG. **71** shows plasma cGMP levels 15 min after the last subcutaneous dose of CNP variant, Day 36, where Gly-wtCNP37 is "CNP38", Pro-Gly-wtCNP37 is "Pro-CNP38", and GHKSEVAHRFK-wtCNP27 (SEQ ID NO: 144) is "HSA-CNP27" in FIGS. **71-73**.

FIG. **72** shows serum levels of cleaved collagen type II from mice treated with Gly-CNP37, Pro-Gly-CNP37 or GHKSEVAHRFK-CNP27 (SEQ ID NO: 144).

FIG. **73** shows serum levels of alkaline phosphatase from mice treated with Gly-CNP37, Pro-Gly-CNP37 or GHKSEVAHRFK-CNP27 (SEQ ID NO: 144).

FIGS. **74**A and B depict desensitization of the cGMP response after acute (A) or chronic (B) treatment with 1 uM Gly-CNP37.

FIG. **75**A demonstrates that daily treatment of wild-type mice with 200 nmol/kg of Pro-Gly-CNP37 ("Pro-CNP38") for 8 days did not desensitize the cGMP response. FIG. **75**B shows that treatment of the mice with 200 nmol/kg of Pro-Gly-CNP37 once a day for two consecutive days potentiated the cGMP response.

FIGS. **76**A-D show that treatment of wild-type mice with 200 nmol/kg of Gly-CNP37 stimulated cGMP secretion in distal femurs (cartilage and bone) (A), femoral cortices (bone) (B), ear pinna (cartilage) (C), and kidney (D). FIGS.

76E-H show that liver (E), heart (F), lung (G) and brain (H) tissues did not exhibit appreciable cGMP secretion in response to Gly-CNP37 relative to vehicle control at the studied time points.

FIGS. **77-82** show results from an on-going study in normal juvenile cynomolgus monkeys subcutaneously injected daily with vehicle or 10 or 36 ug/kg of Pro-Gly-CNP37. Both doses of Pro-Gly-CNP37 have increased growth plate width (FIG. **77**), increased right and left tibia lengths (FIGS. **78**A and B), increased leg length (FIG. **79**), increased arm length (FIG. **80**), increased body length (FIG. **81**), and increased the serum level of alkaline phosphatase (FIG. **82**).

FIG. **83** depicts the observed plot of degradation rate constant (K_{obs}) vs. pH at pH 3-8 and 5° C., 25° C. and 40° C. for Gly-CNP37 formulations.

DETAILED DESCRIPTION OF THE DISCLOSURE

The present disclosure relates to novel variants of CNP having reduced affinity to NEP and/or NPR-C, and reduced susceptibility to cleavage by NEP and/or clearance by NPR-C, pharmaceutical compositions comprising such CNP variants, and methods of using such

CNP variants to treat disorders responsive to CNP, including but not limited to bone-related disorders such as achondroplasia and disorders associated with vascular smooth muscle cells and tissues.

A. Definitions

Unless otherwise stated, the following terms used in this application, including the specification and claims, have the definitions given below.

As used in the specification and the appended claims, the indefinite articles "a" and "an" and the definite article "the" include plural as well as singular referents unless the context clearly dictates otherwise.

The term "about" or "approximately" means an acceptable error for a particular value as determined by one of ordinary skill in the art, which depends in part on how the value is measured or determined. In certain embodiments, the term "about" or "approximately" means within 1, 2, 3, or 4 standard deviations. In certain embodiments, the term "about" or "approximately" means within 30%, 25%, 20%, 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, or 0.05% of a given value or range. Whenever the term "about" or "approximately" precedes the first numerical value in a series of two or more numerical values, it is understood that the term "about" or "approximately" applies to each one of the numerical values in that series.

The terms "ambient temperature" and "room temperature" are used interchangeably herein and refer to the temperature of the surrounding environment (e.g., the room in which a reaction is conducted or a composition is stored). In certain embodiments, ambient temperature or room temperature is a range from about 15° C. to about 28° C., or from about 15° C. to about 25° C., or from about 20° C. to about 28° C., or from about 20° C. to about 25° C., or from about 22° C. to about 28° C., or from about 22° C. to about 25° C. In other embodiments, ambient temperature or room temperature is about 15° C., 16° C., 17° C., 18° C., 19° C., 20° C., 21° C., 22° C., 23° C., 24° C., 25° C., 26° C., 27° C. or 28° C.

Definition of standard chemistry terms may be found in reference works, including Carey and Sundberg, Advanced Organic Chemistry, 3rd Edition, Vols. A and B (Plenum Press, New York 1992). The practice of the present disclosure may employ, unless otherwise indicated, certain conventional methods of synthetic organic chemistry, mass spectrometry, preparative and analytical chromatography, protein chemistry, biochemistry, recombinant DNA technology and pharmacology, within the skill of the art. See, e.g., T. E. Creighton, Proteins: Structures and Molecular Properties (W.H. Freeman and Company, 1993); A. L. Lehninger, Biochemistry (Worth Publishers, Inc., 4th Edition, 2004); Sambrook, et al., Molecular Cloning: A Laboratory Manual (2nd Edition, 1989); Methods In Enzymology (S. Colowick and N. Kaplan eds., Academic Press, Inc.); Remington's Pharmaceutical Sciences, 18th Edition (Easton, Pa.: Mack Publishing Company, 1990).

All publications, patents and patent applications cited herein, whether supra or infra, are hereby incorporated by reference in their entirety.

The following amino acid abbreviations are used throughout the text:

Alanine: Ala (A)	Arginine: Arg (R)
Asparagine: Asn (N)	Aspartic acid: Asp (D)
Cysteine: Cys (C)	Glutamine: Gln (Q)
Glutamic acid: Glu (E)	Glycine: Gly (G)
Histidine: His (H)	Isoleucine: Ile (I)
Leucine: Leu (L)	Lysine: Lys (K)
Methionine: Met (M)	Phenylalanine: Phe (F)
Proline: Pro (P)	Serine: Ser (S)
Threonine: Thr (T)	Tryptophan: Trp (W)
Tyrosine: Tyr (Y)	Valine: Val (V)

"Polypeptide" and "protein" refer to a polymer composed of amino acid residues, related naturally occurring structural variants, and synthetic non-naturally occurring analogs thereof, linked via peptide bonds or peptide bond isosteres. Synthetic polypeptides can be synthesized, for example, using an automated polypeptide synthesizer. The terms "polypeptide" and "protein" are not limited to a minimum length of the product. The term "protein" typically refers to large polypeptides. The term "peptide" typically refers to short polypeptides. Thus, peptides, oligopeptides, dimers, multimers, and the like, are included within the definition. Both full-length proteins and fragments thereof are encompassed by the definition. The terms "polypeptide" and "protein" also include postexpression modifications of the polypeptide or protein, for example, glycosylation, acetylation, phosphorylation and the like. Furthermore, for purposes of the present disclosure, a "polypeptide" can include "modifications," such as deletions, additions, substitutions (which may be conservative in nature or may include substitutions with any of the 20 amino acids that are commonly present in human proteins, or any other naturally or non-naturally-occurring or atypical amino acids), and chemical modifications (e.g., addition of or substitution with peptidomimetics), to the native sequence. These modifications may be deliberate, as through site-directed mutagenesis, or through chemical modification of amino acids to remove or attach chemical moieties, or may be accidental, such as through mutations arising with hosts that produce the proteins or through errors due to PCR amplification.

Conventional notation is used herein to portray polypeptide sequences: the left-hand end of a polypeptide sequence is the amino-terminus; the right-hand end of a polypeptide sequence is the carboxyl-terminus.

"Conservative substitution" refers to substitution of an amino acid in a polypeptide with a functionally, structurally or chemically similar natural or unnatural amino acid. In one

embodiment, the following groups each contain natural amino acids that are conservative substitutions for one another:

(1), Alanine (A) Serine (S), Threonine (T);

(2) Aspartic acid (D), Glutamic acid (E);

(3) Asparagine (N), Glutamine (Q);

(4) Arginine (R), Lysine (K);

(5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); and

(6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W).

In another embodiment, the following groups each contain natural amino acids that are conservative substitutions for one another:

(1) Glycine (G), Alanine (A);

(2) Aspartic acid (D), Glutamic acid (E);

(3) Asparagine (N), Glutamine (Q);

(4) Arginine (R), Lysine (K);

(5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V), Alanine (A);

(6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W); and

(7) Serine (S), Threonine (T), Cysteine (C).

In a further embodiment, amino acids may be grouped as set out below.

(1) hydrophobic: Met, Ala, Val, Leu, Ile, Phe, Trp;

(2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;

(3) acidic: Asp, Glu;

(4) basic: His, Lys, Arg;

(5) residues that influence backbone orientation: Gly, Pro; and

(6) aromatic: Trp, Tyr, Phe, His.

In one embodiment, the peptides or polypeptides described herein are generated via recombinant means, using a polynucleotide encoding a CNP variant. The disclosure thus encompasses polynucleotides encoding any of the CNP variants described herein, host cells or vectors comprising such polynucleotides, optionally linked to expression control sequences, and methods of using such polynucleotides, vectors or host cells to produce CNP variants of the disclosure. CNP variants expressed by such polynucleotides may be produced by methods including growing host cells in culture medium under conditions suitable for expression of the polynucleotide encoding a CNP variant, and isolating the expression product from the host cells or culture medium. Actual expression products may vary slightly from the encoded protein product depending on any post-translational processing.

"Polynucleotide" refers to a polymer composed of nucleotide units. Polynucleotides include naturally occurring nucleic acids, such as deoxyribonucleic acid ("DNA") and ribonucleic acid ("RNA") as well as nucleic acid analogs. The term "nucleic acid" typically refers to large polynucleotides. The term "oligonucleotide" typically refers to short polynucleotides, generally no greater than about 50 nucleotides. It will be understood that when a nucleotide sequence is represented by a DNA sequence (i.e., A, T, G, C), the nucleotide sequence also encompasses an RNA sequence (i.e., A, U, G, C) in which "U" replaces "T." "cDNA" refers to a DNA that is complementary or identical to an mRNA, in either single stranded or double stranded form.

"Expression control sequence" refers to a nucleotide sequence that regulates the expression of a nucleotide sequence operatively linked thereto. "Operatively linked" refers to a functional relationship between two parts in which the activity of one part (e.g., the ability to regulate transcription) results in an action on the other part (e.g., transcription of the sequence). Expression control sequences can include, for example and without limitation, sequences of promoters (e.g., inducible or constitutive), enhancers, transcription terminators, a start codon (i.e., ATG), splicing signals for introns, and stop codons.

"Recombinant polynucleotide" refers to a polynucleotide having sequences that are not naturally joined together. An amplified or assembled recombinant polynucleotide may be included in a suitable vector, and the vector can be used to transform a suitable host cell. A host cell that comprises the recombinant polynucleotide is referred to as a "recombinant host cell." The gene is then expressed in the recombinant host cell to produce, e.g., a "recombinant polypeptide." A recombinant polynucleotide may serve a non-coding function (e.g., promoter, origin of replication, ribosome-binding site, etc.) as well.

"Chimera" as used herein refers to a polynucleotide or polypeptide comprising at least two heterologous polynucleotide or polypeptide sequences (i.e. derived from different sources or not associated with each other as a naturally-occurring sequence) which are directly or indirectly attached or linked together using techniques commonly known in the art, e.g., recombinant expression or chemical crosslinking. In one embodiment, the heterologous sequence can comprise a protein or peptide directly or indirectly linked to a CNP peptide or variant, including proteins or peptides which are cleavable from the CNP peptide or variant. In a related embodiment, CNP variants are chimera as described herein.

In certain embodiments, chimeras include CNP fusion proteins comprising a cleavable carrier protein or peptide tag. The term "cleavable carrier protein" or "cleavable peptide tag" refers to a peptide or polypeptide sequence that may be fused, directly or indirectly via a linker, to a heterologous polypeptide sequence, and is removable from the heterologous sequence using an agent that cleaves or separates the cleavable peptide or polypeptide from the heterologous polypeptide or protein. In some embodiments, the cleavable carrier protein or peptide tag improves generation, purification and/or detection of the fusion protein or the heterologous polypeptide. Exemplary cleavable carrier proteins and peptide tags include, but are not limited to, human transcription factor TAF12 (TAF12), ketosteroid isomerase (KSI), maltose-binding protein (MBP), β-galactosidase (β-Gal), glutathione-S-transferase (GST), thioredoxin (Trx), chitin-binding domain (CBD), BMP-2 mutation (BMPM), SUMO, CAT, TrpE, staphylococcal protein A, streptococcal proteins, starch-binding protein, cellulose-binding domain of endoglucanase A, cellulose-binding domain of exoglucanase Cex, biotin-binding domain, recA, Flag, c-Myc, poly(His), poly (Arg), poly(Asp), poly(Gln), poly(Phe), poly(Cys), green fluorescent protein, red fluorescent protein, yellow fluorescent protein, cyan fluorescent protein, biotin, avidin, streptavidin, antibody epitopes, and fragments thereof.

A "cleaving agent" is an agent that is useful to cleave or separate, e.g., a cleavable peptide or polypeptide from a heterologous polypeptide or protein. Exemplary cleaving agents include, but are not limited to, palladium, cyanogen bromide (CNBr), formic acid, hydroxylamine, clostripain, thrombin, chymotrypsin, trypsin, trypsin-like proteases, carboxypeptidase, enterokinase (enteropeptidase), Kex 2 protease, Omp T protease, Factor Xa protease, subtilisin, proTEV, SUMO protease, V8 protease, HIV protease, rhinovirus protease, furilisin protease, IgA proteases, human Pace protease, collagenase, Nia protease, poliovirus 2Apro protease, poliovirus 3C protease, genenase, furin, elastase, Proteinase K, pepsin, rennin (chymosin), microbial aspartic proteases, papain, calpain, chymopapain, ficin (ficain), bromelain (bromelase), cathespisin B, caspases, thermolysin, Endoprotease Arg-C, Endoprotease Glu-C, Endoprotease Lys-C, kallikrein, and plasmin.

The terms "identical" and percent "identity", in the context of two or more polynucleotide or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of nucleotides or amino acid residues that are the same, when compared and aligned for maximum correspondence, as measured using one of the following sequence comparison algorithms or by visual inspection.

The phrase "substantially homologous" or "substantially identical", in the context of two nucleic acids or polypeptides, generally refers to two or more sequences or subsequences that have at least 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 98% nucleotide or amino acid residue identity, when compared and aligned for maximum correspondence, as measured using one of the following sequence comparison algorithms or by visual inspection. In certain embodiments, the substantial homology or identity exists over regions of the sequences that are at least about 25, 50, 100 or 150 residues in length. In another embodiment, the sequences are substantially homologous or identical over the entire length of either or both comparison biopolymers.

For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are inputted into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, Adv. Appl. Math., 2: 482 (1981), by the homology alignment algorithm of Needleman & Wunsch, J. Mol. Biol., 48: 443 (1970), by the search for similarity method of Pearson & Lipman, Proc. Natl. Acad. Sci. USA, 85: 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis.), or by visual inspection. One example of a useful algorithm is PILEUP, which uses a simplification of the progressive alignment method of Feng & Doolittle, J. Mol. Evol., 35: 351-360 (1987) and is similar to the method described by Higgins & Sharp, CABIOS, 5: 151-153 (1989). Another algorithm useful for generating multiple alignments of sequences is Clustal W (Thompson et al., Nucleic Acids Research, 22: 4673-4680 (1994)). An example of an algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm (Altschul et al., J. Mol. Biol., 215: 403-410 (1990); Henikoff & Henikoff, Proc. Natl. Acad. Sci. USA, 89: 10915 (1989); Karlin & Altschul, Proc. Natl. Acad. Sci. USA, 90: 5873-5787 (1993)). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information.

A further indication that two nucleic acid sequences or polypeptides are substantially homologous or identical is that the polypeptide encoded by the first nucleic acid is immunologically cross reactive with the polypeptide encoded by the second nucleic acid, as described below. Thus, a polypeptide is typically substantially identical to a second polypeptide, for example, where the two polypeptides differ only by conservative substitutions. Another indication that two nucleic acid sequences are substantially identical is that the two molecules hybridize to each other under stringent conditions, as described herein.

"Substantially pure" or "isolated" means an object species is the predominant species present (i.e., on a molar basis, more abundant than any other individual macromolecular species in the composition), and a substantially purified fraction is a composition wherein the object species comprises at least about 50% (on a molar basis) of all macromolecular species present. In one embodiment, a substantially pure composition means that the species of interest comprises at least about 70%, 75%, 80%, 85%, 90%, 95%, 98% or more of the macromolecular species present in the composition on a molar or weight basis. The object species is purified to essential homogeneity (contaminant species cannot be detected in the composition by conventional detection methods) if the composition consists essentially of a single macromolecular species. Solvent species, small molecules (<500 Daltons), stabilizers (e.g., BSA), and elemental ion species are not considered macromolecular species for purposes of this definition. In an embodiment, the compounds of the disclosure are substantially pure or isolated. In another embodiment, the compounds of the disclosure are substantially pure or isolated with respect to the macromolecular starting materials used in their production. In yet another embodiment, the pharmaceutical compositions of the disclosure comprise a substantially pure or isolated CNP variant admixed with one or more pharmaceutically acceptable excipients, carriers or diluents, and optionally with another biologically active agent.

"Naturally occurring" as applied to an object refers to the fact that the object can be found in nature. For example, a polypeptide or polynucleotide sequence that is present in an organism (including viruses) and which has not been intentionally modified by man in the laboratory is naturally occurring. In one embodiment, a "naturally occurring" substance is of human origin.

"Wild-type" (wt) is a term referring to the natural form, including sequence, of a polynucleotide, polypeptide or protein in a species. A wild-type form is distinguished from a mutant form of a polynucleotide, polypeptide or protein arising from genetic mutation(s).

In one embodiment, a first polypeptide that is an "analog" or "variant" or "derivative" of a second polypeptide is a polypeptide having at least about 50%, 60% or 70% sequence homology, but less than 100% sequence homology, with the second polypeptide. Such analogs, variants or derivatives may be comprised of non-naturally occurring amino acid residues, including without limitation, homoarginine, ornithine, penicillamine, and norvaline, as well as naturally occurring amino acid residues. Such analogs, variants or derivatives may also be composed of one or a plurality of D-amino acid residues, and may also contain peptidomimetics or peptide bond isosteres such as non-peptide linkages between two or more amino acid or peptidomimetic residues. In another embodiment, a first polypeptide is an "analog", "variant" or "derivative" of a second polypeptide if the first polypeptide is not a known cleavage product of the second polypeptide or is not a known precursor of the second polypeptide, even if the first polypeptide has 100% sequence homology to the second polypeptide or has a wild-type sequence.

In an embodiment, the term "derived from" as used herein refers to a polypeptide or peptide sequence that is based on a wild type or naturally occurring polypeptide or peptide sequence and can have one or more deletions, additions, and/or substitutions with natural amino acids, unnatural amino acids or peptidomimetics. In one embodiment, the derivative sequence shares at least about 40%, 50%, 60% or 70%, but less than 100%, sequence similarity to the wild-type or naturally occurring sequence. In another embodiment, the

derivative may be a fragment of a polypeptide, wherein the fragment is substantially homologous (e.g., at least about 70%, 75%, 80%, 85%, 90%, or 95% homologous) to the wild-type polypeptide over a length of at least about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 amino acids. In still another embodiment, a polypeptide is "derived from" a wild-type polypeptide if it has a moiety (e.g., a polymer such as, e.g., PEG) directly or indirectly attached to it which is not present on the wild-type polypeptide, even if both polypeptides share 100% homology in their amino acid sequence.

As used herein, an "NPPC-derived" polypeptide refers to a polypeptide derived from the natriuretic peptide precursor C(NPPC) polypeptide, which is a single chain 126-amino acid pre-pro polypeptide, and which upon cleavage ultimately results in wtCNP22. Removal of the signal peptide from NPPC yields pro-CNP, and further cleavage by the endoprotease furin generates an active 53-amino acid peptide (CNP-53), which is secreted and cleaved again by an unknown enzyme to produce the mature 22-amino acid peptide (CNP, or CNP-22). Therefore, CNP22 itself is an "NPPC-derived" polypeptide by virtue of being derived from NPPC. In one embodiment, an NPPC-derived polypeptide is at least about 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, or 95% homologous to the wild type NPPC over the same number of amino acid residues. It is further contemplated that an NPPC-derived peptide may comprise from about 1 to about 53, or 1 to 37, or 1 to 35, or 1 to 31, or 1 to 27, or 1 to 22, or to 35, or about 15 to about 37 residues of the NPPC polypeptide. In one embodiment, an NPPC-derived peptide may comprise a sequence of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, or 53 amino acids derived from the NPPC polypeptide.

The term "effective amount" means a dosage sufficient to produce a desired result on a health condition, pathology, or disease of a subject or for a diagnostic purpose. The desired result may comprise a subjective or objective improvement in the recipient of the dosage. "Therapeutically effective amount" refers to that amount of an agent effective to produce the intended beneficial effect on health. An appropriate "effective" amount in any individual case may be determined by one of ordinary skill in the art using routine experimentation. It will be understood that the specific dose level and frequency of dosage for any particular patient may be varied and will depend upon a variety of factors, including the activity of the specific compound employed; the bioavailability, metabolic stability, rate of excretion and length of action of that compound; the mode and time of administration of the compound; the age, body weight, general health, sex, and diet of the patient; and the severity of the particular condition.

"Treatment" refers to prophylactic treatment or therapeutic treatment or diagnostic treatment. In certain embodiments, "treatment" refers to administration of a compound or composition to a subject for therapeutic, prophylactic or diagnostic purposes.

A "prophylactic" treatment is a treatment administered to a subject who does not exhibit signs of a disease or exhibits only early signs of the disease, for the purpose of decreasing the risk of developing pathology. The compounds or compositions of the disclosure may be given as a prophylactic treatment to reduce the likelihood of developing a pathology or to minimize the severity of the pathology, if developed.

A "therapeutic" treatment is a treatment administered to a subject who exhibits signs or symptoms of pathology for the purpose of diminishing or eliminating those signs or symptoms. The signs or symptoms may be biochemical, cellular, histological, functional or physical, subjective or objective. The compounds of the disclosure may also be given as a therapeutic treatment or for diagnosis.

"Diagnostic" means identifying the presence, extent and/or nature of a pathologic condition. Diagnostic methods differ in their specificity and selectivity. While a particular diagnostic method may not provide a definitive diagnosis of a condition, it suffices if the method provides a positive indication that aids in diagnosis.

"Bone- or cartilage-associated biomarker" or "bone- or cartilage-associated marker" refers to a growth factor, enzyme, protein, or other detectable biological substance or moiety whose level is increased or decreased in association with, e.g., cartilage turnover, cartilage formation, cartilage growth, bone resorption, bone formation, bone growth, or combinations thereof. Such biomarkers may be measured before, during and/or after administration of a CNP variant as described herein. Exemplary bone- or cartilage-associated biomarkers include, but are not limited to, CNP, cGMP, propeptides of collagen type II and fragments thereof, collagen type II and fragments thereof, propeptides of collagen type I and fragments thereof, collagen type 1 and fragments thereof, osteocalcin, proliferating cell nuclear antigen (PCNA), aggrecan chondroitin sulfate, and alkaline phosphatase. Cartilage- and bone-associated biomarkers can be measured in any appropriate biological sample, including but not limited to tissues, blood, serum, plasma, cerebrospinal fluid, synovial fluid and urine. In some embodiments, the biomarkers are measured in blood, plasma or serum from animals undergoing efficacy/pharmacodynamic in vivo studies and/or from the conditioned media of ex vivo studies.

In certain embodiments, the level of at least one bone- or cartilage-associated biomarker is measured and the amount or frequency of administration of CNP variant administered to a subject can be adjusted according to the level of the biomarker measured. In some embodiments, the level of biomarker is "below a target level" or "above a target level." A target level of a biomarker is a level or range of levels of the biomarker at which a therapeutic effect is observed in the subject receiving the CNP variant. In certain embodiments, the target level of a biomarker for a subject having a CNP-responsive disorder or condition is the level or range of levels of the biomarker observed in a normal, non-affected subject. In other embodiments, to indicate a therapeutic effect, the target level of a biomarker need not be equivalent to the level or range of levels of the biomarker observed in a normal subject, but can be within, e.g., 100%, 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10% or 5% of the "normal" level or range of levels of the biomarker observed in a non-affected subject.

For example, if the level of a biomarker increases in association with bone or cartilage formation or growth, the target level of the biomarker indicating a therapeutic effect may be higher than the level of the biomarker in patients suffering from a CNP-responsive disorder who have not been administered a CNP variant, and may optionally be lower than the "normal" level(s), at about the "normal" level(s), or above the "normal" level(s) of the biomarker in subjects not suffering from that disorder. In one embodiment, if the level of a biomarker is below a target level, it indicates an inadequate therapeutic effect, which may require an increase in the amount or frequency of administration of CNP variant administered. In a related embodiment, if the biomarker is above a target level, it indicates that more CNP variant than necessary has been administered, which may require a decrease in the amount or frequency of administration of the CNP variant administered.

As another example, if the level of a biomarker decreases in association with bone or cartilage formation or growth, the

target level of the biomarker indicating a therapeutic effect may be lower than the level of the biomarker in patients suffering from a CNP-responsive disorder who have not been administered a CNP variant, and may optionally be higher than the "normal" level(s), at about the "normal" level(s), or below the "normal" level(s) of the biomarker in subjects not suffering from that disorder. In such a case, the converse of the above adjustments in CNP variant amount and frequency of administration may apply.

"Pharmaceutical composition" refers to a composition suitable for pharmaceutical use in subject animal, including humans and mammals. A pharmaceutical composition comprises a therapeutically effective amount of a CNP variant, optionally another biologically active agent, and optionally a pharmaceutically acceptable excipient, carrier or diluent. In an embodiment, a pharmaceutical composition encompasses a composition comprising the active ingredient(s), and the inert ingredient(s) that make up the carrier, as well as any product that results, directly or indirectly, from combination, complexation or aggregation of any two or more of the ingredients, or from dissociation of one or more of the ingredients, or from other types of reactions or interactions of one or more of the ingredients. Accordingly, the pharmaceutical compositions of the present disclosure encompass any composition made by admixing a compound of the disclosure and a pharmaceutically acceptable excipient, carrier or diluent.

"Pharmaceutically acceptable carrier" refers to any of the standard pharmaceutical carriers, buffers, and the like, such as a phosphate buffered saline solution, 5% aqueous solution of dextrose, and emulsions (e.g., an oil/water or water/oil emulsion). Non-limiting examples of excipients include adjuvants, binders, fillers, diluents, disintegrants, emulsifying agents, wetting agents, lubricants, glidants, sweetening agents, flavoring agents, and coloring agents. Suitable pharmaceutical carriers, excipients and diluents are described in Remington's Pharmaceutical Sciences, 19th Ed. (Mack Publishing Co., Easton, 1995). Preferred pharmaceutical carriers depend upon the intended mode of administration of the active agent. Typical modes of administration include enteral (e.g., oral) or parenteral (e.g., subcutaneous, intramuscular, intravenous or intraperitoneal injection; or topical, transdermal, or transmucosal administration).

A "pharmaceutically acceptable salt" is a salt that can be formulated into a compound for pharmaceutical use, including but not limited to metal salts (e.g., sodium, potassium, magnesium, calcium, etc.) and salts of ammonia or organic amines.

By "pharmaceutically acceptable" or "pharmacologically acceptable" is meant a material that is not biologically or otherwise undesirable, i.e., the material may be administered to an individual without causing any undesirable biological effects or without interacting in a deleterious manner with any of the components of the composition in which it is contained or with any components present on or in the body of the individual.

The term "unit dosage form" refers to physically discrete units suitable as unitary dosages for human and animal subjects, each unit containing a predetermined quantity of a compound of the disclosure calculated in an amount sufficient to produce the desired effect, optionally in association with a pharmaceutically acceptable excipient, diluent, carrier or vehicle. The specifications for the novel unit dosage forms of the present disclosure depend on the particular compound employed and the effect to be achieved, and the pharmacodynamics associated with each compound in the host.

"Physiological conditions" refer to conditions in the body of an animal (e.g., a human). Physiological conditions include, but are not limited to, body temperature and an aqueous environment of physiologic ionic strength, pH and enzymes. Physiological conditions also encompass conditions in the body of a particular subject which differ from the "normal" conditions present in the majority of subjects, e.g., which differ from the normal human body temperature of approximately 37° C. or differ from the normal human blood pH of approximately 7.4.

By "physiological pH" or a "pH in a physiological range" is meant a pH in the range of approximately 7.0 to 8.0 inclusive, more typically in the range of approximately 7.2 to 7.6 inclusive.

As used herein, the term "subject" encompasses mammals and non-mammals. Examples of mammals include, but are not limited to, any member of the mammalian class: humans, non-human primates such as chimpanzees, and other apes and monkey species; farm animals such as cattle, horses, sheep, goats, swine; domestic animals such as rabbits, dogs, and cats; laboratory animals including rodents, such as rats, mice and guinea pigs, and the like. Examples of non-mammals include, but are not limited to, birds, fish, and the like. The term does not denote a particular age or gender.

The terms "polyethylene glycol", "PEG", "polyethylene oxide" and "PEO" are used interchangeably herein unless indicated otherwise. A CNP peptide (CNP22 or a variant thereof) conjugated via an amino group to a "PEOn" polymer associated with the number n, in general has the formula: CH_3—[—O—CH_2CH_2—]$_n$—C(=O)—NHR, where n is the number of ethylene oxide units and R denotes the rest of the peptide. The "PEOn" polymer can optionally have an alkylene group, $(CH_2)_m$, where m is an integer from 1 to 5, between the carbonyl carbon and the repeating ethylene oxide units. Such a "PEOn" (e.g., PEO12 or PEO24) polymer is monodispersed, i.e., is a single discrete polymer of a particular molecular weight. Similarly, a CNP peptide conjugated via an amino group to a "PEGnK" polymer associated with the number nK, in general has the formula: CH_3—[—O—CH_2CH_2—]$_p$—C(=O)—NHR, where p is an integer greater than 1. The "PEGnK" polymer also can optionally have an alkylene group, $(CH_2)_m$, where m is an integer from 1 to 5, between the carbonyl carbon and the repeating ethylene oxide units. However, such a "PEGnK" (e.g., PEG1K, PEG2K, PEG5K or PEG20K) polymer is polydispersed, i.e., contains a mixture of polymers having a distribution of molecular weights, where the number nK denotes the polymer number-average molecular weight (M_n) in kilo Daltons. For example, "PEG2K" conjugated to a CNP peptide denotes a polydispersed PEG polymer having a polymer number-average molecular weight of around 2 kDa.

When a range of the mass of a polymer (e.g., PEG) is given (e.g., in units of kDa), the range refers to a range of polymer number-average molecular weights, not to a range of molecular weights of multiple polymers in a polydispersed mixture, unless expressly indicated otherwise.

The term "halogen", "halide" or "halo" refers to fluorine, chlorine, bromine, and/or iodine.

The term "alkyl" refers to a linear or branched saturated monovalent hydrocarbon radical, wherein the alkyl may optionally be substituted with one or more substituents Q as described herein. In certain embodiments, the alkyl is a linear saturated monovalent hydrocarbon radical that has 1 to 20 (C_{1-20}), 1 to 15 (C_{1-15}), 1 to 12 (C_{1-12}), 1 to 10 (C_{1-10}), or 1 to 6 (C_{1-6}) carbon atoms, or a branched saturated monovalent hydrocarbon radical of 3 to 20 (C_{3-20}), 3 to 15 (C_{3-15}), 3 to 12 (C_{3-12}), 3 to 10 (C_{3-10}), or 3 to 6 (C_{3-6}) carbon atoms. As used herein, linear C_{1-6} and branched C_{3-6} alkyl groups are also referred as "lower alkyl." Examples of alkyl groups include,

but are not limited to, methyl, ethyl, propyl (including all isomeric forms, including n-propyl and isopropyl), butyl (including all isomeric forms, including n-butyl, isobutyl, sec-butyl and tert-butyl), pentyl (including all isomeric forms), and hexyl (including all isomeric forms). For example, C_{1-6} alkyl refers to a linear saturated monovalent hydrocarbon radical of 1 to 6 carbon atoms or a branched saturated monovalent hydrocarbon radical of 3 to 6 carbon atoms.

The term "alkoxy" refers to an —O-alkyl group. In certain embodiments, an alkoxy group may optionally be substituted with one or more substituents Q as described herein.

The term "haloalkyl" refers to an alkyl group that is substituted with one or more halide atoms. In certain embodiments, a haloalkyl group is substituted with one, two, three, four, five or six halide atoms. In certain embodiments, a haloalkyl group may optionally be substituted with one or more additional substituents Q as described herein.

The term "cycloalkyl" refers to a cyclic saturated bridged and/or non-bridged monovalent hydrocarbon radical, which may be optionally substituted with one or more substituents Q as described herein. In certain embodiments, the cycloalkyl has from 3 to 20 (C_{3-20}), from 3 to 15 (C_{3-15}), from 3 to 12 (C_{3-12}), from 3 to 10 (C_{3-10}), or from 3 to 7 (C_{3-7}) carbon atoms. Examples of cycloalkyl groups include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, decalinyl, and adamantyl.

The term "heterocyclyl" or "heterocyclic" refers to a monocyclic non-aromatic ring system or a multicyclic ring system that contains at least one non-aromatic ring, wherein one or more of the non-aromatic ring atoms are heteroatoms independently selected from O, S, or N, and the remaining non-aromatic ring atoms are carbon atoms. In certain embodiments, the heterocyclyl or heterocyclic group has from 3 to 20, from 3 to 15, from 3 to 10, from 3 to 8, from 4 to 7, or from 5 to 6 ring atoms. In certain embodiments, the heterocyclyl is a monocyclic, bicyclic, tricyclic, or tetracyclic ring system, which may include a fused or bridged ring system, and in which the nitrogen or sulfur atoms may be optionally oxidized, the nitrogen atoms may be optionally quaternized, and some rings may be partially or fully saturated, or aromatic. The heterocyclyl may be attached to the main structure at any heteroatom or carbon atom which results in the creation of a stable compound. Examples of heterocyclic groups include, but are not limited to, acridinyl, azepinyl, benzimidazolyl, benzindolyl, benzoisoxazolyl, benzisoxazinyl, benzodioxanyl, benzodioxolyl, benzofuranonyl, benzofuranyl, benzonaphthofuranyl, benzopyranonyl, benzopyranyl, benzotetrahydrofuranyl, benzotetrahydrothienyl, benzothiadiazolyl, benzothiazolyl, benzothiophenyl, benzotriazolyl, benzothiopyranyl, benzoxazinyl, benzoxazolyl, benzothiazolyl, β-carbolinyl, carbazolyl, chromanyl, chromonyl, cinnolinyl, coumarinyl, decahydroisoquinolinyl, dibenzofuranyl, dihydrobenzisothiazinyl, dihydrobenzisoxazinyl, dihydrofuryl, dihydropyranyl, dioxolanyl, dihydropyrazinyl, dihydropyridinyl, dihydropyrazolyl, dihydropyrimidinyl, dihydropyrrolyl, dioxolanyl, 1,4-dithianyl, furanonyl, furanyl, imidazolidinyl, imidazolinyl, imidazolyl, imidazopyridinyl, imidazothiazolyl, indazolyl, indolinyl, indolizinyl, indolyl, isobenzotetrahydrofuranyl, isobenzotetrahydrothienyl, isobenzothienyl, isochromanyl, isocoumarinyl, isoindolinyl, isoindolyl, isoquinolinyl, isothiazolidinyl, isothiazolyl, isoxazolidinyl, isoxazolyl, morpholinyl, naphthyridinyl, octahydroindolyl, octahydroisoindolyl, oxadiazolyl, oxazolidinonyl, oxazolidinyl, oxazolopyridinyl, oxazolyl, oxiranyl, perimidinyl, phenanthridinyl, phenathrolinyl, phenarsazinyl, phenazinyl, phenothiazinyl, phenoxazinyl, phthalazinyl, piperazinyl, piperidinyl, 4-piperidonyl, pteridinyl, purinyl, pyrazinyl, pyrazolidinyl, pyrazolyl, pyridazinyl, pyridinyl, pyridopyridinyl, pyrimidinyl, pyrrolidinyl, pyrrolinyl, pyrrolyl, quinazolinyl, quinolinyl, quinoxalinyl, quinuclidinyl, tetrahydrofuryl, tetrahydrofuranyl, tetrahydroisoquinolinyl, tetrahydropyranyl, tetrahydrothienyl, tetrazolyl, thiadiazolopyrimidinyl, thiadiazolyl, thiamorpholinyl, thiazolidinyl, thiazolyl, thienyl, triazinyl, triazolyl, and 1,3,5-trithianyl. In certain embodiments, a heterocyclic group may optionally be substituted with one or more substituents Q as described herein.

The term "aryl" refers to a monocyclic aromatic group or a multicyclic monovalent aromatic group that contain at least one aromatic hydrocarbon ring. In certain embodiments, the aryl has from 6 to 20 (C_{6-20}), from 6 to 15 (C_{6-15}), or from 6 to 10 (C_{6-10}) ring atoms. Examples of aryl groups include, but are not limited to, phenyl, naphthyl, fluorenyl, azulenyl, anthryl, phenanthryl, pyrenyl, biphenyl, and terphenyl. Aryl also refers to bicyclic or tricyclic carbon rings, where at least one of the rings is aromatic and the others may be saturated, partially unsaturated, or aromatic, for example, dihydronaphthyl, indenyl, indanyl, and tetrahydronaphthyl (tetralinyl). In certain embodiments, an aryl group may optionally be substituted with one or more substituents Q as described herein.

The term "heteroaryl" refers to a monocyclic aromatic group or a multicyclic aromatic group that contain at least one aromatic ring, wherein at least one aromatic ring contains one or more heteroatoms independently selected from O, S, and N. Each ring of a heteroaryl group can contain one or two O atoms, one or two S atoms, and/or one to four N atoms, provided that the total number of heteroatoms in each ring is four or less and each ring contains at least one carbon atom. The heteroaryl may be attached to the main structure at any heteroatom or carbon atom which results in the creation of a stable compound. In certain embodiments, the heteroaryl has from 5 to 20, from 5 to 15, or from 5 to 10 ring atoms. Examples of monocyclic heteroaryl groups include, but are not limited to, pyrrolyl, pyrazolyl, pyrazolinyl, imidazolyl, oxazolyl, isoxazolyl, thiazolyl, thiadiazolyl, isothiazolyl, furanyl, thienyl, oxadiazolyl, pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, and triazinyl. Examples of bicyclic heteroaryl groups include, but are not limited to, indolyl, benzothiazolyl, benzoxazolyl, benzothienyl, quinolinyl, tetrahydroisoquinolinyl, isoquinolinyl, benzimidazolyl, benzopyranyl, indolizinyl, benzofuranyl, isobenzofuranyl, chromonyl, coumarinyl, cinnolinyl, quinoxalinyl, indazolyl, purinyl, pyrrolopyridinyl, furopyridinyl, thienopyridinyl, dihydroisoindolyl, and tetrahydroquinolinyl. Examples of tricyclic heteroaryl groups include, but are not limited to, carbazolyl, benzindolyl, phenanthrollinyl, acridinyl, phenanthridinyl, and xanthenyl. In certain embodiments, a heteroaryl group may optionally be substituted with one or more substituents Q as described herein.

The term "optionally substituted" is intended to mean that a group, including alkyl, alkoxy, haloalkyl, cycloalkyl, heterocyclyl, aryl and heteroaryl, may be substituted with one or more substituents Q (in one embodiment, one, two, three or four substituents Q), where each Q is independently selected from the group consisting of cyano, halo, oxo, nitro, C_{1-6} alkyl, C_{1-6} alkoxy, halo-C_{1-6} alkyl, C_{3-7} cycloalkyl, heterocyclyl, C_{6-14} aryl, heteroaryl, $—C(O)R^e$, $—C(O)OR^e$, $—C(O)NR^fR^g$, $—C(NR^e)NR^fR^g$, $—OR^e$, $—OC(O)R^e$, $—OC(O)OR^e$, $—OC(O)NR^fR^g$, $—OC(=NR^e)NR^fR^g$, $—OS(O)R^e$, $—OS(O)_2R^e$, $—OS(O)NR^fR^g$, $—OS(O)_2NR^fR^g$, $—NR^fR^g$, $—NR^eC(O)R^f$, $—NR^eC(O)OR^f$, $—NR^eC(O)NR^fR^g$, $—NR^eC(=NR^h)NR^fR^g$, $—NR^eS(O)R^f$, $—NR^eS(O)_2R^f$, $—NR^eS(O)NR^fR^g$, $—NR^eS(O)_2NR^fR^g$, $—SR^e$, $—S(O)R^e$, $—S(O)_2R^e$, and $—S(O)_2NR^fR^g$, wherein each R^e, R^f, R^g, and R^h is independently hydrogen, C_{1-6} alkyl, C_{3-7} cycloalkyl, heterocyclyl, C_{6-14} aryl, or heteroaryl; or R^f and R^g, together with the N atom to which they are attached, form heterocyclyl.

B. CNP Variants

The use of CNP22 as a therapeutic is limited by its short half-life in plasma (J. Clin. Endocrinol. Metab., 78: 1428-35 (1994)). In human plasma, the concentration of CNP22 typically is less than five picomolar. CNP22 is degraded and cleared from circulation by NEP and NPR-C in humans (Growth Hormone & IGF Res., 16: S6-S14). In all human and animal studies using systemically administered CNP22, continuous infusion has been used to increase the CNP22 concentration in the subjects. A CNP peptide having a longer half-life and at least a similar level of functionality would be beneficial to a CNP-based therapeutic strategy. CNP variants are also disclosed in related International Application No. PCT/US08/84270, specifically incorporated herein by reference.

The present disclosure provides CNP variants which have reduced affinity to NEP and/or NPR-C, and reduced susceptibility to cleavage by NEP and/or clearance by NPR-C, but which have substantially similar or better functionality than wild-type CNP22. Reduced susceptibility of CNP variants to cleavage by NEP and/or clearance by NPR-C would increase the plasma or serum half-life of the variants, thereby increasing the opportunity for the variants to distribute to the target tissues and sites and effectuate the desired pharmacological effects. In certain embodiments, the CNP variants described herein have reduced susceptibility to cleavage by NEP and/or clearance by NPR-C in vitro or in vivo by at least about 1.5-fold, 2-fold, 2.5-fold, 3-fold, 3.5-fold, 4-fold, 4.5-fold, or 5-fold compared to wtCNP22, and have increased plasma or serum half-life in vivo by at least about 1.5-fold, 2-fold, 2.5-fold, 3-fold, 3.5-fold, 4-fold, 4.5-fold, or 5-fold compared to wtCNP22, while retaining at least about 50%, 60%, 70%, 80%, 90% or 100% of the functionality of wtCNP22, or having at least about 1.5-fold, 2-fold, 2.5-fold, 3-fold, 3.5-fold, 4-fold, 4.5-fold, or 5-fold greater functionality than wtCNP22. CNP functionality can be evaluated in terms of, e.g., the level of one or more biomarkers (e.g., cGMP) associated with cartilage or bone formation or growth in an in vitro or in vivo study, the length of particular bones in an ex vivo or in vivo study, etc.

Natural substrates of NEP are small and natriuretic peptides (about 2.2 to about 3.2 kDa) are the largest of the natural substrates. According to X-ray crystallographic analyses, the NEP active-site is buried deep inside a central cavity, effectively restricting the size of substrate molecules to no more than about 3 kDa (Oefner et al., J. Mol. Biol., 296: 341-349 (2000)). Based on NPR-B signaling studies, variants of CNP-22, such as CNP-17 (retaining only the cyclic domain, Cys6-Cys22, of CNP22) and CNP-53 (CNP-22 with a 31-amino acid extension at the N-terminus), can still bind and activate NPR-B similarly to the 2.2 kDa wtCNP-22. Accordingly, the disclosure encompasses CNP variants conjugated to a natural (e.g., peptide) and/or synthetic (e.g., PEG) polymer at the N-terminus and/or C-terminus of CNP22 or variants thereof, which exhibit increased NEP resistance but retain the ability to bind and activate the NPR-B signaling receptor.

In one embodiment, the disclosure encompasses CNP variants represented by the general formula:

(x)-Cys_6-Phe_7-Gly_8-Leu_9-Lys_{10}-Leu_{11}-Asp_{12}-Arg_{13}-Ile_{14}-Gly_{15}-Ser_{16}-Met_{17}-Ser_{18}-Gly_{19}-Leu_{20}-Gly_{21}-Cys_{22}-(z) (SEQ ID NO:139), or

(x)-Gly_1-Leu_2-Ser_3-Lys_4-Gly_5-Cys_6-Phe_7-Gly_8-Leu_9-Lys_{10}-Leu_{11}-Asp_{12}-Arg_{13}-Ile_{14}-Gly_{15}-Ser_{16}-Met_{17}-Ser_{18}-Gly_{19}-Leu_{20}-Gly_{21}-Cys_{22}-(z) (SEQ ID NO:140), wherein:

(x) and (z) each independently are a natural polymer (e.g., a peptide sequence containing at least one amino acid) and/or a synthetic polymer (e.g., PEG) as described herein, such that the total mass of the CNP variant is characterized by the ranges described generally herein, e.g., in the range from about 2.6 kDa or 2.8 kDa to about 6 or 7 kDa. In one embodiment, the residues from Cys6 to Cys22 form a cyclic portion. In an embodiment, (x) and/or (z) comprise an amino acid extension derived from NPPC or a non-CNP polypeptide (e.g., ANP, BNP, IgG, etc.), wherein the extension contains 1 to 40, 1 to 35, 1 to 31, 5 to 35, 5 to 31 or 5 to 15 amino acids. In another embodiment, the CNP variants comprise one or more modifications and/or substitutions with another natural amino acid, an unnatural amino acid, a peptidomimetic and/or a peptide bond isostere at one or more of the following positions of CNP22: Gly1, Lys4, Gly5, Cys6, Phe7, Gly8, Leu9, Lys10, Leu11, Ile14, Gly15, Ser16, Met17, Gly19, Leu20 and Gly21.

In another embodiment, CNP variants having a total mass characterized by the ranges described generally herein, e.g., from about 2.6 kDa or 2.8 kDa to about 6 or 7 kDa, designed for increased resistance to NEP degradation, are represented by the general formula:

(x)-Cys_6-Phe_7-Gly_8-Leu_9-$(h)_{10}$-Leu_{11}-Asp_{12}-Arg_{13}-Ile_{14}-Gly_{15}-Ser_{16}-Met_{17}-Ser_{18}-Gly_{19}-Leu_{20}-Gly_{21}-Cys_{22}-(z) (SEQ ID NO: 141), or

(x)-Gly_1-Leu_2-Ser_3-$(b)_4$-Gly_5-Cys_6-Phe_7-Gly_8-Leu_9-$(h)_{10}$-Leu_{11}-Asp_{12}-Arg_{13}-Ile_{14}-Gly_{15}-Ser_{16}-Met_{17}-Ser_{18}-Gly_{19}-Leu_{20}-Gly_{21}-Cys_{22}-(z) (SEQ ID NO: 6), wherein:

(x) is a synthetic or natural polymeric group, or a combination thereof, wherein a non-limiting example of a synthetic polymeric group is PEG (or PEO), and a non-limiting example of a natural polymeric group is an amino acid sequence containing from 1 to 35 amino acids and derived from NPPC or variants thereof with substitutions and/or deletions, ANP, BNP, or other non-CNP (poly)peptides such as, e.g., serum albumin, IgG, histidine-rich glycoproteins, fibronectin, fibrinogen, zinc finger-containing polypeptides, osteocrin or fibroblast growth factor 2 (FGF2);

(z) may be absent or may be a synthetic or natural polymeric group, or a combination thereof, wherein a non-limiting example of a synthetic polymeric group is PEG, and a non-limiting example of a natural polymeric group is an amino acid sequence derived from a natriuretic polypeptide (e.g., NPPC, CNP, ANP or BNP) or non-natriuretic polypeptide (e.g., serum albumin or IgG); and

(b) and (h) independently may be the wild type Lys at that position or may be replaced with a conservative amino acid substitution or any natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amine on a side chain, including but not limited to Arg, Gly, 6-hydroxynorleucine, citrulline (Cit), Gln, Glu or Ser. In one embodiment, (b) is Arg. In another embodiment, for improved NEP resistance, (b) is not Gly. In yet another embodiment, (h) is not Arg.

Non-limiting examples of amino acid sequences derived from NPPC or variants thereof include:

```
Arg,

Glu-Arg,

                                        (SEQ ID NO: 7)
Gly-Ala-Asn-Lys-Lys,

                                        (SEQ ID NO: 8)
Gly-Ala-Asn-Arg-Arg,

                                        (SEQ ID NO: 9)
Gly-Ala-Asn-Pro-Arg,
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-continued

(SEQ ID NO: 10)
Gly-Ala-Asn-Gln-Gln,

(SEQ ID NO: 11)
Gly-Ala-Asn-Ser-Ser,

(SEQ ID NO: 12)
Gly-Ala-Asn-Arg-Gln,

(SEQ ID NO: 13)
Gly-Ala-Asn-Arg-Met,

(SEQ ID NO: 14)
Gly-Ala-Asn-Arg-Thr,

(SEQ ID NO: 15)
Gly-Ala-Asn-Arg-Ser,

(SEQ ID NO: 16)
Ala-Ala-Trp-Ala-Arg-Leu-Leu-Gln-Glu-His-Pro-Asn-Ala,

(SEQ ID NO: 17)
Ala-Ala-Trp-Ala-Arg-Leu-Leu-Gln-Glu-His-Pro-Asn-Ala-Arg,

(SEQ ID NO: 18)
Asp-Leu-Arg-Val-Asp-Thr-Lys-Ser-Arg-Ala-Ala-Trp-Ala-Arg,

(SEQ ID NO: 19)
Gln-Glu-His-Pro-Asn-Ala-Arg-Lys-Tyr-Lys-Gly-Ala-Asn-Lys-Lys,

(SEQ ID NO: 20)
Gln-Glu-His-Pro-Asn-Ala-Arg-Lys-Tyr-Lys-Gly-Ala-Asn-Arg-Arg,

(SEQ ID NO: 21)
Asp-Leu-Arg-Val-Asp-Thr-Lys-Ser-Arg-Ala-Ala-Trp-Ala-Arg-Leu-Leu-Gln-Glu-His-Pro-Asn-Ala-Arg-Lys-Tyr-Lys-Gly-Ala-Asn-Lys-Lys,
and

(SEQ ID NO: 22)
Asp-Leu-Arg-Val-Asp-Thr-Lys-Ser-Arg-Ala-Ala-Trp-Ala-Arg-Leu-Leu-Gln-Glu-His-Pro-Asn-Ala-Arg-Lys-Tyr-Lys-Gly-Ala-Asn-Arg-Arg.

Non-limiting examples of amino acid sequences derived from non-CNP polypeptides such as, e.g., ANP, BNP, serum albumin and IgG include:

(SEQ ID NO: 23)
Ser-Leu-Arg-Arg-Ser-Ser;

(SEQ ID NO: 24)
Asn-Ser-Phe-Arg-Tyr;

(SEQ ID NO: 25)
Ser-Pro-Lys-Met-Val-Gln-Gly-Ser-Gly;

(SEQ ID NO: 26)
Met-Val-Gln-Gly-Ser-Gly;

(SEQ ID NO: 27)
Lys-Val-Leu-Arg-Arg-Tyr;

(SEQ ID NO: 28)
Lys-Val-Leu-Arg-Arg-His;

(SEQ ID NO: 29)
Gly-Gln-His-Lys-Asp-Asp-Asn-Pro-Asn-Leu-Pro-Arg;

(SEQ ID NO: 30)
Gly-Val-Pro-Gln-Val-Ser-Thr-Ser-Thr;

-continued

(SEQ ID NO: 31)
Gly-Glu-Arg-Ala-Phe-Lys-Ala-Trp-Ala-Val-Ala-Arg-Leu-Ser-Gln;

(SEQ ID NO: 32)
Gly-Gln-Pro-Arg-Glu-Pro-Gln-Val-Tyr-Thr-Leu-Pro-Pro-Ser.

In an embodiment, the N-terminal (x) group and/or the C-terminal (z) group of any of the CNP variants having an (x) and/or (z) group, as described herein, independently comprise an amino acid sequence that contains a small number of, if any, acidic natural or unnatural amino acids (e.g., Asp or Glu). In another embodiment, (x) and/or (z) are enriched in basic natural or unnatural amino acids (e.g., Lys, Arg or His) to maintain an alkaline pI similar to the pI of CNP22 (pI 8.9). In one embodiment, the pI of the CNP variants is in the range from about 8 to about 10.5, designed so that the CNP variants can diffuse more readily through the extracellular matrix surrounding chondrocytes of bone growth plates. In narrower embodiments, the pI of the CNP variants is from about 8.5 to about 10.5, or from about 8.5 to about 10, or from about 9 to about 10.

In yet another embodiment, (x) and/or (z) are enriched in polar natural or unnatural amino acids, designed for increased aqueous solubility. In still another embodiment, (x) and/or (z) contain a small number of, if any, hydrophobic natural or unnatural amino acids (e.g., Ala, Val, Leu, Ile or Met).

In a further embodiment, the N-terminus of the CNP variants terminates in at least one glycine residue, designed for increased serum half-life. In a related embodiment, to prevent pyroglutamine formation, the N-terminus of CNP variants terminates in a glycine residue if it would otherwise terminate in glutamine. In one embodiment, the (x) group contains an amino acid extension whose N-terminus terminates in at least one glycine residue. In another embodiment, (x) and/or (z) do not contain two adjacent basic natural or unnatural amino acids (e.g., Lys-Lys or Arg-Arg), designed to reduce susceptibility to cleavage by the protease furin. In an embodiment, (x) does not contain two adjacent basic amino acids immediately preceding the position corresponding to Gly1 of CNP22.

In still another embodiment, the (x) group and/or the (z) group of the CNP variants comprise an amino acid sequence derived from NPPC (e.g., derived from CNP53). In an embodiment, (x) comprises an amino acid sequence derived from the N-terminal tail of ANP or BNP. In another embodiment, (z) comprises an amino acid sequence derived from the C-terminal tail of ANP or BNP. In a further embodiment, (x) and/or (z) comprise an amino acid sequence derived from a non-natriuretic polypeptide such as, e.g., IgG, human serum albumin (HSA), histidine-rich glycoproteins, fibronectin, fibrinogen, zinc finger-containing polypeptides, FGF-2, and bone-targeting proteins (e.g., osteocrin, osteopontin, osteocalcin, and sialoprotein).

In any embodiment described herein in which CNP22 or a variant thereof can have an N-terminal (x) group and/or a C-terminal (z) group, (x) and/or (z) independently can contain an amino acid sequence derived from the functional domain of a bone morphogenetic protein (BMP). An N-terminal and/or C-terminal amino acid extension derived from the functional domain of a BMP can increase the NEP resistance, and hence the serum half-life of the CNP variant, by increasing the total mass of the CNP variant to characterized by the ranges described generally herein, e.g., a range from about 2.6 kDa or 2.8 kDa to about 6 or 7 kDa. In addition, since certain BMPs are growth factors and cytokines that

induce the formation of bone and cartilage, a fragment derived from the functional domain of a BMP can promote chondrocyte, cartilage or bone growth by a mechanism distinct from activation of the guanylyl cyclase function of NPR-B by the cyclic domain of CNP22 or a variant thereof. Non-limiting examples of BMPs that promote bone formation and development, cartilage formation and development, and/or osteoblast differentiation include BMP1, BMP2, BMP3, BMP5, BMP7 and BMP8a. In an embodiment, the N-terminus and/or C-terminus of CNP22 or a variant thereof independently are conjugated to an amino acid sequence derived from the last 140 amino acids in the C-terminal portion of BMP1, BMP2, BMP3, BMP5, BMP7 or BMP8a.

In one embodiment, the CNP variants contain an amino acid extension at the N-terminus and/or C-terminus of CNP22 or CNP17, including but not limited to:

(SEQ ID NO: 4)
DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMS
GLGC (CNP-53);

(SEQ ID NO: 60)
QEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC
(CNP-37, Analog BL);

(SEQ ID NO: 61)
AAWARLLQEHPNAGLSKGCFGLKLDRIGSMSGLGC (Analog CA);

(SEQ ID NO: 62)
AAWARLLQEHPNARGLSKGCFGLKLDRIGSMSGLGC (Analog CB);

(SEQ ID NO: 63)
DLRVDTKSRAAWARGLSKGCFGLKLDRIGSMSGLGC (Analog CC);

(SEQ ID NO: 40)
RGLSKGCFGLKLDRIGSMSGLGC;

(SEQ ID NO: 38)
ERGLSKGCFGLKLDRIGSMSGLGC;

(SEQ ID NO: 64)
GANQQGLSKGCFGLKLDRIGSMSGLGC;

(SEQ ID NO: 65)
GANRRGLSKGCFGLKLDRIGSMSGLGC;

(SEQ ID NO: 66)
GANPRGLSKGCFGLKLDRIGSMSGLGC;

(SEQ ID NO: 67)
GANSSGLSKGCFGLKLDRIGSMSGLGC;

(SEQ ID NO: 144)
GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC;
and

(SEQ ID NO: 68)
SPKMVQGSG-CNP17-KVLRRH (Analog CD) (CNP17 having
N-terminal and C-terminal tails derived from BNP).

In another embodiment, the CNP variants have a K4R substitution at position 4 of CNP22. Non-limiting examples of CNP(K4R) variants include:

(SEQ ID NO: 36)
GANRRGLSRGCFGLKLDRIGSMSGLGC (Analog AY);

(SEQ ID NO: 37)
GANPRGLSRGCFGLKLDRIGSMSGLGC (Analog CI);

(SEQ ID NO: 41)
RGLSRGCFGLKLDRIGSMSGLGC (Analog AZ);

(SEQ ID NO: 39)
ERGLSRGCFGLKLDRIGSMSGLGC (Analog BA);

-continued

(SEQ ID NO: 69)
GANQQGLSRGCFGLKLDRIGSMSGLGC (Analog CH);
and

(SEQ ID NO: 70)
GANSSGLSRGCFGLKLDRIGSMSGLGC (Analog CG).

In further embodiments, the CNP variants are chimeras comprising CNP22, or a variant thereof having amino acid addition(s), deletion(s) and/or substitution(s), and a peptide fragment derived from a polypeptide or protein other than CNP, or the whole non-CNP polypeptide or protein, to the N-terminus of the CNP peptide, wherein CNP22 or the variant thereof may optionally have an N-terminal amino acid extension of one or more amino acid residues. In certain embodiments, the CNP chimeras comprise CNP22 or a variant thereof that has an N-terminal amino acid extension of one or more amino acid residues. In certain embodiments, the CNP chimeras contain lysine-lysine (KK) residues or GANKK residues immediately preceding the first position of CNP22 (Gly in the case of CNP22) or a variant thereof. In other embodiments, the CNP chimeras contain one or two residues different from lysine-lysine immediately preceding the first position of CNP22 or a variant thereof. Non-limiting examples of residues that can immediately precede the first position of CNP22 or a variant thereof include KP, PK, PR, PQ, QK, QQ, RR, SS, GANKP(SEQ ID NO: 200), GANPK (SEQ ID NO: 201), GANPR (SEQ ID NO: 9), GANPQ (SEQ ID NO: 202), GANQK (SEQ ID NO: 203), GANQQ (SEQ ID NO: 10), GANRR (SEQ ID NO: 8), and GANSS (SEQ ID NO: 11).

In another embodiment, the CNP variants are chimera comprising CNP22 and an N-terminal peptide fragment, including but not limited to:

(SEQ ID NO: 76)
GHHSHEQHPHGANQQGLSKGCFGLKLDRIGSMSGLGC (Analog CQ)
(histidine-rich glycoprotein (HRGP) fragment-CNP22
chimera);

(SEQ ID NO: 77)
GAHHPHEHDTHGANQQGLSKGCFGLKLDRIGSMSGLGC (Analog CR)
(HRGP fragment-CNP22 chimera);

(SEQ ID NO: 78)
GHHSHEQHPHGANPRGLSKGCFGLKLDRIGSMSGLGC (Analog CX)
(HRGP fragment-CNP22 chimera);

(SEQ ID NO: 79)
GQPREPQVYTLPPSGLSKGCFGLKLDRIGSMSGLGC (Analog CF)
(IgG$_1$(F$_c$) fragment-CNP22 chimera);

(SEQ ID NO: 80)
GQHKDDNPNLPRGANPRGLSKGCFGLKLDRIGSMSGLGC
(Analog CY) (human serum albumin (HSA) fragment-
CNP22 chimera);

(SEQ ID NO: 81)
GERAFKAWAVARLSQGLSKGCFGLKLDRIGSMSGLGC
(Analog CE) (HSA fragment-CNP22 chimera);

(SEQ ID NO: 82)
FGIPMDRIGRNPRGLSKGCFGLKLDRIGSMSGLGC (Analog CZ)
(osteocrin "NPR C inhibitor" fragment-CNP22
chimera);
and

(SEQ ID NO: 83)
GKRTGQYKLGSKTGPGPKGLSKGCFGLKLDRIGSMSGLGC
(Analog DA) (FGF2 "heparin-binding domain"
fragment-CNP22 chimera).

In yet another embodiment, the CNP variants are chimera comprising an N-terminal peptide fragment and CNP22 in which arginine is substituted for Lys4 of CNP22 ("CNP22 (K4R)"), including but not limited to:

(SEQ ID NO: 84)
GQPREPQVYTGANQQGLSRGCFGLKLDRIGSMSGLGC (Analog CK) ($IgG_1(F_c)$ fragment-CNP22(K4R) chimera);

(SEQ ID NO: 85)
GVPQVSTSTGANQQGLSRGCFGLKLDRIGSMSGLGC (Analog CL) (HSA fragment-CNP22(K4R) chimera);

(SEQ ID NO: 86)
GQPSSSSQSTGANQQGLSRGCFGLKLDRIGSMSGLGC (Analog CM) (fibronectin fragment-CNP22(K4R) chimera);

(SEQ ID NO: 87)
GQTHSSGTQSGANQQGLSRGCFGLKLDRIGSMSGLGC (Analog CN) (fibrinogen fragment-CNP22(K4R) chimera);

(SEQ ID NO: 88)
GSTGQWHSESGANQQGLSRGCFGLKLDRIGSMSGLGC (Analog CO) (fibrinogen fragment-CNP22(K4R) chimera);
and

(SEQ ID NO: 89)
GSSSSSSSSSGANQQGLSRGCFGLKLDRIGSMSGLGC (Analog CP) (zinc finger fragment-CNP22(K4R) chimera).

Chimera comprising IgG and CNP22 or a variant thereof are designed for, inter alia, increased resistance to NEP degradation and reduced binding to serum albumin. CNP chimera comprising a surface fragment of HSA are designed for, inter alia, reduced immunogenicity and reduced binding to serum albumin. HRGP-CNP22 and HRGP-CNP22(K4R) chimera containing a cationic, histidine-rich, non-lysine, non-arginine sequence at the N-terminus are designed for, inter alia, increased stability to proteases. Chimera containing an osteocrin fragment are designed to release, upon protease (e.g., furin) cleavage, the osteocrin fragment at bone growth plates, where the fragment would inhibit the clearance receptor NPR-C. With respect to chimera comprising an FGF2 heparin-binding fragment, heparin binding to the fragment is designed to protect the chimera from degradation, thereby providing a longer serum half-life. Chimera containing a fibronectin, fibrinogen, or zinc-finger fragment are designed for reduced binding to serum albumin, among other features.

Not intending to be bound by theory, a CNP variant of molecular weight from about 2.6 or 2.8 kDa to about 6 or 7 kDa which has increased resistance to NEP degradation and has similar or improved functionality (e.g., binding to NPR-B and stimulation of cGMP signaling) as compared to wtCNP22, may be more effective if it does not bind tightly to plasma proteins such as serum albumin. A CNP variant that does not bind tightly to plasma proteins (e.g., serum albumin) may be more effective in diffusing through cartilage, getting to chondrocytes of bone growth plates, and binding to and activating NPR-B for cGMP signaling. In one embodiment, CNP variants designed for reduced binding to plasma proteins (e.g., serum albumin) are chimeras comprising CNP22 or a variant thereof and a peptide fragment from IgG. In another embodiment, CNP variants designed for reduced binding to plasma proteins are chimeras comprising CNP22 or CNP22(K4R) and a fragment from a polypeptide (e.g., IgG, HSA, fibronectin, fibrinogen, a zinc finger-containing polypeptide, etc.). In yet another embodiment, CNP variants designed for reduced binding to plasma proteins comprise CNP22 or a variant thereof conjugated to a hydrophilic or water-soluble polymer. In one embodiment, the hydrophilic or water-soluble polymer is PEG (or PEO). In another embodiment, the hydrophilic or water-soluble polymer (e.g., PEG) is functionalized with one or more functional groups that impart a negative charge to the polymer under physiological conditions, such as, e.g, carboxyl, sulfate or phosphate groups, or a combination thereof.

In a further embodiment, CNP variants of the disclosure include truncated CNP peptides ranging from human CNP-17 (hCNP-17) to human CNP-53 (hCNP-53), and having wild-type amino acid sequences derived from hCNP-53. Such truncated CNP peptides include:

(SEQ ID NO: 4)
DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-53);

(SEQ ID NO: 146)
LRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-52);

(SEQ ID NO: 147)
RVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-51);

(SEQ ID NO: 148)
VDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-50);

(SEQ ID NO: 149)
DTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-49);

(SEQ ID NO: 150)
TKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-48);

(SEQ ID NO: 151)
KSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-47);

(SEQ ID NO: 152)
SRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-46);

(SEQ ID NO: 153)
RAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-45);

(SEQ ID NO: 154)
AAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-44);

(SEQ ID NO: 155)
AWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-43);

(SEQ ID NO: 156)
WARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-42);

(SEQ ID NO: 157)
ARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-41);

(SEQ ID NO: 158)
RLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-40);

(SEQ ID NO: 159)
LLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-39);

(SEQ ID NO: 160)
LQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-38);

(SEQ ID NO: 60)
QEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-37);

(SEQ ID NO: 161)
EHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-36);

-continued

(SEQ ID NO: 162)
HPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-35);

(SEQ ID NO: 163)
PNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-34);

(SEQ ID NO: 164)
NARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-33);

(SEQ ID NO: 165)
ARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-32);

(SEQ ID NO: 166)
RKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-31);

(SEQ ID NO: 167)
KYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-30);

(SEQ ID NO: 168)
YKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-29);

(SEQ ID NO: 169)
KGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-28);

(SEQ ID NO: 170)
GANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-27);

(SEQ ID NO: 171)
ANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-26);

(SEQ ID NO: 172)
NKKGLSKGCFGLKLDRIGSMSGLGC (CNP-25);

(SEQ ID NO: 173)
KKGLSKGCFGLKLDRIGSMSGLGC (CNP-24);

(SEQ ID NO: 174)
KGLSKGCFGLKLDRIGSMSGLGC (CNP-23);

(SEQ ID NO: 1)
GLSKGCFGLKLDRIGSMSGLGC (CNP-22);

(SEQ ID NO: 175)
LSKGCFGLKLDRIGSMSGLGC (CNP-21);

(SEQ ID NO: 176)
SKGCFGLKLDRIGSMSGLGC (CNP-20);

(SEQ ID NO: 177)
KGCFGLKLDRIGSMSGLGC (CNP-19);

(SEQ ID NO: 178)
GCFGLKLDRIGSMSGLGC (CNP-18);
and

(SEQ ID NO: 2)
CFGLKLDRIGSMSGLGC (CNP-17).

In certain embodiments, CNP variants do not include CNP-17, CNP-22 or CNP-53.

In another embodiment, the truncated CNP peptides ranging from hCNP-17 to hCNP-53 can contain amino acid addition(s), deletion(s) and/or substitution(s) with natural or unnatural amino acid(s) or peptidomimetic(s) (e.g., peptide bond isostere(s)), as described herein, at any one or more of the amino acid positions of the particular truncated CNP peptides. In yet another embodiment, the truncated CNP peptides having wild-type sequences or amino acid addition(s), deletion(s) and/or substitution(s), can be conjugated at the N-terminus, C-terminus and/or internal site(s) to any of the moieties described herein, including but not limited to bone- or cartilage-targeting moieties (e.g., bisphosphonates, bone- or cartilage-targeting peptide sequences (e.g., polyAsp, polyGlu), peptide sequences derived from bone-targeting domains of bone proteins (e.g., osteopontin, osteocalcin, sialoprotein)), peptide sequences derived from the functional domains of bone morphogenetic proteins (e.g., BMP2, BMP3, BMP5, BMP7, BMP8a), peptide sequences derived from natriuretic polypeptides (e.g., NPPC, ANP, BNP), peptide sequences derived from polypeptides of non-natriuretic origin (e.g., serum albumin, IgG, histidine-rich glycoproteins, fibronectin, fibrinogen, zinc finger-containing polypeptides, FGF-2, osteocrin), moieties that reduce renal clearance (e.g., negatively charged PEG moieties), hydrophilic polymers (e.g., PEG), carbohydrates (e.g., carbohydrates recognized by receptors on the surface of cells at bone growth plates), hydrophobic acids (e.g., C_5-C_{12} carboxylic acids, natural fatty acids), phospholipids, and combinations thereof. In an embodiment, the truncated CNP peptides having wild-type sequences or amino acid addition(s), deletion(s) and/or substitution(s), and optionally conjugated to one or more moieties at the N-terminus, C-terminus and/or internal site(s), have a total mass characterized by the ranges described generally herein, e.g., from about 2.6 kDa or 2.8 kDa to about 6 or 7 kDa.

In a further embodiment, the CNP variants are derivatives of CNP37, which is QEHPNARKYKGANKK-CNP22 (SEQ ID NO: 60). The CNP37 variants can contain amino acid addition(s), deletion(s), and/or substitution(s) with natural or unnatural amino acid(s) or peptidomimetic(s) (e.g., peptide bond isostere(s)) at any one or more of the 37 positions of CNP37. Non-limiting examples of substitutions that can be made in CNP37, based on the numbering of CNP22, include K4R, G5S, G5R, G8S, K10R, G15S, S16Q, M17N, G19R, and combinations thereof. In an embodiment, the CNP37 derivatives contain a substitution of Met17 to a natural (e.g., asparagine) or unnatural amino acid or peptidomimetic, designed in part to avoid oxidation of the sulfur atom of methionine. In another embodiment, the CNP37 variants contain substitution(s) of Lys8, Lys10, Lys14 and/or Lys15 (based on numbering from the N-terminus of CNP37) to non-basic natural or unnatural amino acid(s) or peptiomimetic(s), designed in part to reduce albumin binding.

In addition or alternatively to amino acid addition(s), deletion(s) and/or substitution(s), the CNP37 derivatives can be conjugated at the N-terminus, C-terminus, and/or an internal site to any of the moieties described herein, including but not limited to bone- or cartilage-targeting moieties (e.g., bone-targeting peptide domains), moieties that reduce renal clearance (e.g., negatively charged PEG moieties), hydrophilic polymers (e.g., PEG), amino acid sequences comprising one or more amino acids (e.g., osteocrin "NPR-C inhibitor" fragment), carbohydrates (e.g., carbohydrates recognized by receptors on the surface of cells at bone growth plates), hydrophobic acids (e.g., C_5-C_{12} carboxylic acids and natural fatty acids), and combinations thereof.

In one embodiment, the CNP variants are modified CNP37 peptides having mutation(s)/substitution(s) at the furin cleavage site (underlined), designed to improve in vivo resistance to the furin protease, and/or containing glycine (underlined) at the N-terminus, designed to improve plasma stability and prevent pyroglutamine formation. Such CNP37 variants include but are not limited to:

(SEQ ID NO: 71)
GQEHPNARKYKGANPKGLSKGCFGLKLDRIGSMSGLGC (An. CS);

(SEQ ID NO: 72)
GQEHPNARKYKGANQKGLSKGCFGLKLDRIGSMSGLGC (An. CT);

(SEQ ID NO: 73)
GQEHPNARKYKGANQQGLSKGCFGLKLDRIGSMSGLGC (An. CU);

-continued

(SEQ ID NO: 74)
GQEHPNARKYKGANKPGLSKGCFGLKLDRIGSMSGLGC (An. CW);

(SEQ ID NO: 75)
GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC
(Gly-CNP37, An. DB);
and

(SEQ ID NO: 145)
PGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC
(Pro-Gly-CNP37)

In a further embodiment, the CNP variants of the disclosure include CNP peptides and variants thereof that can be produced by the fusion protein process described herein. Non-limiting examples of CNP variants that can be produced by the fusion protein process described herein, using chemical or proteolytic cleavage or protein self-cleavage, include:

(SEQ ID NO: 179)
GDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMS
GLGC (Gly-wtCNP53);

(SEQ ID NO: 75)
GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC
(Gly-wtCNP37);

(SEQ ID NO: 60)
QEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (wtCNP37);

(SEQ ID NO: 144)
GHKSEVAHRFK-GANKKGLSKGCFGLKLDRIGSMSGLGC
(HSA fragment-wtCNP27);

(SEQ ID NO: 36)
GANRRGLSRGCFGLKLDRIGSMSGLGC [CNP27(K4,5,9R)];

(SEQ ID NO: 180)
DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSG
LGC [CNP53(M48N)];

(SEQ ID NO: 181)
GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC
[Gly-CNP37(M32N)];

(SEQ ID NO: 182)
QEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC
[CNP37(M32N)];

(SEQ ID NO: 183)
GHKSEVAHRFK-GANKKGLSKGCFGLKLDRIGSNSGLGC
[HSA-CNP27(M22N)];

(SEQ ID NO: 184)
GANRRGLSRGCFGLKLDRIGSNSGLGC [CNP27(K4,5,9R,
M22N)];

(SEQ ID NO: 185)
PDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMS
GLGC (Pro-wtCNP53);

(SEQ ID NO: 145)
PGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC
(Pro-Gly-wtCNP37);

(SEQ ID NO: 186)
PQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC
(Pro-wtCNP37);

(SEQ ID NO: 187)
PNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (wtCNP34);

(SEQ ID NO: 188)
P-GHKSEVAHRFK-GANKKGLSKGCFGLKLDRIGSMSGLGC
(Pro-HSA-wtCNP27);

(SEQ ID NO: 189)
PGANRRGLSRGCFGLKLDRIGSMSGLGC [Pro-CNP27(K4,5,9R)];

(SEQ ID NO: 190)
MDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMS
GLGC (Met-wtCNP53);

(SEQ ID NO: 191)
MGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC
(Met-Gly-wtCNP37);

(SEQ ID NO: 192)
MQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC
(Met-wtCNP37);

(SEQ ID NO: 193)
M-GHKSEVAHRFK-GANKKGLSKGCFGLKLDRIGSMSGLGC
(Met-HSA-wtCNP27);

(SEQ ID NO: 194)
MGANRRGLSRGCFGLKLDRIGSMSGLGC [Met-CNP27(K4,5,9R)].

Other CNP variants, including truncated CNP peptides ranging from hCNP-17 to hCNP-53 and having wild-type sequences or amino acid addition(s), deletion(s) and/or substitution(s), can also be produced by the fusion protein process described herein, so long as the intended site of chemical or proteolytic cleavage of the fusion protein is not present within the amino acid sequence of the target CNP variant itself. As a non-limiting example, the fusion protein process described herein can be employed to produce truncated wtCNP34 using formic acid cleavage.

In additional embodiments, for any of the CNP peptides and CNP variants described herein that have asparagine (Asn/N) residue(s) and/or glutamine (Gln/Q) residue(s), whether they have a wild-type sequence or a non-natural amino acid sequence, any Asn residue(s) and/or any Gln residue(s) can independently be substituted with any other natural or unnatural amino acids, including conservative substitutions such as Asn to Gln. Such substitution(s) are designed in part to minimize or avoid any potential deamidation of asparagine and/or glutamine. Non-limiting examples of CNP peptides and variants in which any Asn residue(s) and/or any Gln residue(s) can independently be substituted with any other natural or unnatural amino acids, including conservative substitutions such as Asn to Gln, include wtCNP34, wtCNP37, Gly-wtCNP37, Pro-wtCNP37, Pro-Gly-wtCNP37, GHKSEVAHRFK-wtCNP27 (SEQ ID NO: 144), Pro-GHKSEVAHRFK-wtCNP27 (SEQ ID NO: 188), PEO12-GANRR-CNP22(K4R) (SEQ ID NO: 36), and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36). In certain embodiments, an asparagine residue of the CNP peptides and CNP variants described herein is not substituted with glutamine, aspartic acid or glutamic acid. In certain embodiments, a glutamine residue of the CNP peptides and CNP variants described herein is not substituted with asparagine, aspartic acid or glutamic acid. As a non-limiting example, asparagine residues 7 and/or 15 of Pro-Gly-wtCNP37 (PGQEHP NARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC) (SEQ ID NO: 145) can independently be substituted with any other natural or unnatural amino acids, including glutamine, to avoid any potential deamidation of the asparagine residue(s) to aspartic acid or isoaspartic acid. In certain embodiments, asparagine residues 7 and/or 15 of Pro-Gly-wtCNP37 are not substituted with glutamine, aspartic acid or glutamic acid.

It is understood, however, that the present disclosure encompasses CNP variants in which any one or more, up to all, residues susceptible to deamidation or a deamidation-like reaction (e.g., isomerization) may be converted to other residue(s) via deamidation or a deamidation-like reaction to any

extent, up to 100% conversion per converted residue. In certain embodiments, the disclosure encompasses CNP variants in which:

(1) any one or more, up to all, asparagine (Asn/N) residues may be converted to aspartic acid or aspartate, and/or to isoaspartic acid or isoaspartate, via deamidation up to about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% conversion per converted residue; or

(2) any one or more, up to all, glutamine (Gln/Q) residues may be converted to glutamic acid or glutamate, and/or to isoglutamic acid or isoglutamate, via deamidation up to about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% conversion per converted residue; or

(3) any one or more, up to all, aspartic acid or aspartate (Asp/D) residues may be converted to isoaspartic acid or isoaspartate via a deamidation-like reaction (also called isomerization) up to about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% conversion per converted residue; or

(4) any one or more, up to all, glutamic acid or glutamate (Glu/E) residues may be converted to isoglutamic acid or isoglutamate via a deamidation-like reaction (also called isomerization) up to about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% conversion per converted residue; or

(5) a combination of the above.

As a non-limiting example, the disclosure encompasses CNP variants in which any one or more, up to all, asparagine, glutamine, aspartic acid, and/or glutamic acid residues of Pro-Gly-wtCNP37 [PGQEHPNARKYKGA NKKGLSKGCFGLKLDRIGSMSGLGC] (SEQ ID NO: 145) may be converted to (1) aspartic acid/aspartate and/or isoaspartic acid/isoaspartate, (2) glutamic acid/glutamate and/or isoglutamic acid/isoglutamate, (3) isoaspartic acid/isoaspartate, and/or (4) isoglutamic acid/isoglutamate, respectively, via deamidation or a deamidation-like reaction up to about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% conversion per converted residue, as described above.

As a further example, the disclosure encompasses CNP variants in which any one or more, up to all, asparagine and/or aspartic acid residues of Pro-Gly-wtCNP37 [PGQEHP NARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC] (SEQ ID NO: 145) may be converted to (1) aspartic acid/aspartate and/or isoaspartic acid/isoaspartate, and/or (2) isoaspartic acid/isoaspartate, respectively, via deamidation or a deamidation-like reaction up to about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% conversion per converted residue.

As another example, the present disclosure encompasses CNP variants in which any one or more, up to all, asparagine, glutamine, aspartic acid, and/or glutamic acid residues of Gly-wtCNP37 [GQEHPNARKYKGA NKKGLSKGCFGLKLDRIGSMSGLGC (SEQ ID NO: 75)] may be converted to (1) aspartic acid/aspartate and/or isoaspartic acid/isoaspartate, (2) glutamic acid/glutamate and/or isoglutamic acid/isoglutamate, (3) isoaspartic acid/isoaspartate, and/or (4) isoglutamic acid/isoglutamate, respectively, via deamidation or a deamidation-like reaction up to about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% conversion per converted residue.

As yet another example, the disclosure encompasses CNP variants in which any one or more, up to all, asparagine, glutamine, aspartic acid, and/or glutamic acid residues of wtCNP37 [QEHPNARKYKGANKKGLSKGCFGLKL DRIGSMSGLGC (SEQ ID NO: 60)] may be converted to (1) aspartic acid/aspartate and/or isoaspartic acid/isoaspartate, (2) glutamic acid/glutamate and/or isoglutamic acid/isoglutamate, (3) isoaspartic acid/isoaspartate, and/or (4) isoglutamic acid/isoglutamate, respectively, via deamidation or a deamidation-like reaction up to about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% conversion per converted residue.

As a further example, the present disclosure encompasses CNP variants in which any one or more, up to all, asparagine, aspartic acid, and/or glutamic acid residues of an HSA-wtCNP27 chimera, GHKSEVAHRFKGA NKKGLSKGCFGLKLDRIGSMSGLGC (SEQ ID NO: 144), may be converted to (1) aspartic acid/aspartate and/or isoaspartic acid/isoaspartate, (2) isoaspartic acid/isoaspartate, and/or (3) isoglutamic acid/isoglutamate, respectively, via deamidation or a deamidation-like reaction up to about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% conversion per converted residue.

As a still further example, the disclosure encompasses CNP variants in which any one or more, up to all, asparagine, aspartic acid, and/or glutamic acid residues of a Pro-HSA-wtCNP27 chimera, PGHKSEVAHRFKGA NKKGLSKGCFGLKLDRIGSMSGLGC (SEQ ID NO: 188), may be converted to (1) aspartic acid/aspartate and/or isoaspartic acid/isoaspartate, (2) isoaspartic acid/isoaspartate, and/or (3) isoglutamic acid/isoglutamate, respectively, via deamidation or a deamidation-like reaction up to about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% conversion per converted residue.

In addition, the present disclosure encompasses CNP variants in which any one or more, up to all, methionine (Met/M) residues may be oxidized to any chemically feasible oxidized form (e.g., sulfoxide and/or sulfone) up to about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% transformation per oxidized residue.

In another embodiment, the CNP variants comprise CNP22 or variants thereof conjugated at the N-terminus and/or C-terminus to moiet(ies) that facilitate translocation of the variants across a cell membrane or cell barrier. In one embodiment, the CNP variants are conjugated at the N-terminus and/or C-terminus to peptide sequence(s) that facilitate transport of the variants across a cell membrane or cell barrier, including via active peptide transporters.

In a further embodiment, the N-terminus and/or C-terminus of CNP22 or a variant thereof are conjugated to chemical moieties such as, e.g., natural and/or synthetic polymers, to increase the total mass of the modified CNP peptide to the ranges described generally herein, e.g., a range from about 2.6 or 2.8 kDa to about 6 or 7 kDa. In one embodiment, the chemical moieties are biocompatible hydrophilic or water-soluble natural (e.g., peptides, carbohydrates) or synthetic (e.g., PEG (or PEO)) polymers.

In a particular embodiment, the N-terminus and/or C-terminus of CNP22 or a variant thereof are conjugated to PEG (or PEO) polymers to result in a total mass characterized by the ranges described generally herein, e.g., from about 2.6 or 2.8 kDa to about 6 or 7 kDa. Pegylation of CNP22 or a variant thereof is designed, inter alia, to reduce immunogenicity and improve half-life by reducing renal clearance and increasing protease resistance. A PEG moiety can be attached to the N- and/or C-terminus of CNP22 or any variant described herein, including but not limited to CNP-17 (the Cys6-Cys22 cyclized portion of CNP22), CNP37, and variants of CNP17, CNP22 or CNP37 having N- and/or C-terminal amino acid extension(s), amino acid substitution(s) and/or amino acid deletion(s). In an embodiment, the Lys4 and/or Lys10 residues of CNP17, CNP22 or CNP37, or variants thereof, are substituted with a natural or unnatural amino acid (e.g., Arg,

Gly, Ser, Gln, Glu or Cit) or peptidomimetic that does not contain a reactive primary amine on a side chain, to preclude any potential PEGylation of these lysine residues. In one embodiment, the Lys4 and/or Lys10 residues of the CNP peptides are substituted with Arg. In another embodiment, the Lys10 residue is not substituted with Arg.

In a further embodiment, CNP variants (including CNP22 and variants thereof) having a PEG (or PEO) moiety and an amino acid extension at the N-terminus contain arginine at the position immediately preceding the position corresponding to Gly1 of CNP22. Such PEGylated CNP variants are designed for increased resistance to NEP degradation, reduced binding to serum albumin, and enhanced CNP functional activity (e.g., activation of cGMP signaling). Non-limiting examples of PEGylated CNP variants include PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36), PEO12-GANRR-CNP22(K4R) (SEQ ID NO: 36), PEO24-GANRR-CNP22(SEQ ID NO: 36), PEO12-GANRR-CNP22(SEQ ID NO: 36), PEO24-GANPR-CNP22(K4R) (SEQ ID NO: 37), PEO12-GANPR-CNP22(K4R) (SEQ ID NO: 37), PEO24-GANPR-CNP22(SEQ ID NO: 37), PEO12-GANPR-CNP22 (SEQ ID NO: 37), PEO24-GANQQ-CNP22(SEQ ID NO: 64), PEO12-GANQQ-CNP22(SEQ ID NO: 64), PEO24-ER-CNP22(K4R) (SEQ ID NO: 39), PEO12-ER-CNP22(K4R) (SEQ ID NO: 39), PEO24-ER-CNP22(SEQ ID NO: 39), PEO12-ER-CNP22(SEQ ID NO: 39), PEO24-R-CNP22 (K4R) (SEQ ID NO: 41), PEO12-R-CNP22(K4R) (SEQ ID NO: 41), PEO24-R-CNP22(SEQ ID NO: 41), and PEO12-R-CNP22(SEQ ID NO: 41), wherein PEO24 is a monodispersed 1.2 kDa PEG polymer and PEO12 is a monodispersed 0.6 kDa PEG polymer. In one embodiment, the PEG (or PEO) polymer is conjugated to the N-terminus of the CNP variants.

The disclosure contemplates use of hydrophilic or water soluble polymers (e.g., PEG) that can vary in type (e.g., homopolymer or copolymer; random, alternating or block copolymer; linear or branched; monodispersed or polydispersed), linkage (e.g., hydrolysable or stable linkage such as, e.g., amide, imine, aminal, alkylene, or ester bond), conjugation site (e.g., at the N-terminus and/or C-terminus, preferably not at any of the residues in the cyclized region of CNP (corresponding to residues 6-22 of CNP22)), and length (e.g., from about 0.2, 0.4 or 0.6 kDa to about 2, 3, 4 or 5 kDa). The hydrophilic or water-soluble polymer can be conjugated to the CNP peptide by means of N-hydroxy succinimide (NHS)- or aldehyde-based chemistry or other chemistry, as is known in the art. Such CNP variants can be generated using, e.g., wtCNP22 (2.2 kDa), CNP17 retaining only the cyclized region (residues 6-22) of wtCNP22, CNP variants having an amino acid extension at the N-terminus and/or C-terminus of CNP22 or CNP17, or variants having amino acid substitutions, additions and/or deletions such as, e.g., GANRR-CNP22(K4R) (SEQ ID NO: 36), GANPR-CNP22(K4R) (SEQ ID NO: 37), R-CNP22(SEQ ID NO: 40), R-CNP22 (K4R) (SEQ ID NO: 41), ER-CNP22(SEQ ID NO: 38) and ER-CNP22(K4R) (SEQ ID NO: 39). In an embodiment, the PEG-CNP variants having a total mass characterized by the ranges described generally herein, e.g., from about 2.6 or 2.8 kDa to about 6 or 7 kDa, contain a monodispersed, linear PEG (or PEO) moiety conjugated at the N-terminus and/or C-terminus via NHS- or aldehyde-based chemistry, or a two-arm or three-arm branched PEG moiety conjugated at the N-terminus and/or C-terminus via NHS-based chemistry. The disclosure also encompasses negatively charged PEG-CNP variants designed for reduced renal clearance, including but not limited to carboxylated, sulfated and phosphorylated compounds (Caliceti, Adv. Drug Deliv. Rev., 55: 1261-77 (2003); Perlman, J. Clin. Endo. Metab., 88: 3227-35 (2003); Pitkin, Antimicrob. Ag. Chemo., 29: 440-444 (1986); Vehaskari, Kidney Int'l, 22: 127-135 (1982)). In one embodiment, the PEG (or PEO) moiety contains carboxyl group(s), sulfate group(s), and/or phosphate group(s).

In another embodiment, the PEG (or PEO) moieties conjugated to the N-terminus, C-terminus and/or internal site(s) of CNP variants described herein contain one or more functional groups that are positively charged under physiological conditions. Such PEG moieties are designed, inter alia, to improve distribution of such PEGylated CNP variants to cartilage tissues. In one embodiment, such PEG moieties contain one or more primary, secondary or tertiary amino groups, quaternary ammonium groups, and/or other amine-containing (e.g., urea) groups.

In an embodiment, the disclosure encompasses CNP22 or variants thereof conjugated via NHS- or aldehyde-based chemistry to PEG (or PEO) of the formula $(CH_2CH_2O)_n$, wherein n is an integer from about 6 to about 100, and the PEG polymer is from about 0.3 kDa to about 5 kDa. In another embodiment, n is an integer from about 12 to about 50, and the PEG polymer is from about 0.6 kDa to about 2.5 kDa. In yet another embodiment, n is from about 12 to about 24, and the PEG polymer is from about 0.6 kDa to about 1.2 kDa. In still another embodiment, the terminal hydroxyl group of the PEG polymer is capped with a non-reactive group. In a particular embodiment, the end-capping group is an alkyl group, e.g., a lower alkyl group such as methyl.

In a further embodiment, the disclosure provides CNP variants having one or more peptide bonds or peptide bond isosteres that have reduced susceptibility to cleavage by peptidases including neutral endopeptidase (NEP). NEP is a membrane-bound zinc-dependent endopeptidase that cleaves a substrate peptide bond at the amino end of large hydrophobic residues. Thus, modification of a peptide bond at a cleavage site for NEP to an unnatural peptide or non-peptide bond may preclude or decrease the efficiency of NEP cleavage.

For ANP and CNP, NEP cleavage is reported to occur first at the Cys6-Phe7 bond within the cyclized region, then elsewhere throughout the remainder of the structures. For BNP, cleavage is reported to occur first at the peptide N-terminus, then within the cyclic structure. Although the primary NEP cleavage site on CNP is reported to be the Cys6-Phe7 bond, when wtCNP22 was exposed to NEP digestion for 2.5 minutes in vitro, all possible sites were unexpectedly hydrolyzed, with the Cys6-Phe7 and Gly8-Leu9 peptide bonds being slightly most labile, as described in Example 2.

Substrate specificity of NEP is primarily determined by two substrate-binding subsites, S1' and S2' (Oefner et al., J. Mol. Biol. 296:341-349 (2000)). The S1' site accepts a large hydrophobic P1' residue of which the N-terminal peptide bond is subjected to hydrolysis (e.g., Phe, Leu, Be and Met). The S2' site generally prefers a smaller residue, termed P2' (e.g., Gly or Ser). In the case of CNP, Phe7 is reported to be the preferred P1' residue for the NEP S1' site, while Gly8 is the preferred P2' residue for the S2' site. Because these two subsites can together accommodate only a certain total side chain size, any increase in the total size of the P1'-P2' residues of CNP can potentially disrupt NEP binding. For example, addition of a chloride atom at the 3-position of the P1' Phe7 aromatic ring (i.e., 3-Cl-Phe7) can potentially modify (e.g., destabilize) interactions between CNP and the NEP cleavage sites, for example at the S1' subsite. Addition of a tertiary butyl group to the smaller P2' residue Gly8 (i.e., tBu-Gly8) can potentially disrupt the interaction between CNP and the S2' subsite.

Accordingly, in one embodiment, CNP variants of the disclosure include CNP having an increase in the size of the

P1'-P2' residues, such as Phe7-Gly8, to interfere with substrate recognition at the active site, thereby reducing susceptibility to NEP cleavage. Natural amino acids, unnatural amino acids and/or peptidomimetic moieties are substituted for one or more large P1' hydrophobic residues, including but not limited to Phe7, Leu9, Leu11, Ile14, Met17 and Leu20, and/or for one or more smaller P2' residues, including but not limited to Cys6, Gly8, Gly15, Ser16 and Gly19.

The disclosure encompasses CNP variants comprising at least one modified amino acid and/or at least one modified peptide bond, at at least one residue involved in substrate recognition and/or cleavage by NEP, wherein the modified amino acids and modified peptide bonds can be natural amino acids, unnatural amino acids, peptidomimetics and/or peptide bond isosteres. In one embodiment, the NEP cleavage site on CNP between Cys6 and Phe7 is modified. In a related embodiment, the peptide bond (—C(=O)—NH—) between Cys6 and Phe7 is replaced with one of the following peptide bond isosteres:

—CH_2—NH—,

—C(=O)—N(R)—, where the amide group is alkylated with any of the following R groups: methyl, ethyl, n-propyl, isopropyl, cyclopropyl, n-butyl, isobutyl, sec-butyl or tert-butyl,

—C(=O)—NH—CH_2—,

—CH_2—S—,

—CH_2—$S(O)_n$—, where n is 1 or 2,

—CH_2—CH_2—,

—CH=CH—,

—C(=O)—CH_2—,

—CH(CN)—NH—,

—CH(OH)—CH_2—,

—O—C(=O)—NH—, or

—NHC(=O)NH—.

In another embodiment, the CNP variants are represented by the formula:

(x)-Gly_1-Leu_2-Ser_3-Lys_4-Gly_5-(b)$_6$-(c)$_7$-(d)$_8$-Leu_9-Lys_{10}-Leu_{11}-Asp_{12}-Arg_{13}-Ile_{14}-Gly_{15}-Ser_{16}-Met_{17}-Ser_{18}-Gly_{19}-Leu_{20}-Gly_{21}-Cys_{22}-(z) (SEQ ID NO: 90), wherein:

(x) and (z) independently may be absent or may be selected from the group consisting of synthetic bone-targeting compounds such as, e.g., bisphosphonates; amino acid sequences useful in bone or cartilage targeting such as, e.g., polyAsp and polyGlu; amino acid sequences derived from bone-targeting domains of bone proteins such as, e.g., osteopontin, osteocalcin, and sialoprotein (Wang et al., Adv. Drug Delivery Rev., 57: 1049-76 (2005)); polymeric and non-polymeric molecules that reduce renal clearance such as, e.g., negatively charged PEGs; and natural polymers (e.g., those containing amino acids, fatty acids and/or carbohydrates) and synthetic polymers (e.g., PEGs) that increase resistance of the CNP variant to NEP degradation by increasing the total mass of the CNP variant to the ranges described generally herein, e.g., from about 2.6 or 2.8 kDa to about 6 or 7 kDa;

(b) and (c) may be the wild-type Cys6 and Phe7, another natural amino acid or an unnatural amino acid, or may contain a peptide bond isostere as described herein to increase resistance to NEP cleavage; and

(d) may be the wild-type Gly8, or may be a larger natural or unnatural (e.g., t-Bu-Gly) amino acid or peptidomimetic to reduce binding to NEP.

In one embodiment, such CNP variants contain at least one modified amino acid at (b), (c) and/or (d).

Other peptide bonds within CNP may be cleaved even if CNP22 or a variant thereof has an NEP-resistant peptide bond or peptide bond isostere at Cys6-Phe7, including the Gly8-Leu9, Lys10-Leu11, Arg13-Ile14, Ser16-Met17, and Gly19-Leu20 bonds. Therefore, the disclosure encompasses CNP variants having peptide bond isostere(s) at one or more other NEP cleavages sites in addition to the Cys6-Phe7 bond, wherein the peptide bond isosteres include those described herein.

In another embodiment, the disclosure encompasses CNP variants having a cysteine analog at Cys6 and/or Cys22, including but not limited to homocysteine, penicillamine, 2-mercaptopropionic acid, and 3-mercaptopropionic acid. In an embodiment, such CNP variants have a cyclic domain formed by a disulfide bond between the wild-type Cys6 or analog and Cys22 or analog.

In yet another embodiment, one or more residues of CNP22 or a variant thereof, up to all the residues, are substituted with a D-amino acid. Substitution of an L-amino acid with a D-amino acid essentially moves the side chain about 120 degrees from its original position, thereby potentially disrupting the binding of the CNP peptide to NEP. In a specific embodiment, L-Phe at Phe7 is substituted with its D-enantiomer, D-Phe.

In still another embodiment, a beta amino acid such as, e.g., 3-amino-2-phenylpropionic acid (or 2-phenyl-beta-alanine), replaces the wild-type alpha-amino acid Phe7. Use of a beta-amino acid effectively increases the peptide backbone length by one methylene unit. Protease resistance can result from the change in substrate conformation or the increased distance between amino acid side chains.

Non-limiting examples of variants of CNP22 having an unnatural alpha-amino acid, a beta-amino acid or a peptide bond isostere include:

(SEQ ID NO: 56)
GLSKGC(CH_2NH)FGLKLDRIGSMSGLGC (Analog A),

(SEQ ID NO: 57)
GLSKGC-(N-Me-Phe)-GLKLDRIGSMSGLGC (Analog B),

(SEQ ID NO: 136)
GLSKGC-(D-Phe)-GLKLDRIGSMSGLGC (Analog E),

(SEQ ID NO: 58)
GLSKGCF-(tBu-Gly)-LKLDRIGSMSGLGC (Analog F),

(SEQ ID NO: 137)
GLSKGC-(3-Cl-Phe)-GLKLDRIGSMSGLGC (Analog G),
and

(SEQ ID NO: 59)
GLSKGC-[NHCH_2CH(Ph)CO]-GLKLDRIGSMSGLGC (Analog H, formed using 3-amino-2-phenylpropionic acid).

In a further embodiment, the CNP variants have a total mass characterized by the ranges described generally herein, e.g., from about 2.6 or 2.8 kDa to about 6 or 7 kDa, designed for increased resistance to NEP degradation, and are represented by the formula:

(x)-Gly_1-Leu_2-Ser_3-(a)$_4$-Gly_5-(b)$_6$-(c)$_7$-(d)$_8$-(e)$_9$-(f)$_{10}$-(g)$_{11}$-Asp_{12}-Arg_{13}-(h)$_{14}$-Gly_{15}-Ser_{16}-(i)$_{17}$-Ser_{18}-Gly_{19}-(j)$_{20}$-Gly_{21}-Cys_{22}-(z) (SEQ ID NO: 46), wherein:

(x) and (z) independently may be absent or may be selected from the group consisting of synthetic bone-targeting compounds such as, e.g., bisphosphonates; amino acid sequences useful in bone or cartilage targeting such as, e.g., polyAsp and polyGlu; amino acid sequences derived from bone-targeting domains of bone proteins such as, e.g., osteopontin, osteocalcin, and sialoprotein; polymeric and non-polymeric moieties that reduce renal clearance such as, e.g., negatively charged PEGs; polymers containing, e.g., amino acids, hydrophobic acids, and/or carbohydrates; and synthetic hydrophilic polymers such as, e.g., PEGs;

(a) may be the wild-type Lys at that position or may be replaced with a conservative amino acid substitution or a natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amine on a side chain, including but not limited to Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu, wherein in one embodiment (a) is Arg;

(b) is selected from the group consisting of Cys and peptide-bond isosteres between Cys6 and Phe7 such as, e.g., Cys-CH_2—NH;

(c) is selected from the group consisting of L-Phe; D-Phe; 3-amino-2-phenylpropionic acid; N-alkylated derivatives of Phe, wherein the N-alkyl group is methyl, ethyl, n-propyl, isopropyl, cyclopropyl, n-butyl, isobutyl, sec-butyl or tert-butyl; and Phe analogs, wherein one or more ortho-, meta-, and/or para-positions of the benzene ring of the Phe analog are substituted with one or more substituents selected from the group consisting of halogen, hydroxyl, cyano, straight or branched C_{1-6} alkyl, straight or branched C_{1-6} alkoxy, straight or branched halo-C_{1-6} alkyl, C_{3-10} cycloalkyl, heterocyclyl, C_{6-14} aryl and heteroaryl (examples include, but are not limited to, tyrosine, 3-chlorophenylalanine, 2,3-chloro-phenylalanine, 3-chloro-5-fluoro-phenylalanine, 2-chloro-6-fluoro-3-methyl-phenylalanine), or wherein the benzene ring of the Phe analog can be replaced with another aryl group (non-limiting examples include 1- and 2-naphthylalanine) or with a heteroaryl group (non-limiting examples include pyridylalanine, thienylalanine and furylalanine);

(d) is selected from the group consisting of Gly, tert-butyl-Gly (tBu-Gly), Thr, Ser, Val and Asn;

(e) is selected from the group consisting of Leu, Ser, Thr and peptide-bond isosteres such as, e.g., N-Me-Leu;

(f) may be the wild type Lys at that position or may be replaced with a conservative amino acid substitution or a natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amine on a side chain, including but not limited to Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu, wherein in one embodiment (f) is not Arg;

(g) is selected from the group consisting of Leu and peptide-bond isosteres such as, e.g., N-Me-Leu;

(h) is selected from the group consisting of Ile, tBu-Gly, and peptide-bond isosteres such as, e.g., N-Me-Ile;

(i) is selected from the group consisting of Met, Val, Asn, beta-Cl-Ala, 2-aminobutyric acid (Abu) and 2-amino-isobutyric acid (Aib); and

(j) is selected from the group consisting of Leu, norleucine (Nle), homoleucine (Hleu), Val, tert-butyl-Ala (tBu-Ala), Ser, Thr, Arg, and peptide-bond isosteres such as, e.g., N-Me-Leu.

In another embodiment, the CNP variants have a total mass characterized by the ranges described generally herein, e.g., from about 2.6 or 2.8 kDa to about 6 or 7 kDa, designed for increased resistance to NEP cleavage, and are represented by the formula:

(x)-Gly_1-Leu_2-Ser_3-$(a)_4$-Gly_5-$(b)_6$-$(c)_7$-$(d)_8$-$(e)_9$-$(f)_{10}$-$(g)_{11}$-Asp_{12}-Arg_{13}-$(h)_{14}$-$(i)_{15}$-Ser_{16}-$(j)_{17}$-Ser_{18}-Gly_{19}-$(k)_{20}$-Gly_{21}-Cys_{22}-(z) (SEQ ID NO:143), wherein:

(x) and (z) independently may be absent or may be selected from the group consisting of synthetic bone-targeting compounds such as, e.g., bisphosphonates; amino acid sequences useful in bone or cartilage targeting such as, e.g., polyAsp and polyGlu; amino acid sequences derived from bone-targeting domains of bone proteins and derivatives thereof, such as, e.g., fusion proteins or peptide sequences of osteopontin, osteocalcin, sialoprotein, etc.; moieties that reduce renal clearance, including but not limited to hydrophilic or water-soluble polymers such as, e.g., charged PEG molecules; and moieities comprising, e.g., PEGs, amino acids, carbohydrates, and/or hydrophobic acids;

(a) may be the wild type Lys at that position or may be replaced with a conservative amino acid substitution or a natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amine on a side chain, including but not limited to Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu, wherein in one embodiment (a) is Arg;

(b) is selected from the group consisting of Cys and peptide bond isosteres between Cys6 and Phe7 such as, e.g., Cys-CH_2—NH;

(c) is selected from the group consisting of L-Phe; D-Phe; 3-amino-2-phenylpropionic acid; N-alkylated derivatives of Phe, wherein the N-alkyl group is methyl, ethyl, n-propyl, isopropyl, cyclopropyl, n-butyl, isobutyl, sec-butyl or tert-butyl; and Phe analogs, wherein one or more ortho-, meta-, and/or para-positions of the benzene ring of the Phe analog are substituted with one or more substituents selected from the group consisting of halogen, hydroxyl, cyano, straight or branched C_{1-6} alkyl, straight or branched C_{1-6} alkoxy, straight or branched halo-C_{1-6} alkyl, C_{3-10} cycloalkyl, C_{6-14} aryl, heterocyclyl and heteroaryl (examples include, but are not limited to, tyrosine, 3-chlorophenylalanine, 2,3-chloro-phenylalanine, 3-chloro-5-fluoro-phenylalanine, 2-chloro-6-fluoro-3-methyl-phenylalanine), or wherein the benzene ring of the Phe analog can be replaced with another aryl group (non-limiting examples include 1- and 2-naphthylalanine) or with a heteroaryl group (non-limiting examples include pyridylalanine, thienylalanine and furylalanine);

(d) is selected from the group consisting of Gly, tert-butyl-Gly, Thr, Ser, Val and Asn;

(e) is selected from the group consisting of Leu, Ser, Thr, and peptide bond isosteres such as, e.g., N-Me-Leu;

(f) is selected from the group consisting of Lys, Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln and Ser;

(g) is selected from the group consisting of Leu, Asn, and peptide bond isosteres such as, e.g., N-Me-Leu;

(h) is selected from the group consisting of Ile, tert-butyl-Gly (tBu-Gly), Asn, and peptide bond isosteres such as, e.g., N-Me-Ile;

(i) is selected from the group consisting of Gly, Arg, Ser and Asn;

(j) is selected from the group consisting of Met, Val, Asn, beta-Cl-Ala, 2-aminobutyric acid (Abu) and 2-amino-isobutyric acid (Aib); and

(k) is selected from the group consisting of Leu, norleucine (Nle), homoleucine (Hleu), Val, tert-butyl-Ala (tBu-Ala), Arg, Thr, Ser, and peptide bond isosteres such as, e.g., N-Me-Leu.

To improve the delivery of the CNP variants to the target sites of bone-related disorders (e.g., skeletal dysplasias), the CNP variants can be attached (e.g., at the N-terminus and/or C-terminus) to bone- or cartilage-targeting moieties. Non-limiting examples of bone- or cartilage-targeting moieties include bisphosphonates; hydroxyapatite; glucosamine; collagen (e.g., collagen type X); polyAsp; polyGlu; and amino acid sequences derived from bone-targeting domains of bone proteins such as, e.g., osteocrin, osteopontin, osteocalcin, and sialoprotein.

In addition to being less susceptible to NEP cleavage, the CNP variants potentially have reduced affinity to the NPR-C clearance receptor, while retaining CNP functionality. Besides NEP-mediated degradation, the half-life of CNP22 is influenced by the clearance receptor, NPR-C, which shares 58% sequence homology with the extracellular peptide-binding domain of NPR-B. CNP22 binds tightly to not only NPR-B (7-30 pM affinity), but also NPR-C (11-140 pM)

(Bennett, B. D. et al., J. Biol. Chem., 266: 23060-67 (1991); Koller K. J. & Goeddel, D. V., Circulation, 86: 1081-88 (1992); Suga, S. et al., Endocrinology, 130: 229-39 (1992)). Even though the NPR-B crystal structure has yet to be reported, sequence homology as well as similarities between the NPR-C and NPR-A crystal structures (He, X.-L. et al., Science, 293(5535): 1657-62 (2001); Ogawa, H. et al., J. Biol. Chem., 279(27): 28625-31 (2004); He, X.-L., J. Mol. Biol., 361(4): 698-714 (2006)) suggest that NPR-B likely assumes a similar overall structural fold.

Therefore, an NPR-B homology model was built based on structure-based sequence alignment and crystallographic structures of the following related systems: CNP bound to NPR-C, ANP bound to NPR-A, and ANP bound to NPR-C (He, X.-L. et al., Science, 293(5535): 1657-62 (2001); Ogawa, H. et al., J. Biol. Chem., 279(27): 28625-31 (2004); He, X.-L., J. Mol. Biol., 361(4): 698-714 (2006)). Based on observations that the receptor appears to determine the bound peptide conformation, and that NPR-B most closely resembles NPR-A with respect to primary structure and functional properties, the NPR-B/CNP homology model was built with the NPR-A/ANP crystal structure as a model. Published signaling data of CNP variants (U.S. Pat. No. 5,434,133 and US Patent Application Publication No. 2004/0138134 A1), and of functional ANP variants that no longer bind to NPR-C (Cunningham, EMBO 13(11) 2508-15, 1994) were used to refine and interpret the NPR-B/CNP model.

The present disclosure encompasses CNP variants designed for improved NPR-B selectivity based on a homology-based structural model of the NPR-B/CNP complex. Combining the experimental and computational structure data of natriuretic peptides bound to the various receptors with the published functional data, CNP variants were generated that continue to bind to NPR-B, but can potentially have reduced affinity to the NPR-C clearance receptor. For example, NPR-C has a unique insertion in a loop structure in the peptide-binding site, placing its loop residues closer to such peptide residues as CNP Gly8 (or ANP Gly9), compared to respective loop residues in NPR-A and NPR-B. Earlier studies indicated that the G9T mutation in ANP contributes to reduce affinity to NPR-C, thereby improving NPR-A selectivity (Cunningham, EMBO J., 13(11): 2508-15 (1994)). Accordingly, CNP variants were generated to replace the corresponding Gly8 residue with a larger residue (Ser, Val, Thr or Asn) to disrupt the CNP binding to NPR-C without affecting its binding to NPR-B. Further, one or more mutations were introduced at the C-terminal end of CNP, encompassing Gly15 to Gly21, which is predicted to interact with receptor-specific residues, based on the detailed structural analyses of the receptor/peptide complexes. For example, a G19R mutation in CNP22 does not result in a significant loss of NPR-B signaling activity. This mutation, however, cannot be modeled into the available crystal structure of NPR-C/CNP without altering the conformations of neighboring residues. These observations suggest that the G19R mutation may selectively disrupt the binding of CNP to a particular receptor, such as NPR-C.

In an embodiment, the CNP variants have substitution(s) at one or more Gly sites at positions 1, 5, 8, 15, 19 and 21, to reduce conformational flexibility and thereby increase receptor specificity. Comparative analyses of crystal structures of ANP bound to NPR-C and NPR-A (Ogawa, H. et al., J. Biol. Chem., 279: 28625-31 (2004); He, X.-L., J. Mol. Biol., 361: 698-714 (2006)) indicate that the conformational flexibility of ANP may play an important role in determining the receptor selectivity.

In one embodiment, functional CNP variants with potentially reduced affinity to NPR-C have one or more of the following amino acid substitutions: G1R, G1E, G5R, G5Q, G5S, F7Y, G8T, G8S, G8V, G8N, L9S, L9T, K10Cit, K10Q, K10S, 114N, G15R, G15S, G15N, G15Cit, S16Q, M17V, M17N, G19S, G19R, G19N, L20V, L20R, L20T, L20S, G21S, G21T and G21R. In an embodiment, the CNP variants have multipoint substitutions at positions 1, 5, 7, 8, 9, 10, 14, 15, 16, 17, 19, 20 and/or 21, and may optionally have modifications at any of the other positions in the peptide sequence of the variant.

In a further embodiment, the CNP variants described herein may be conjugated to moieties, up to a total mass characterized by the ranges described generally herein, e.g., from about 2.6 or 2.8 kDa to about 6 or 7 kDa, at the N-terminus, the C-terminus and/or an internal site, to facilitate bone/cartilage targeting, reduce NPR-C and renal clearance, increase resistance to NEP degradation, and/or improve CNP functionality. In one embodiment, the CNP variants are not conjugated to a polymeric moiety at a site within the cyclic region (corresponding to Cys6 to Cys22 of CNP22). Non-limiting examples of polymeric or non-polymeric moieties that can be conjugated to the CNP variants include synthetic bone-targeting compounds such as, e.g., bisphosphonates; bone/cartilage targeting peptide sequences such as, e.g., polyAsp and polyGlu; peptide sequences derived from bone-targeting domains of bone proteins such as, e.g., osteopontin, osteocalcin and sialoprotein; peptide sequences derived from the functional domains of bone morphogenetic proteins such as, e.g., BMP2, BMP3, BMP5, BMP7 and BMP8a; peptide sequences derived from polypeptides of natriuretic origin such as, e.g., NPPC, ANP and BNP; other natural polymeric or non-polymeric moieties such as, e.g., carbohydrates, fatty acids and phospholipids; biocompatible synthetic hydrophilic polymers such as, e.g., PEG (or PEO); hydrophobic polymeric or non-polymeric moieties such as, e.g., heptanoic acid and pentanoic acid; and combinations thereof.

The CNP variants described herein can have substantially similar or better functional activity than CNP22, e.g., with respect to stimulation of cGMP production and signaling. In one embodiment, the CNP variants in vitro or in vivo stimulate the production of at least about 50% of the cGMP level produced under the same concentration of wtCNP22 (e.g., 1 uM). In certain embodiments, the CNP variants retain at least about 50%, 60%, 70%, 80%, 90%, 95% or 100% of the cGMP-stimulation activity of wild-type CNP22 in vitro or in vivo. In another embodiment, the CNP variants have improved cGMP-stimulation activity compared to CNP22. In certain embodiments, the CNP variants in vitro or in vivo stimulate the production of at least about 110%, 120%, 130%, 140%, 150%, 200%, 250%, 300%, 350%, 400%, 450%, 500% or more of the cGMP level produced under the same concentration of wtCNP22 (e.g., 1 uM).

Optionally excluded from the present disclosure are any of the natriuretic (e.g., CNP) peptides, fragments and variants specifically disclosed, and any of the natriuretic (e.g., CNP) peptides, fragments and variants actually produced, in any of the prior publications referenced herein, including but not limited to, U.S. Pat. Nos. 5,434,133, 6,034,231, 6,020,168, 6,743,425, 7,276,481, WO 94/20534, WO 02/047871, WO 2005/098490, WO 2004/047871, EP 0497368, EP 0466174, and Furuya et al., Biochem. Biophys. Res. Comm. 183: 964-969 (1992)). All such documents are incorporated by reference herein in their entirety.

In one embodiment, the present disclosure optionally excludes all known wild-type CNP-53, wild-type CNP-22, wild-type CNP-17, wild-type BNP, and wild-type ANP of

human origin and non-human origin. For example, in an embodiment the disclosure optionally excludes human CNP-17, human CNP-22, chicken CNP-22 (corresponding to hCNP-22(Leu9Val)), trout and eel CNP-22 (corresponding to hCNP-22(Leu2Trp, Ser3Asn, Lys4Arg)), frog CNP22-I (corresponding to hCNP-22(Leu2Tyr, Lys4Arg, Leu9Val, Ser16Ala, Met17Phe)), frog CNP22-II (corresponding to hCNP-22(Leu2Thr, Ser16Ala)), human CNP-53, and pig and rat CNP-53 (corresponding to hCNP-53(Gln17His, Ala28Gly)). In another embodiment, the disclosure optionally excludes fragments of NPPC, proCNP and CNP-53 which are produced by proteolytic cleavage in vivo in humans and non-human animals. In yet another embodiment, optionally excluded from the disclosure are the following truncated fragments of wild-type human CNP-53: CNP-50, CNP-46, CNP-44, CNP-39, CNP-30, CNP-29, CNP-28, CNP-27 and CNP-26.

In a further embodiment, the present disclosure optionally excludes CNP peptides and fragments thereof isolated or sought from the shark species *Triakis scyllia* and *Scyliorhinus canicula* (see, e.g., M. Takano et al., Zool. Sci., 11: 451-454 (1994)), including:

(SEQ ID NO: 204)
RLLKDLSNNPLRFRGRSKKGPSRGCFGVKLDRIGAMSGLGC (CNP-41);

(SEQ ID NO: 205)
LKDLSNNPLRFRGRSKKGPSRGCFGVKLDRIGAMSGLGC (CNP-39);

(SEQ ID NO: 206)
KDLSNNPLRFRGRSKKGPSRGCFGVKLDRIGAMSGLGC (CNP-38);
and

(SEQ ID NO: 207)
GPSRGCFGVKLDRIGAMSGLGC (CNP-22).

In another embodiment, optionally excluded from the disclosure are CNP peptides and fragments thereof isolated or sought from the shark species *Lamna ditropis* (see, e.g., M. Takano et al., Zool. Sci., 11: 451-454 (1994)), including:

(SEQ ID NO: 208)
RLLKDLSNNPLRFKGRSKKGPSRGCFGVKLDRIGAMSGLGC (CNP-41);

(SEQ ID NO: 209)
LKDLSNNPLRFKGRSKKGPSRGCFGVKLDRIGAMSGLGC (CNP-39);

(SEQ ID NO: 210)
KDLSNNPLRFKGRSKKGPSRGCFGVKLDRIGAMSGLGC (CNP-38);

(SEQ ID NO: 211)
FKGRSKKGPSRGCFGVKLDRIGAMSGLGC (CNP-29);
and

(SEQ ID NO: 212)
GPSRGCFGVKLDRIGAMSGLGC (CNP-22).

In still another embodiment, optionally excluded from the disclosure are CNP peptides and fragments thereof isolated from the shark species *Squalus acanthias* (see, e.g., M. Takano et al., Zool. Sci., 11: 451-454 (1994)), including:

(SEQ ID NO: 213)
RLLQDLSNNPLRFKGRSKKGPSRSCFGLKLDRIGAMSGLGC (CNP-41);
and

(SEQ ID NO: 214)
GPSRSCFGLKLDRIGAMSGLGC (CNP-22).

In a further embodiment, the present disclosure optionally excludes CNP peptides isolated from medaka and puffer fish, designated "CNP-1", "CNP-2", "CNP-3" and "CNP-4" in K. Inoue et al., Proc. Nat. Acad. Sci., 100(17): 10079-10084 (2003):

(SEQ ID NO: 215)
GWNRGCFGLKLDRIGSMSGLGC (medaka and puffer fish CNP-1);

(SEQ ID NO: 216)
PMVAGGGCFGMKMDRIGSISGLGC (medaka CNP-2);

(SEQ ID NO: 217)
GRSSMVGGRGCFGMKIDRIGSISGLGC (puffer fish CNP-2);

(SEQ ID NO: 218)
GGMRSCFGVRLERIGSFSGLGC (medaka CNP-3);

(SEQ ID NO: 219)
GGLRSCFGVRLARIGSFSGLGC (puffer fish CNP-3);

(SEQ ID NO: 220)
GGSTSRSGCFGHKMDRIGTISGMGC (medaka CNP-4);
and

(SEQ ID NO: 221)
GGSSRSGCFGHKMDRIGTISGMGC (puffer fish CNP-4).

In a still further embodiment, optionally excluded from the disclosure are CNP-39 isolated from platypus venom and the CNP-22 fragment thereof, designated "ovCNP-39" and "ovCNP-39(18-39)" in G. de Plater et al., Toxicon., 36(6): 847-857 (1998):

(SEQ ID NO: 222)
LLHDHPNPRKYKPANKKGLSKGCFGLKLDRIGSTSGLGC (ovCNP-39);
and

(SEQ ID NO: 223)
GLSKGCFGLKLDRIGSTSGLGC (ovCNP-39(18-39).

In another embodiment, the present disclosure optionally excludes the following peptides as specifically disclosed in US 2007/0197434:

(SEQ ID NO: 224)
Gly-Leu-Ser-Lys-Gly-Cys-Phe-Gly-Leu-Lys-Leu-Asp-Arg-Ile-Gly-Ala-Met-Ser-Gly-Leu-Gly-Cys;

(SEQ ID NO: 225)
Gly-Leu-Ser-Lys-Gly-Cys-Phe-Gly-Leu-Lys-Leu-Asp-Arg-Ile-Gly-Ser-Gln-Ser-Gly-Leu-Gly-Cys;

(SEQ ID NO: 226)
Gly-Leu-Ser-Lys-Gly-Cys-Phe-Gly-Leu-Lys-Leu-Asp-Arg-Ile-Gly-Ser-Ala-Ser-Gly-Leu-Gly-Cys;

(SEQ ID NO: 227)
Ser-Leu-Arg-Arg-Ser-Ser-Cys-Phe-Gly-Leu-Lys-Leu-Asp-Arg-Ile-Gly-Ser-Met-Ser-Gly-Leu-Gly-Cys;

(SEQ ID NO: 228)
Gly-Leu-Ser-Lys-Gly-Cys-Phe-Gly-Leu-Lys-Leu-Asp-Arg-Ile-Gly-Ser-Met-Ser-Gly-Leu-Gly-Cys-Asn-Ser-Phe-Arg-Tyr;
and

(SEQ ID NO: 229)
Cys-Phe-Gly-Leu-Lys-Leu-Asp-Arg-Ile-Gly-Ser-Gln-Ser-Gly-Leu-Gly-Cys-Asn-Ser-Phe-Arg-Tyr.

In yet another embodiment, the disclosure optionally excludes peptides of SEQ ID NO:10, disclosed generically in US 2007/0197434, wherein such peptides are CNP-17 vari-

ants having certain natural amino acid substitution(s) at position(s) 4, 5, 6, 11, 12, 14 and/or 15. In still another embodiment, optionally excluded from the disclosure are peptides corresponding to hCNP-53(Ser47Ala), hCNP-53 (Met48Gln), hCNP-53(Met48Ala), and hCNP-53(C-term.)-Asn-Ser-Phe-Arg-Tyr.

In an embodiment, the present disclosure optionally excludes the peptides of SEQ ID NOs 1-4 and 6-71 as specifically disclosed in U.S. Pat. No. 7,276,481. In another embodiment, the disclosure optionally excludes peptides of SEQ ID NO 5, disclosed generically in U.S. Pat. No. 7,276,481, wherein such peptides are variants of CNP17 having at least one natural amino acid substitution at Leu9, Lys10, Leu11, Ser16, Met17, Gly19, and/or Leu20. In still another embodiment, optionally excluded are CNP17 variants in which CNP17 or variants thereof contain N-Me-Phe7, or N-Me-Phe7 and N-Me-Leu11. In a further embodiment, the disclosure optionally excludes CNP17 variants of SEQ ID NO 5, as disclosed in U.S. Pat. No. 7,276,481, which are fused or conjugated to growth hormone (GH), insulin-like growth factor 1 (IGF-1), or thyroid hormone (TH). In yet another embodiment, optionally excluded are CNP22 variants in which CNP22 is fused to GH, IGF-1 or TH, or attached to GH, IGF-1 or TH via a linker (e.g., a peptide linker). In still another embodiment, optionally excluded are CNP17 variants in which CNP17 or variants thereof are conjugated to biotin or fluorescein at the N-terminus or the C-terminus.

In a further embodiment, the present disclosure optionally excludes the peptides of Compound Nos. 1-27, and SEQ ID NOs 1-17, 22-24, 30, 31 and 40-42 as specifically disclosed in U.S. Pat. No. 5,434,133. In another embodiment, the disclosure optionally excludes peptides of SEQ ID NOs 18-21 and 25-29, disclosed generically in U.S. Pat. No. 5,434,133. In still another embodiment, the disclosure optionally excludes the peptides of SEQ ID NOs 1-4 and 9 as specifically disclosed in WO 94/20534.

In some embodiments, however, the disclosure still encompasses methods of use of the natriuretic (e.g., CNP) peptides, fragments and variants optionally excluded herein, as well as pharmaceutical compositions (including sterile pharmaceutical compositions) comprising such natriuretic (e.g., CNP) peptides, fragments and variants.

C. Synthesis and Purification of CNP Variants

In some embodiments, the CNP variants described herein are produced by recombinant expression, using certain techniques known in the art in certain embodiments. See, e.g., Sambrook, Fritsch & Maniatis, Molecular Cloning: A Laboratory Manual, Second Edition. Cold Spring Harbor Laboratory Press (Cold Spring Harbor, N.Y. (1989)); DNA Cloning: A Practical Approach, Volumes I and II, D. N. Glover, Ed. (1985); and Current Protocols in Molecular Biology, John Wiley & Sons, Inc. (1994).

In certain embodiments, the CNP variants described herein are produced by a recombinant process that comprises culturing in a medium a host cell comprising a first polynucleotide encoding a CNP variant polypeptide linked to a second polynucleotide encoding a cleavable peptide or protein under conditions that result in expression of a fusion polypeptide encoded by the polynucleotides, wherein the fusion polypeptide comprises the CNP variant polypeptide directly linked to the cleavable peptide or protein or indirectly linked thereto via a linker. In some embodiments, the host cell is transformed with an expression vector comprising the polynucleotide encoding the CNP variant polypeptide linked to the polynucleotide encoding the cleavable peptide or protein. In certain embodiments, the fusion polypeptide is expressed as a soluble protein or as an inclusion body. The expressed fusion polypeptide can be isolated from the host cell or culture medium, and the isolated fusion polypeptide can be contacted with a cleaving agent to release the CNP variant.

Host cells used to produce CNP variants can be bacterial, yeast, insect, non-mammalian vertebrate, or mammalian cells. Bacterial cells include without limitation *E. coli* cell lines and strains. Non-limiting examples of *E. coli* cell lines and strains include BL21, BL21(DE3), BL21(DE3)pLysS, BL21(DE3)pGro7, ArcticExpress(DE3), C41 [also called C41(DE3)], C43 [also called C43(DE3)], Origami B(DE3), Origami B(DE3)pLysS, KRX, and Tuner(DE3). In an embodiment, CNP variants and CNP fusion proteins are produced using BL21(DE3) cells. Mammalian cells include, but are not limited to, hamster, monkey, chimpanzee, dog, cat, bovine, porcine, mouse, rat, rabbit, sheep and human cells. The host cells can be immortalized cells (a cell line) or non-immortalized (primary or secondary) cells and can be any of a wide variety of cell types, such as, but not limited to, fibroblasts, keratinocytes, epithelial cells (e.g., mammary epithelial cells, intestinal epithelial cells), ovary cells (e.g., Chinese hamster ovary or CHO cells), endothelial cells, glial cells, neural cells, formed elements of the blood (e.g., lymphocytes, bone marrow cells), chondrocytes and other bone-derived cells, and precursors of these somatic cell types. Host cells containing the CNP variant DNA or RNA are cultured under conditions appropriate for growth of the cells, expression of the DNA or RNA and identification/selection of cells expressing the CNP variant.

In some embodiments, the host cells are grown or cultured for a time period at a temperature from about 10° C. to about 40° C., or from about 20° C. to about 40° C., or from about 30° C. to about 40° C. In certain embodiments, the host cells are grown or cultured for a time period at about 20° C., 22° C., 25° C., 28° C., 30° C., 35° C. or 37° C. In certain embodiments, the host cells are grown or cultured for a time period at about 35° C. or 37° C.

Recombinant polynucleotides encoding CNP variant polypeptides (including CNP fusion proteins) are expressed in an expression vector comprising a recombinant polynucleotide comprising expression control sequences operatively linked to a nucleotide sequence to be expressed. An expression vector comprises sufficient cis-acting elements for expression; other elements for expression can be supplied by the host cell or in vitro expression system. Expression vectors include all those known in the art, including without limitation cosmids, plasmids (e.g., naked or contained in liposomes) and viruses that incorporate the recombinant polynucleotide. The expression vector is inserted into an appropriate host cell, via transformation or transfection, for expression of the polynucleotide encoding the polypeptide (see, e.g., Sambrook et al. (supra)).

Non-limiting examples of expression vectors contemplated for production of CNP variants, including cleavable CNP fusion proteins, include pJexpress, pJexpress401, pJexpress404, pET-15b, pET-21a, pET-22b, pET-31b, pET-32a, pET-41a, pMAL, pMAL-c2X, pQE-30, pET-SUMO, and pTYB11. Expression of particular constructs can generate soluble CNP variants (including CNP fusion proteins) or insoluble CNP variants (including CNP fusion proteins) in the form of inclusion bodies.

In some embodiments, expression of the polynucleotide(s) encoding a CNP variant or CNP fusion protein is enhanced using an isopropyl β-D-1-thiogalactopyranoside (IPTG)-inducible vector. In some embodiments, the host cells are grown or cultured for a time period at a temperature from

about 10° C. to about 40° C., or from about 20° C. to about 40° C., or from about 30° C. to about 40° C., in the presence of IPTG. In certain embodiments, the host cells are grown or cultured for a time period at about 20° C., 22° C., 25° C., 28° C., 30° C., 35° C. or 37° C. in the presence of IPTG. In certain embodiments, the host cells are grown or cultured for a time period at about 35° C. or 37° C. in the presence of 1 mM IPTG.

In further embodiments, the host cells are cultured with IPTG at a concentration from about 0.4 mM to about 2 mM, or from about 0.4 mM to about 1.5 mM, or from about 0.4 mM to about 1 mM. In certain embodiments, the IPTG is at a concentration of about 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9 or 2 mM. In an embodiment, the concentration of IPTG is about 1 mM.

In certain embodiments, the CNP variants described herein are recombinantly expressed as fusion proteins comprising a CNP variant polypeptide and a cleavable carrier protein or cleavable tag (e.g., peptide tag), wherein the fusion protein comprises the CNP variant polypeptide directly linked to the cleavable carrier protein or tag or indirectly linked thereto via a linker. Use of a carrier protein or tag facilitates, e.g., detection, isolation and/or purification of the fusion protein. Cleavable carrier proteins and tags include, but are not limited to, histidine (e.g., hexa-His) tags; human transcription factor TAF12 (TAF12), TAF12 fragments, TAF12 histone fold domain, mutants of TAF12 and fragments thereof, TAF12(C/A), TAF12(D/E), TAF12(4D/4E), TAF12(6D/6E), TAF12(10D/10E), TAF12(C/A & D/E), TAF12(C/A & 4D/4E), TAF12(C/A & 6D/6E), TAF12(C/A & 10D/10E); ketosteroid isomerase (KSI); maltose-binding protein (MBP); β-galactosidase (β-Gal); glutathione-S-transferase (GST); thioredoxin (Trx); chitin binding domain (CBD); BMP-2, BMP-2 mutants, BMP-2(C/A); SUMO; and mutants and fragments thereof.

An expression construct can express a fusion protein comprising a CNP variant and a carrier protein or tag. The tag can be an amino acid sequence that confers a useful property to the fusion protein. In one embodiment, the tag is a ligand-binding domain that can be used to purify the fusion protein by applying the fusion protein to separation media containing the ligand. For example, a fusion protein comprising a glutathione-S-transferase (GST) domain can be applied to a chromatographic column containing glutathione-linked separation media. As another example, a fusion protein comprising maltose-binding protein (MBP) as a tag can be applied to separation media containing maltose. As a further example, a fusion protein comprising a polyhistidine tag may be applied to a nickel column, whereby chelation of the polyhistidine tag to the nickel column facilitates purification of the fusion protein. In another embodiment, the tag is a ligand. For example, a fusion protein can comprise glutathione as a tag and be applied to a chromatographic column containing glutathione-S-transferase-linked separation media. Non-limiting examples of carrier proteins and tags for use in fusion proteins include human transcription factor TAF12 (TAF12), ketosteroid isomerase (KSI), maltose-binding protein (MBP), β-galactosidase (β-Gal), glutathione-S-transferase (GST), thioredoxin (Trx), chitin-binding domain (CBD), BMP-2 mutation (BMPM), SUMO, CAT, TrpE, staphylococcal protein A, streptococcal proteins, starch-binding protein, cellulose-binding domain of endoglucanase A, cellulose-binding domain of exoglucanase Cex, biotin-binding domain, recA, Flag, poly(His), poly(Arg), poly(Asp), poly (Gln), poly(Phe), poly(Cys), green fluorescent protein, red fluorescent protein, yellow fluorescent protein, cyan fluorescent protein, biotin, avidin, streptavidin, antibody epitopes, and mutants and fragments thereof.

To generate the target CNP variant, the carrier protein or tag can be cleaved from the fusion protein by means of chemical cleavage, protease cleavage, or protein self-cleavage. Exemplary chemical and proteolytic cleavage agents (cleavage sites in parenthesis) include, but are not limited to, formic acid (Asp-Pro), cyanogen bromide (CNBr) (Met-X), hydroxylamine (Asn-Gly), Factor Xa (IEGR-X) (SEQ ID NO: 230), Enterokinase (DDDDK-X) (SEQ ID NO: 231), ProTEV (EXXYXQ-G) (SEQ ID NO: 232), and SUMO protease. Due to the nature of the particular kinds of chemical cleavage, cleavage using formic acid may generate Pro-CNP, CNBr may generate CNP having Met-to-Asn substitution, and hydroxylamine may generate Gly-CNP. Alternatively, chemical or protease cleavage may be avoided by using particular constructs (e.g., pET-21a-CNP) that express CNP variants not as fusion proteins. Expression of pET-21a-CNP may produce Met-CNP. Or certain fusion proteins (e.g., those containing intein-CBD) can undergo self-cleavage to generate CNP.

In further embodiments, a fusion protein comprises a cleavable peptide linker between a CNP variant and a carrier protein or tag (e.g., peptide tag). In certain embodiments, the cleavable peptide linker is selected from the group consisting of Asp-Pro, Asn-Gly, Met-X, Val-Asp-Asp-Arg (SEQ ID NO: 233), Gly-Ser-Asp-Arg (SEQ ID NO: 234), Ile-Thr-Asp-Arg (SEQ ID NO: 235), Pro-Gly-Asp-Arg (SEQ ID NO: 236), Ile-Glu-Gly-Arg-X (SEQ ID NO: 230), Asp-Asp-Asp-Asp-Lys-X (SEQ ID NO: 231), Glu-X-X-Tyr-X-Gln-Gly (SEQ ID NO: 232), Ala-Phe-Leu-Gly-Pro-Gly-Asp-Arg (SEQ ID NO: 237), and MGSSHHHHHHSSGLVPRGSHTGD-DDDKHMD (pET-15b linker) (SEQ ID NO: 95), where X denotes an amino acid. In some embodiments, the cleavable peptide linker is cleaved by a cleaving agent selected from the group consisting of palladium, cyanogen bromide (CNBr), formic acid, hydroxylamine, clostripain, thrombin, chymotrypsin, trypsin, trypsin-like proteases, carboxypeptidase, enterokinase (enteropeptidase), Kex 2 protease, Omp T protease, Factor Xa protease, subtilisin, proTEV, SUMO protease, V8 protease, HIV protease, rhinovirus protease, furilisin protease, IgA proteases, human Pace protease, collagenase, Nia protease, poliovirus 2Apro protease, poliovirus 3C protease, genenase, furin, elastase, Proteinase K, pepsin, rennin (chymosin), microbial aspartic proteases, papain, calpain, chymopapain, ficin (ficain), bromelain (bromelase), cathespisin B, caspases, thermolysin, Endoprotease Arg-C, Endoprotease Glu-C, Endoprotease Lys-C, kallikrein, and plasmin.

In certain embodiments, the cleavable carrier protein, tag (e.g., peptide tag) or peptide linker is cleaved using formic acid to release the CNP variant from the fusion protein. In some embodiments, the formic acid is at a concentration from about 1% to about 20%, or from about 1% to about 15%, or from about 2% to about 15%, or from about 1% to about 10%, or from about 2% to about 10%, or from about 1% to about 5%, or from about 2% to about 5%. In certain embodiments, the formic acid is at a concentration of about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14% or 15%. In certain embodiments, the formic acid is at a concentration of about 2%, 5% or 10%.

In further embodiments, cleavage of the CNP fusion protein in the presence of formic acid is conducted at a temperature from about 20° C. to about 80° C., or from about 30° C. to about 75° C., or from about 40° C. to about 75° C., or from about 50° C. to about 75° C., or from about 50° C. to about 70° C., or from about 55° C. to about 70° C., or from about 50° C.

to about 60° C. In some embodiments, cleavage in the presence of formic acid is conducted at about 20° C., 22° C., 25° C., 30° C., 35° C., 37° C., 40° C., 42° C., 45° C., 50° C., 55° C., 60° C., 65° C., 70° C., 75° C. or 80° C. In certain embodiments, cleavage in the presence of formic acid is conducted at about 50° C., 55° C., 60° C., 65° C. or 70° C. In certain embodiments, cleavage in the presence of formic acid is conducted at about 55° C. or 70° C.

In additional embodiments, cleavage of the CNP fusion protein in the presence of formic acid is performed for a time period from about 3 hr to about 48 hr, or from about 5 hr to about 48 hr, or from about 5 hr to about 36 hr, or from about 5 hr to about 24 hr, or from about 5 hr to about 18 hr, or from about 20 hr to about 24 hr, or for about 6 hr to about 10 hr. In certain embodiments, cleavage in the presence of formic acid is performed for about 5 hr, 6 hr, 12 hr, 15 hr, 18 hr, 20 hr or 24 hr.

In some embodiments, cleavage of the CNP fusion protein is conducted in the presence of about 2%, 5% or 10% formic acid at about 55° C. for about 20 hr to about 36 hr, or at about 60° C. for about 15 hr to about 24 hr, or at about 65° C. for about 10 hr to about 21 hr, or at about 70° C. for about 6 hr to about 18 hr. In certain embodiments, cleavage of the CNP fusion protein is conducted in the presence of about 2% formic acid at about 55° C. for about 20 hr to about 24 or 36 hr, or at about 60° C. for about 15 hr to about 24 hr, or at about 65° C. for about 10 hr to about 18 hr, or at about 70° C. for about 6 hr to about 10 hr.

The present disclosure provides mild conditions for cleavage of CNP fusion proteins using formic acid to afford high yields of CNP variants. The conditions described herein for fusion protein cleavage using formic acid are also suitable for cleavage of fusion proteins comprising polypeptides or proteins other than CNP, where the fusion proteins contain an Asp-Pro peptide bond.

In further embodiments, soluble CNP fusion proteins or CNP fusion protein inclusion bodies are treated with a buffer and/or a detergent prior to chemical cleavage (e.g., using formic acid) or proteolytic cleavage of the CNP fusion protein. Non-limiting examples of buffers include B-PER II; diluted B-PER II (e.g., 1/20 dilution); B-PER; B-PER phosphate buffer; buffers containing Tris (e.g., 25 mM Tris, pH 7.5); buffers containing Tris and NaCl (e.g., 25 mM Tris, 150 mM NaCl, pH 7.9); and PBS. In an embodiment, the buffer is B-PER II. Non-limiting examples of detergents include octylsucrose, Triton X-100, Tween-20, NP-40, and CA-630. The detergent can be in a buffer (e.g., 1% detergent in 25 mM Tris buffer, pH 7.5). In certain embodiments, the detergent is Triton X-100 or CA-630.

It is understood that any of the methods and conditions described above may be used in combination with any of the other methods and conditions described above to generate a CNP variant disclosed herein.

In other embodiments, the CNP variants described herein are synthesized using a peptide synthesizer and purified according to methods known in the art, e.g., according to the methods of Atherton and Sheppard, *Solid Phase Peptide Synthesis: a Practical Approach*, IRL Press (Oxford, England (1989)).

Peptides can be synthesized based on, e.g., the following peptide sequence of CNP: G^1LS(K or R)$GC^6F^7G^8L$(K or R or Nle or 6-OH-Nle)$LDRIGSMSGLGC^{22}$.

Exemplary CNP variants include but are not limited to:

Analog A (GLSKGC(CH2NH)FGLKLDRIGSMSGLGC) (SEQ ID NO: 56) was made by converting the backbone "—C═O" group of C^6 to a "—CH_2" group;

Analog B (GLSKGC(N—Me—Phe)GLKLDRIGSMSGLGC) (SEQ ID NO: 57) was made by converting the backbone "—NH" group of F^7 to an "—N—CH_3" group;

Analog E (GLSKGC(D-Phe)GLKLDRIGSMSGLGC) (SEQ ID NO: 136) was made using D-Phe at F^7;

Analog F (GLSKGCF(tBu-Gly)LKLDRIGSMSGLGC) (SEQ ID NO: 58) was made using a tertiary-butyl-Gly at G^8;

Analog G (GLSKGC(3-Cl-Phe)GLKLDRIGSMSGLGC) (SEQ ID NO: 137) was made by adding a chloride atom to a meta position of the phenyl ring of F^7 (similar variants can be generated by making ortho, meta and/or para substitutions of the phenyl ring of Phe7 with Cl, F, Br, OH and/or CH_3);
and

Analog H (GLSKGC[NHCH$_2$CH(Ph)CO]GLKLDRIGSMSGLGC) (SEQ ID NO: 59) was made using (±)-3-(amino)-2-phenylpropionic acid at F^7.

Examples of CNP variants having, e.g., amino acid extensions, substitutions with natural or unnatural amino acids or peptide bond isosteres, and/or conjugations to polymers or hydrophobic moieties, include without limitation:

Analog J (SEQ ID NO: 91)
C6—CH2—NH, N—Me-L9, N—Me-L20

Analog K (SEQ ID NO: 92)
N—Me-L9, N—Me-L20

Analog L (SEQ ID NO: 93)
N—Me-L9, N—Me-L11, N—Me-L20

Analog M (SEQ ID NO: 94)
N—Me-L9, N—Me-L11

Analog Z (SEQ ID NO: 95)
K4R, F7Y

Analog AA (SEQ ID NO: 96)
K4R, G8V

Analog AB (SEQ ID NO: 97)
K4R, G8S

Analog AC (SEQ ID NO: 98)
K4R, G8T

Analog AD (SEQ ID NO: 99)
K4R, L9T

Analog AE (SEQ ID NO: 100)
K4R, G15R

Analog AF (SEQ ID NO: 101)
K4R, G15Cit

-continued

Analog AG K4R, M17V	(SEQ ID NO: 102)
Analog AH K4R	(SEQ ID NO: 35)
Analog AJ K4R, L20V	(SEQ ID NO: 103)
Analog AK K4R, L20t-Bu-Ala	(SEQ ID NO: 104)
Analog AT G1E, K4E	(SEQ ID NO: 105)
Analog AV G1E, K4E - pentanoic acid (attached at the N-terminus)	(SEQ ID NO: 106)
Analog AW G1E, K4E - heptanoic acid (attached at the N-terminus)	(SEQ ID NO: 107)
Analog AX CNP17 (delta N-term)	(SEQ ID NO: 2)
Analog AY GANRR-CNP22(K4R)	(SEQ ID NO: 36)
Analog AZ R-CNP22(K4R)	(SEQ ID NO: 41)
Analog BB G1E - heptanoic acid (attached at the N-terminus)	(SEQ ID NO: 108)
Analog BC G1E - pentanoic acid (attached at the N-terminus)	(SEQ ID NO: 109)
Analog BF K4R, K10Cit	(SEQ ID NO: 110)
Analog BG K4R, K10Q	(SEQ ID NO: 111)
Analog BH K4R, K10R	(SEQ ID NO: 112)
Analog BJ K4R, G15N	(SEQ ID NO: 113)
Analog BK K4R, G15S	(SEQ ID NO: 114)
Analog BL CNP-37	(SEQ ID NO: 60)
CNP-53	(SEQ ID NO: 4)

-continued

Analog CA AAWARLLQEHPNA-CNP22	(SEQ ID NO: 61)
Analog CB AAWARLLQEHPNAR-CNP22	(SEQ ID NO: 62)
Analog CC DLRVDTKSRAAWAR-CNP22	(SEQ ID NO: 63)
Analog CD SPKMVQGSG-CNP17-KVLRRH (N- and C-terminal BNP tails)	(SEQ ID NO: 68)
Analog CE GERAFKAWAVARLSQ-CNP22 (HSA-CNP22)	(SEQ ID NO: 81)
Analog CF GQPREPQVYTLPPS-CNP22	(SEQ ID NO: 79)
PEG(24K)-CNP22 PEG(20K)-CNP22 PEG(5K)-CNP22 PEG(2K)-CNP22 PEG(2K)-CNP17	
PEG(1K)-GANRR-CNP22(K4R)	(SEQ ID NO: 36)
PEG(1K)-CNP22 PEO4-(PEO12)3(branched)-CNP22 PEO12-CNP22	
PEO12-GANRR-CNP22(K4R)	(SEQ ID NO: 36)
PEO24-GANRR-CNP22(K4R); and	(SEQ ID NO: 36)
SEQ ID NOs: 1 to 6 and 34 to 144, and variants thereof that comprise up to 1, 2, 3, 4, or 5 further modifications.	

In one embodiment, the CNP variants are cyclized via formation of a disulfide bond between Cys^6 and Cys^{22}. Cys^6 can be a cysteine analog such as, e.g., homocysteine or penicillamine. In a further embodiment, the CNP variants can be cyclized by a covalent bond formed head-to-tail, side chain-to-side chain, side chain-to-head, or side chain-to-tail. In an embodiment, the covalent bond is formed between an amino acid at or toward the N-terminus and an amino acid at or toward the C-terminus of the peptide (referred to as "terminal" amino acids in this context). In another embodiment, the covalent bond is formed between the side chains of the two terminal amino acids. In yet another embodiment, the covalent bond is formed between the side chain of one terminal amino acid and the terminal group of the other terminal amino acid, or between the terminal groups of the two terminal amino acids.

Head-to-tail cyclization of the terminal amine to the terminal carboxyl group can be carried out using a number of methods, e.g., using p-nitrophenyl ester, 2,4,5-trichlorophenyl ester, pentafluorophenyl ester, the azide method, the mixed anhydride method, HATU, a carbodimide (e.g., DIC, EDC or DCC) with a catalyst such as HOBt, HONSu or HOAt, or on-resin cyclization.

In addition, the cyclic structure can be formed via a bridging group involving the side chains of amino acid residues of the CNP variant and/or the terminal amino acid residues. A bridging group is a chemical moiety that allows cyclization of two portions of the peptide. Non-limiting examples of bridg-

ing groups include amides, thioethers, thioesters, disulfides, ureas, carbamates, sulfonamides, and the like. A variety of methods are known in the art for incorporation of units having such bridging groups. For example, a lactam bridge (i.e., a cyclic amide) can be formed between the N-terminal amino group or an amino group on a side chain and the C-terminal carboxylic acid or a carboxyl group on a side chain, e.g., the side chain of lysine or ornithine and the side chain of glutamic acid or aspartic acid. A thioester can be formed between the C-terminal carboxyl group or a carboxyl group on a side chain and the thiol group on the side chain of cysteine or a cysteine analog.

Alternatively, a cross link can be formed by incorporating a lanthionine (thio-dialanine) residue to link alanine residues that are covalently bonded together by a thioether bond. In another method, a cross-linking agent, such as a dicarboxylic acid (e.g., suberic acid (octanedioic acid)), can link the functional groups of amino acid side chains, such as free amino, hydroxyl, and thiol groups.

Enzyme-catalyzed cyclization can also be used. For example, it has been reported that the thioesterase domain of tyrocidine synthetase can be used to cyclize a thioester precursor, a subtilisin mutant can be utilized to cyclize peptide glycolate phenylalanylamide esters, and the antibody ligase 16G3 can be employed to cyclize a p-nitrophenylester. For a review of peptide cyclization, see Davies, J. Peptide Sci., 9: 471-501 (2003), incorporated herein by reference in its entirety.

In certain embodiments, the final cyclized product has a purity of at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or at least about 99%.

D. Chemically Modified CNP Variants

Chemical modification of CNP22 or variants thereof can potentially impart advantageous properties to the modified CNP peptides, such as increased stability and half-life and reduced immunogenicity (for a general discussion of chemical modification of therapeutic proteins, see Pharmazie, 57(1): 5-29 (2002)). For example, attaching natural or synthetic, polymeric or non-polymeric moieties (e.g., PEG) to CNP peptides, to increase the total mass of the CNP peptides to the ranges described generally herein, e.g., a range from about 2.6 or 2.8 kDa to about 6 or 7 kDa, can reduce the susceptibility of the modified peptides to in vivo cleavage by exopeptidases and/or endopeptidases (e.g., NEP). In addition to PEGylation, glycosylation and other chemical derivatization procedures, e.g., modification by phosphorylation, amidation, carboxylation, acetylation, methylation, and creation of acid-addition salts, amides, esters and N-acyl derivatives, may also mask potentially immunogenic regions and/or proteolytically sensitive regions (Science, 303: 480-482 (2004)).

Examples of chemical modifications include, without limitation, the polymer addition method of Bednarsaki and the cross-linking method of Altus Corporation for improving stability and protease resistance and reducing immunogenicity. Bednarsaki showed that polymer addition can improve protein temperature stability (J. Am. Chem. Soc., 114(1): 378-380 (1992)), and Altus Corporation found that glutaraldehyde cross-linking can improve enzyme stability.

Chemical modification of polypeptides can be performed in a non-specific fashion (leading to mixtures of derivatized species) or in a site-specific fashion (e.g., based on wild-type macromolecule reactivity-directed derivatization and/or site-selective modification using a combination of site-directed mutagenesis and chemical modification) or, alternatively, using expressed protein ligation methods (Curr. Opin. Biotechnol., 13(4): 297-303 (2002)).

Pegylated CNP Variants

In one embodiment, for increased stability (e.g., resistance to NEP degradation), CNP22 or variants thereof (including those having amino acid additions, substitutions and/or deletions) are conjugated to hydrophilic, natural or synthetic polymers, to increase the total mass of the modified CNP peptides to a range from about 2.6 kDa or 2.8 kDa to about 4, 5, 6, 7 or higher kDa. In certain embodiments, the added hydrophilic polymers have a total mass of about 0.6, 0.8, 1, 1.2, 1.4, 1.6, 1.8, 2, 2.2, 2.4, 2.6, 2.8, 3, 3.2, 3.4, 3.6, 3.8, 4, 4.2, 4.4, 4.6, 4.8, or about 5 kDa.

In an embodiment, the hydrophilic polymers are water-soluble so that the CNP peptides conjugated thereto do not precipitate out in an aqueous (e.g., physiological) environment. Further, the hydrophilic polymers are biocompatible, i.e., do not cause injury, toxicity or an immunological reaction in vivo.

The hydrophilic polymers can be branched or unbranched. In one embodiment, the hydrophilic polymers are not branched.

Various sites of conjugation of CNP22 or variants thereof to a hydrophilic polymer are possible, including but not limited to: (1) only at the N-terminus; (2) only at the C-terminus; (3) only at an internal site (e.g., Lys4); (4) at both the N-terminus and the C-terminus; (5) at the N-terminus and an internal site; and (6) at the C-terminus and an internal site. In one embodiment, CNP22 or variants thereof are conjugated to a hydrophilic polymer only at the N-terminus. In another embodiment, conjugation is only at an internal site (e.g., Lys4). In yet another embodiment, conjugation is at the N-terminus and an internal site (e.g., Lys4). In still another embodiment, for better functionality the CNP peptides are not conjugated to a hydrophilic polymer at a site (e.g., Lys10) within the cyclic domain (corresponding to Cys6 to Cys22 of CNP22). If conjugation to a hydrophilic polymer is based on bond formation with a reactive primary amino group on the CNP peptide, conjugation at an internal site (e.g., Lys4 and/or Lys10) can be prevented by substitution of Lys4 and/or Lys10 with a natural or unnatural amino acid or peptidomimetic that does not contain a reactive primary amino group on a side chain, such as, e.g., Gly, Ser, Arg, Asn, Gln, Asp, Glu or citrulline (Cit). In a particular embodiment, Lys4 and/or Lys10 are replaced with Arg. In another embodiment, Lys10 is not replaced with Arg.

Non-limiting examples of hydrophilic polymers include polymers formed from carboxylic acid-bearing monomers (e.g., methacrylic acid (MA) and acrylic acid (AA)), polyvinyl alcohols, polymers formed from hydroxyl-bearing monomers (e.g., hydroxyethyl methacrylate (HEMA), hydroxypropyl methacrylate (HPMA), hydroxypropyl methacrylamide, and 3-trimethylsilylpropyl methacrylate (TMSPMA)), polyalkylene oxides, polyoxyethylated polyols (e.g., glycerol), poly(ethylene glycol) (PEG), poly(propylene glycol), mono-C_1-C_{10} alkoxy-PEGs (e.g., monomethoxy-PEG), tresyl monomethoxy-PEG, aryloxy-PEGs, PEG acrylate (PEGA), PEG methacrylate, PEG propionaldehyde, bis-succinimidyl carbonate PEG, copolymers of 2-methacryloyloxyethyl-phosphorylcholine (MPC) and N-vinyl pyrrolidone (VP), hydroxy functional poly(N-vinyl pyrrolidone) (PVP), SIS-PEG (SIS is polystyrene-polyisobutylene-polystyrene block copolymer), polystyrene-PEG, polyisobutylene-PEG, PCL-PEG (PCL is polycaprolactone), PLA-PEG (PLA is polylactic acid), PMMA-PEG (PMMA is poly(methyl methacrylate)), PDMS-PEG (PDMS is polydimethyloxanone), PVDF-PEG (PVDF is polyvinylidene

fluoride), PLURONIC™ surfactants (polypropylene oxide-co-polyethylene glycol), poly(tetramethylene glycol), poly (L-lysine-g-ethylene glycol) (PLL-g-PEG), poly(L-lysine-g-hyaluronic acid) (PLL-g-HA), poly(L-lysine-g-phosphoryl choline) (PLL-g-PC), poly(L-lysine-g-vinyl pyrrolidone) (PLL-g-PVP), poly(ethylimine-g-ethylene glycol) (PEI-g-PEG), poly(ethylimine-g-hyaluronic acid) (PEI-g-HA), poly (ethylimine-g-phosphoryl choline) (PEI-g-PC), poly(ethylimine-g-vinyl pyrrolidone) (PEI-g-PVP), PLL-co-HA, PLL-co-PC, PLL-co-PVP, PEI-co-PEG, PEI-co-HA, PEI-co-PC, PEI-co-PVP, cellulose and derivatives thereof (e.g., hydroxyethyl cellulose), dextran, dextrins, hyaluronic acid and derivatives thereof (e.g., sodium hyaluronate), elastin, chitosan, acrylic sulfate, acrylic sulfonate, acrylic sulfamate, methacrylic sulfate, methacrylic sulfonate, methacrylic sulfamate, polymers and copolymers thereof, and polymers and copolymers of combinations thereof.

In a particular embodiment, the hydrophilic polymer is poly(ethylene glycol) (PEG), also called poly(ethylene oxide) (PEO). As used herein, the term "PEG" or "PEO" encompasses all the forms of PEG, branched and unbranched, which can be used to derivatize polypeptides, including without limitation mono-(C_1-C_{10}) alkoxy-PEGs and aryloxy-PEGs.

In one embodiment, the PEG-CNP conjugates comprise a PEG (or PEO) polymer of the formula $(CH_2CH_2O)_n$, wherein n is an integer from about 6 to about 100, and the PEG polymer is from about 0.3 kDa to about 5 kDa. In another embodiment, n is an integer from about 12 to about 50, and the PEG polymer is from about 0.6 kDa to about 2.5 kDa. In yet another embodiment, n is from about 12 to about 24, and the PEG polymer is from about 0.6 kDa to about 1.2 kDa. In a further embodiment, the terminal hydroxyl group of the PEG polymer is capped with a non-reactive group. In a particular embodiment, the end-capping group is an alkyl group, e.g., a lower alkyl group such as methyl, so that the PEG polymer terminates in an alkoxy group. In an embodiment, the PEG polymer is not branched. In another embodiment, CNP22 or variants thereof are conjugated to a PEG polymer only at the N-terminus.

PEGs and PEOs potentially include molecules with a distribution of molecular weights, i.e., they are potentially polydispersed, depending on the manner in which they are prepared. The size/mass distribution of a polymeric preparation can be characterized statistically by its weight average molecular weight (M_w) and its number average molecular weight (M_n), the ratio of which is called the polydispersity index (M_w/M_n). M_w and M_n can be measured by mass spectroscopy. PEG-CNP variants conjugated to a PEG moiety larger than 1.5 kDa may exhibit a range of molecular weights due to the polydispersed nature of the parent PEG molecule. For example, in the case of mPEG2K (Sunbright ME-020HS, NOF Co.), the molecular masses of the PEG molecules are distributed over a range from about 1.5 kDa to about 3 kDa, with a polydispersity index of 1.036. By contrast, the PEGs conjugated to CNP22 or variants thereof using MS $(PEG)_n$ reagents (n=4, 8, 12 or 24, denoted as, e.g., "PEO12" or "PEO24") from Pierce Biotechnology (Rockford, Ill.) are monodispersed, having discrete chain length and defined molecular weight.

Methods for generating polypeptides comprising a PEG moiety are known in the art (see, e.g., U.S. Pat. No. 5,824,784). Methods for preparing PEGylated CNP peptides generally comprise the steps of (a) reacting CNP22 or a variant thereof with a PEGylation reagent under conditions suitable for attaching PEG to the CNP peptide (e.g., at the N-terminus), and (b) obtaining the reaction product(s). Because PEGylating a CNP peptide might significantly alter its binding to NPR-B, depending on the size of the PEG moiety and the location of PEGylation, different kinds of PEG and PEGylation reaction conditions can be explored. The chemistry that can be used for PEGylation of a CNP peptide includes acylation of reactive primary amine(s) of the peptide using the NHS-ester of methoxy-PEG (O—[(N-Succinimidyloxycarbonyl)-methyl]-O'-methylpolyethylene glycol). Acylation with methoxy-PEG-NHS or methoxy-PEG-SPA results in an amide linkage that eliminates any charge of the original primary amine. PEG-CNP peptides designated with the symbol "PEO12" or "PEO24", as well as those designated with the symbol "PEG1K", "PEG2K", "PEG5K" or "PEG20K", are PEGylated via reaction of a primary amino group on the peptide with an NHS ester-activated, methoxy-end capped PEG reagent. PEG-CNP variants can also be prepared by other methods, e.g., via reductive amination involving a primary amino group on the peptide and a PEG aldehyde, such as, e.g., PEG-propionaldehyde, or mono-C_1-C_{10} alkoxy or aryloxy derivatives thereof (see U.S. Pat. No. 5,252,714).

Unlike ribosome protein synthesis, synthetic peptide synthesis proceeds from the C-terminus to the N-terminus. Accordingly, Boc-PEG (containing tert-butyloxycarbonyl (Boc)) is one method to attach PEG to the C-terminus of a peptide (R. B. Merrifield, J. Am. Chem. Soc., 85(14): 2149-2154 (1963)). Alternatively, Fmoc (fluorenylmethoxycarbonyl) chemistry can be employed (E. Atherton and R. C. Sheppard, *Solid Phase Peptide Synthesis: a Practical Approach*, IRL Press (Oxford, England (1989)).

The present methods for preparing PEG-CNP variants provide a substantially homogenous mixture of polymer-protein conjugates. After purification, discrete PEG-CNP preparations are sufficiently pure for in vitro and in vivo testing of biological properties. As demonstrated herein, certain PEG-CNP variants exhibit reduced susceptibility to NEP cleavage and substantially similar or better functionality (e.g., stimulation of cGMP production).

As described herein, PEGylation reactions of CNP22 or variants thereof, using appropriate PEGylation reagent/CNP peptide ratios and reaction conditions, provide PEG-CNP derivatives. The nature and extent of PEGylation can be determined using, e.g., PAGE and HPLC analysis. In certain embodiments, at least about 50%, 60%, 70%, 80%, 90%, 95% or 99% of CNP22 or variants thereof are mono-PEGylated at the N-terminus. To optimize the beneficial effects of PEGylation on the biological properties of a CNP peptide, the polymer length, conformation (e.g., branched or linear), and/or functionalization (e.g., adding a negatively charged group) of a PEG moiety can be varied. PEGylated CNP variants are tested for NEP resistance, pharmacokinetics and bioactivity (e.g., the ability to bind to NPR-B and stimulate the generation of cGMP). PEGylated CNP variants that show improved NEP resistance and at least about 50% of the cGMP-stimulating activity of CNP22 can be further tested, e.g., in vitro in a rat chondrosarcoma cell-based achondroplasia model and in vivo in a murine achondroplasia animal model.

E. Methods of Using CNP Variants, Pharmaceutical Compositions of CNP Variants, and Routes of Administration

Methods of Using CNP Variants

Bone-Related Disorders

Fibroblast growth factors (FGFs) play important roles in bone formation, and mutations in FGF receptor genes (FGFR 1, 2 and 3) give rise to a variety of inherited skeletal malfor-

mations (Curr. Biol., 5: 500-507 (1995)). In particular, activating mutations in FGFR-3 are responsible for disorders of the long bones, including achondroplasia, the most common form of human genetic dwarfism (Nature, 371: 252-254 (1994); Cell, 78: 335-342 (1994)), the milder disorder hypochondroplasia (Ann. N.Y. Acad. Sci., 785: 182-187 (1996)), and the more severe and neonatal lethal thanatophoric dysplasia (TD) types I and II (Hum. Mol. Genet., 5: 509-512 (1996); Nat. Genet., 9: 321-328 (1995)). Mouse models overexpressing FGF-2, and consequentially activating FGFR-3, show shortened long bones and macrocephaly (Mol. Biol. Cell, 6: 1861-73 (1995)). Consistent with this model, mice deficient in FGFR-3 show remarkable skeletal overgrowth with wider growth plates (Nature Genet., 12: 390-397 (1996)).

Complementary experiments with CNP, NPR-B and NPR-C suggest a link between the peptide ligand, the corresponding receptors, and bone growth. Activation of NPR-B by elevated plasma concentrations of CNP in transgenic mice causes skeletal overgrowth (Nat. Med., 10: 80-86 (2004)) histologically similar to that of the growth plate cartilage of FGFR-3 knockout mice (Nat. Genet., 4: 390-397 (1996)). In NPR-C knockout mice, NPR-C-mediated clearance of CNP should be eliminated; consistent with this prediction, the knockout animals show elongated long bones and elongated vertebrae with kyphosis (Proc. Natl. Acad. Sci. USA 96: 7403-08 (1999)). Conversely, CNP knockout mice are dwarfed with shorter long bones and vertebrae, a phenotype histologically similar to that of achondroplasia, and have increased mortality as a result of malocclusion and pulmonary restriction from the small rib cage (Proc. Natl. Acad. Sci. USA, 98: 4016-4021 (2001)). Consistent with the proposed role of CNP as an activator of NPR-B, the NPR-B knockout mouse has the same dwarfed skeletal phenotype and increased mortality as the CNP knockout mouse (Proc. Natl. Acad. Sci. USA, 101: 17300-05 (2004)). Furthermore, in a mouse model of achondroplasia with activated FGFR-3 in the cartilage, targeted overexpression of CNP in chondrocytes counteracts dwarfism (Yasoda et al., Nat. Med., 10: 80-86 (2004)). Additionally, CNP has been show to play a role in regulating endochondral bone growth and chondrocyte activity, including but not limited to chondrocyte proliferation and differentiation, inhibition of the mitogen activated protein (MAP) kinase/MEK (Raf-1) kinase signaling pathway, and promotion of endochondral ossification (Yasoda et al., Nat. Med., 10: 80-86 (2004)). These results suggest that activation of the CNP/NPR-B system is a potential therapeutic strategy for treatment of human achondroplasia.

By stimulating matrix production, proliferation and differentiation of chondrocytes and increasing long bone growth, the CNP variants of the disclosure are useful for treating mammals, including humans, suffering from a bone-related disorder, such as a skeletal dysplasia. Non-limiting examples of CNP-responsive bone-related disorders and skeletal dysplasias include achondroplasia, hypochondroplasia, short stature, dwarfism, osteochondrodysplasias, thanatophoric dysplasia, osteogenesis imperfecta, achondrogenesis, chondrodysplasia punctata, homozygous achondroplasia, chondrodysplasia punctata, camptomelic dysplasia, congenital lethal hypophosphatasia, perinatal lethal type of osteogenesis imperfecta, short-rib polydactyly syndromes, hypochondroplasia, rhizomelic type of chondrodysplasia punctata, Jansen-type metaphyseal dysplasia, spondyloepiphyseal dysplasia congenita, atelosteogenesis, diastrophic dysplasia, congenital short femur, Langer-type mesomelic dysplasia, Nievergelt-type mesomelic dysplasia, Robinow syndrome, Reinhardt syndrome, acrodysostosis, peripheral dysostosis, Kniest dysplasia, fibrochondrogenesis, Roberts syndrome, acromesomelic dysplasia, micromelia, Morquio syndrome, Kniest syndrome, metatrophic dysplasia, and spondyloepimetaphyseal dysplasia. Further, the CNP variants are useful as an adjunct or alternative to growth hormone for treating idiopathic short stature and other skeletal dysplasias.

In addition, the CNP variants are useful for treating other bone-related conditions and disorders, such as rickets, hypophosphatemic rickets [including X-linked hypophosphatemic rickets (also called vitamin D-resistant rickets) and autosomal dominant hypophosphatemic rickets], and osteomalacia [including tumor-induced osteomalacia (also called oncogenic osteomalacia or oncogenic hypophosphatemic osteomalacia)].

The CNP variants of the disclosure can also be used to treat osteoarthritis. Osteoarthritis is a degenerative disease of the articular cartilage and occurs frequently in the elderly. Osteoarthritis involves destruction of the cartilage and proliferative change in the bone and cartilage resulting from degeneration of articular components, with the change resulting in a secondary arthritis (e.g., synovitis). The extracellular matrix proteins, which are the functional entity of the cartilage, are reduced, and the number of chondrocytes decreases in osteoarthritis (Arth. Rheum. 46(8): 1986-1996 (2002)). By promoting the matrix production, growth and differentiation of chondrocytes, the CNP variants are useful for countering the undesired effects of FGF-2 and increasing matrix synthesis in subjects suffering from arthritis, including osteoarthritis, thereby treating arthritis, including osteoarthritis.

Vascular Smooth Muscle Disorders

CNP and other vasoactive peptides (including ANP, BNP and urodilatin) have vasodilator and diuretic properties and play an important role in cardiovascular homeostasis (J. Cardiovasc. Pharmacol., 117: 1600-06 (1998); Kidney Int., 49: 1732-37 (1996); Am. J. Physiol., 275: H1826-1833 (1998)). CNP is widely distributed in the cardiovascular system, especially in high concentration in vascular endothelial cells (J. Cardiovasc. Pharmacol., 117: 1600-06 (1998)). CNP is a potent relaxant of vascular smooth muscle, particularly in the coronary circulation (Biochem. Biophys. Res. Commun., 205: 765-771 (1994)), and is an inhibitor of smooth muscle cell proliferation (Biochem. Biophys. Res. Commun., 177: 927-931 (1991)). Although the vasodilator effect of CNP is less potent than that of ANP (about 1:100) (Hypertens. Res., 21: 7-13 (1998); Am. J. Physiol., 275: L645-L652 (1998)), CNP mRNA is increased in response to shear stress (FEBS Lett., 373: 108-110 (1995)) and plasma levels of CNP are elevated in inflammatory cardiovascular pathologies (Biochem. Biophys. Res. Commun., 198: 1177-1182 (1994)). CNP has been shown to suppress inflammation through inhibition of macrophage infiltration in injured carotid arteries of rabbits (Circ. Res., 91: 1063-1069 (2002)) and to directly inhibit cardiac fibroblast proliferation through an NPR-B/cGMP-dependent pathway (Endocrinology, 144: 2279-2284 (2003)).

The cardiovascular actions of CNP are mediated via activation of the NPR subtypes, NPR-B and NPR-C (Endocrinology, 130: 229-239 (1992)), the latter accounting for 95% of NPRs expressed in vivo (Science, 293: 1657-1662 (2001)). The CNP/NPR-B pathway leads to elevation of cGMP, a well-established secondary messenger in the cardiovascular system. NPR-C's 37-amino acid portion from the C-terminus has a consensus sequence that interacts with the heterotrimeric G protein G_i (J. Biol. Chem., 274: 17587-17592 (1999)), which has been shown to regulate adenylate cyclase and phospholipase C activity (J. Biol. Chem., 276: 22064-70 (2001); Am. J. Physiol., 278: G974-980 (2000); J. Biol.

Chem., 271: 19324-19329 (1996)). CNP mediates smooth muscle hyperpolarization and relaxation via activation of NPR-C and the opening of a G protein-regulated, inwardly rectifying K^+ channels (Proc. Natl. Acad. Sci. USA, 100: 1426-1431 (2003)). Likewise, CNP has important anti-proliferative effects in cardiac fibroblasts and, through interaction with NPR-C, regulates local blood flow and systemic blood pressure by hyperpolarizing smooth muscle cells (R. Rose and W. Giles, J. Physiol. 586: 353-366 (2008)).

By binding to NPR-B on vascular smooth muscle cells, CNP22 stimulates the production of cGMP, which acts as an intracellular secondary messenger to cause ultimately the relaxation of blood vessels. Based on the hypotensive actions of CNP, the CNP variants of the disclosure are useful for treating hypertension, congestive heart failure, cardiac edema, nephredema, hepatic edema, acute and chronic renal insufficiency, and so on. In addition, activation of cGMP signaling suppresses the growth of vascular smooth muscle cells. Accordingly, the CNP variants of the disclosure can be used to treat conditions or diseases caused by the abnormal growth of vascular smooth muscle cells, including but not limited to restenosis and arteriosclerosis.

The studies described above suggest that CNP may be a potential therapeutic candidate for vascular smooth muscle relaxation and remodeling. Pharmacological effects of CNP concerning certain disorders have been attributed, in part, to vasoprotective effects rather than to vasodilator activity (Am. J. Respir. Crit. Care Med., 170: 1204-1211 (2004)). Therefore, the CNP variants of the present disclosure are useful for treating conditions, e.g., vascular smooth muscle disorders, in which CNP may have a vasoprotective effect, including without limitation inducing smooth muscle relaxation and inhibiting infiltration of macrophages into cardiac tissue. In one embodiment, the CNP variants are used to treat heart failure, including but not limited to acute decompensated heart failure and acute congestive heart failure. In another embodiment, the CNP variants are used to treat asthma, cardiomyopathy, and restenosis of coronary arteries (by increasing smooth muscle cell relaxation and decreasing proliferation of smooth muscle cells).

Pharmaceutical Compositions of CNP Variants

In additional embodiments, the disclosure provides pharmaceutical compositions comprising a CNP variant, and one or more pharmaceutically acceptable excipients, carriers and/or diluents. In certain embodiments, the compositions further comprise one or more other biologically active agents (e.g., inhibitors of proteases, receptor tyrosine kinases, and/or the clearance receptor NPR-C).

In some embodiments, the compositions comprise the desired CNP variant in at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% purity. In certain embodiments, the compositions contain less than about 10%, 5%, 4%, 3%, 2%, 1% or 0.5% of macromolecular contaminants, such as other mammalian (e.g., human) proteins and other CNP variants.

Non-limiting examples of excipients, carriers and diluents include vehicles, liquids, buffers, isotonicity agents, additives, stabilizers, preservatives, solubilizers, surfactants, emulsifiers, wetting agents, adjuvants, and so on. The compositions can contain liquids (e.g., water, ethanol); diluents of various buffer content (e.g., Tris-HCl, phosphate, acetate buffers, citrate buffers), pH and ionic strength; detergents and solubilizing agents (e.g., Polysorbate 20, Polysorbate 80); anti-oxidants (e.g., methionine, ascorbic acid, sodium metabisulfite); preservatives (e.g., Thimerosol, benzyl alcohol, m-cresol); and bulking substances (e.g., lactose, mannitol, sucrose). The use of excipients, diluents and carriers in the formulation of pharmaceutical compositions is known in the art; see, e.g., Remington's Pharmaceutical Sciences, 18th Edition, pages 1435-1712, Mack Publishing Co. (Easton, Pa. (1990)), which is incorporated herein by reference in its entirety.

For example, carriers include without limitation diluents, vehicles and adjuvants, as well as implant carriers, and inert, non-toxic solid or liquid fillers and encapsulating materials that do not react with the active ingredient(s). Non-limiting examples of carriers include phosphate buffered saline, physiological saline, water, and emulsions (e.g., oil/water emulsions). A carrier can be a solvent or dispersing medium containing, e.g., ethanol, a polyol (e.g., glycerol, propylene glycol, liquid polyethylene glycol, and the like), a vegetable oil, and mixtures thereof.

In some embodiments, the compositions are liquid formulations. In certain embodiments, the formulations comprise a CNP variant in a concentration range from about 0.1 mg/ml to about 20 mg/ml, or from about 0.5 mg/ml to about 20 mg/ml, or from about 1 mg/ml to about 20 mg/ml, or from about 0.1 mg/ml to about 10 mg/ml, or from about 0.5 mg/ml to about 10 mg/ml, or from about 1 mg/ml to about 10 mg/ml.

In further embodiments, the compositions comprise a buffer solution or buffering agent to maintain the pH of a CNP-containing solution or suspension within a desired range. Non-limiting examples of buffer solutions include phosphate buffered saline, Tris buffered saline, and Hank's buffered saline. Buffering agents include without limitation sodium acetate, sodium phosphate, and sodium citrate. Mixtures of buffering agents can also be used. In certain embodiments, the buffering agent is acetic acid/acetate or citric acid/citrate. The amount of buffering agent suitable in a composition depends in part on the particular buffer used and the desired pH of the solution or suspension. For example, acetate is a more efficient pH buffer at pH 5 than pH 6, so less acetate may be used in a solution at pH 5 than at pH 6. In some embodiments, the buffering agent has a concentration of about 10 mM±5 mM. In certain embodiments, the pH of a composition is from about pH 3 to about pH 7.5, or from about pH 3.5 to about pH 7, or from about pH 3.5 to about pH 6.5, or from about pH 4 to about pH 6, or from about pH 4 to about pH 5, or is at about pH 5.0±1.0.

In other embodiments, the compositions contain an isotonicity-adjusting agent to render the solution or suspension isotonic and more compatible for injection. Non-limiting examples of isotonicity agents include NaCl, dextrose, glucose, glycerin, sorbitol, xylitol, and ethanol. In certain embodiments, the isotonicity agent is NaCl. In certain embodiments, NaCl is in a concentration of about 160±20 mM, or about 140 mM±20 mM, or about 120±20 mM, or about 100 mM±20 mM, or about 80 mM±20 mM, or about 60 mM±20 mM.

In yet other embodiments, the compositions comprise a preservative. Preservatives include, but are not limited to, m-cresol and benzyl alcohol. In certain embodiments, the preservative is in a concentration of about 0.4%±0.2%, or about 1%±0.5%, or about 1.5%±0.5%, or about 2.0%±0.5%.

In still other embodiments, the compositions contain an anti-adsorbent (e.g., to mitigate adsorption of a CNP variant to glass or plastic). Anti-adsorbents include without limitation benzyl alcohol, Polysorbate 20, and Polysorbate 80. In certain embodiments, the anti-adsorbent is in a concentration from about 0.001% to about 0.5%, or from about 0.01% to about 0.5%, or from about 0.1% to about 1%, or from about 0.5% to about 1%, or from about 0.5% to about 1.5%, or from about 0.5% to about 2%, or from about 1% to about 2%.

In additional embodiments, the compositions comprise a stabilizer. Non-limiting examples of stabilizers include glycerin, glycerol, thioglycerol, methionine, and ascorbic acid and salts thereof. In some embodiments, when the stabilizer is thioglycerol or ascorbic acid or a salt thereof, the stabilizer is in a concentration from about 0.1% to about 1%. In other embodiments, when the stabilizer is methionine, the stabilizer is in a concentration from about 0.01% to about 0.5%, or from about 0.01% to about 0.2%. In still other embodiments, when the stabilizer is glycerin, the stabilizer is in a concentration from about 5% to about 100% (neat).

In further embodiments, the compositions contain an anti-oxidant. Exemplary anti-oxidants include without limitation methionine and ascorbic acid. In certain embodiments, the molar ratio of antioxidant to CNP variant is from about 0.1:1 to about 15:1, or from about 1:1 to about 15:1, or from about 0.5:1 to about 10:1, or from about 1:1 to about 10:1 or from about 3:1 to about 10:1.

Pharmaceutically acceptable salts can be used in the compositions, including without limitation mineral acid salts (e.g., hydrochloride, hydrobromide, phosphate, sulfate), salts of organic acids (e.g., acetate, propionate, malonate, benzoate, mesylate, tosylate), and salts of amines (e.g., isopropylamine, trimethylamine, dicyclohexylamine, diethanolamine). A thorough discussion of pharmaceutically acceptable salts is found in *Remington's Pharmaceutical Sciences,* 18th Edition, Mack Publishing Company, (Easton, Pa. (1990)).

The pharmaceutical compositions can be administered in various forms, such as tablets, capsules, granules, powders, solutions, suspensions, emulsions, ointments, and transdermal patches. The dosage forms of the compositions can be tailored to the desired mode of administration of the compositions. For oral administration, the compositions can take the form of, e.g., a tablet or capsule (including softgel capsule), or can be, e.g., an aqueous or nonaqueous solution, suspension or syrup. Tablets and capsules for oral administration can include one or more commonly used excipieints, diluents and carriers, such as mannitol, lactose, glucose, sucrose, starch, corn starch, sodium saccharin, talc, cellulose, magnesium carbonate, and lubricating agents (e.g., magnesium stearate, sodium stearyl fumarate). If desired, flavoring, coloring and/or sweetening agents can be added to the solid and liquid formulations. Other optional ingredients for oral formulations include without limitation preservatives, suspending agents, and thickening agents. Oral formulations can also have an enteric coating to protect the CNP variant from the acidic environment of the stomach. Methods of preparing solid and liquid dosage forms are known, or will be apparent, to those skilled in this art (see, e.g., Remington's Pharmaceutical Sciences, referenced above).

Formulations for parenteral administration can be prepared, e.g., as liquid solutions or suspensions, as solid forms suitable for solubilization or suspension in a liquid medium prior to injection, or as emulsions. For example, sterile injectable solutions and suspensions can be formulated according to techniques known in the art using suitable diluents, carriers, solvents (e.g., buffered aqueous solution, Ringer's solution, isotonic sodium chloride solution), dispersing agents, wetting agents, emulsifying agents, suspending agents, and the like. In addition, sterile fixed oils, fatty esters, polyols and/or other inactive ingredients can be used. As further examples, formulations for parenteral administration include aqueous sterile injectable solutions, which can contain anti-oxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient; and aqueous and nonaqueous sterile suspensions, which can contain suspending agents and thickening agents.

Compositions comprising a CNP variant can also be lyophilized formulations. In certain embodiments, the lyophilized formulations comprise a buffer and bulking agent, and optionally an antioxidant. Exemplary buffers include without limitation acetate buffers and citrate buffers. Exemplary bulking agents include without limitation mannitol, sucrose, dexran, lactose, trehalose, and povidone (PVP K24). In certain embodiments, mannitol is in an amount from about 3% to about 10%, or from about 4% to about 8%, or from about 4% to about 6%. In certain embodiments, sucrose is in an amount from about 6% to about 20%, or from about 6% to about 15%, or from about 8% to about 12%. Exemplary anti-oxidants include, but are not limited to, methionine and ascorbic acid.

The disclosure also provides kits containing, e.g., bottles, vials, ampoules, tubes, cartridges and/or syringes that comprise a liquid (e.g., sterile injectable) formulation or a solid (e.g., lyophilized) formulation. The kits can also contain pharmaceutically acceptable vehicles or carriers (e.g., solvents, solutions and/or buffers) for reconstituting a solid (e.g., lyophilized) formulation into a solution or suspension for administration (e.g., by injection), including without limitation reconstituting a lyophilized formulation in a syringe for injection or for diluting concentrate to a lower concentration. Furthermore, extemporaneous injection solutions and suspensions can be prepared from, e.g., sterile powder, granules, or tablets comprising a CNP-containing composition. The kits can also include dispensing devices, such as aerosol or injection dispensing devices, pen injectors, autoinjectors, needleless injectors, syringes, and/or needles.

As a non-limiting example, a kit can include syringes having a single chamber or dual chambers. For single-chamber syringes, the single chamber can contain a liquid CNP formulation ready for injection, or a solid (e.g., lyophilized) CNP formulation or a liquid formulation of a CNP variant in a relatively small amount of a suitable solvent system (e.g., glycerin) that can be reconstituted into a solution or suspension for injection. For dual-chamber syringes, one chamber can contain a pharmaceutically acceptable vehicle or carrier (e.g., solvent system, solution or buffer), and the other chamber can contain a solid (e.g., lyophilized) CNP formulation or a liquid formulation of a CNP variant in a relatively small amount of a suitable solvent system (e.g., glycerin) which can be reconstituted into a solution or suspension, using the vehicle or carrier from the first chamber, for injection.

As a further example, a kit can include one or more pen injector or autoinjector devices, and dual-chamber cartridges. One chamber of a cartridge can contain a pharmaceutically acceptable vehicle or carrier (e.g., solvent system, solution or buffer), and the other chamber can contain a solid (e.g., lyophilized) CNP formulation or a liquid formulation of a CNP variant in a relatively small amount of a suitable solvent system (e.g., glycerin) which can be reconstituted into a solution or suspension, using the vehicle or carrier from the first chamber, for injection. A cartridge can comprise an amount of the CNP variant that is sufficient for dosing over a desired time period (e.g., 1 day, 2 days, 3 days, 1 week, 2 weeks, 3 weeks, 4 weeks, etc.). The pen injector or autoinjector can be adjusted to administer a desired amount of the CNP formulation from a cartridge.

In addition, pharmaceutical compositions comprising a CNP variant can be formulated as a slow release, controlled release or sustained release system for maintaining a relatively constant level of dosage over a desired time period, such as 1 week, 2 weeks, 3 weeks, 1 month, 2 months, or 3 months. Slow release, controlled release and sustained release formulations can be prepared using, e.g., biodegrad-

able polymeric systems {which can comprise, e.g., hydrophilic polymers [e.g., polylactide, polyglycolide, poly(lactide-glycolide)]}, and can take the form of, e.g., microparticles, microspheres or liposomes, as is known in the art.

Dosages and Frequency of Dosing

As used herein, the term "therapeutically effective amount" of an active agent (e.g., a CNP variant) refers to an amount that provides therapeutic benefit to a patient. The amount may vary from one individual to another and may depend upon a number of factors, including the overall physical condition of the patient. A therapeutically effective amount of a CNP variant can be readily ascertained by one skilled in the art, using publicly available materials and procedures. For example, the amount of a CNP variant used for therapy should give an acceptable rate of growth based on growth charts for children ages 0-17 years with achondroplasia (214 females and 189 males), which list height for age, head circumference, and segmental growth (Horton W A et al., Standard growth curves for achondroplasia, J. Pediatr., 93: 435-8 (1978)). CDC charts can be used to assess weight for age and weight for height or BMI for age. Secondary outcomes with courses that are more chronic in nature can also be measured.

Having a longer serum half-life than wild-type CNP22, the CNP variants can potentially be administered less frequently than CNP22. The dosing frequency for a particular individual may vary depending upon various factors, including the disorder being treated and the condition and response of the individual to the therapy. In certain embodiments, a pharmaceutical composition containing a CNP variant is administered to a subject about one time per day, one time per two days, one time per three days, or one time per week. In one embodiment, for treatment of bone-related disorders (e.g., skeletal dysplasias, including achondroplasia), a daily or weekly dose of a CNP variant is administered to patients until and/or through adulthood.

The CNP variants described herein can be administered to patients at therapeutically effective doses to treat, ameliorate or prevent bone-related disorders (e.g., skeletal dysplasias, including achondroplasia) and conditions (e.g., vascular smooth muscle disorders) in which CNP can provide a vasoprotective effect. The safety and therapeutic efficacy of the CNP variants can be determined by standard pharmacological procedures in cell cultures or experimental animals, such as, for example, by determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50}. Active agents exhibiting a large therapeutic index are normally preferred.

Data obtained from cell culture assays and animal studies can be used to formulate a range of dosage for use in humans. The dosage normally lies within a range of circulating concentrations that include the ED_{50}, with little or minimal toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized. The therapeutically effective dose can be determined from cell culture assays and animal studies.

In certain embodiments, the CNP variants described herein are administered at a dose in the range from about 5 or 10 nmol/kg to about 300 nmol/kg, or from about 20 nmol/kg to about 200 nmol/kg. In some embodiments, the CNP variants are administered at a dose of about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 125, 130, 140, 150, 160, 170, 175, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 350, 400, 450, 500, 750, 1000, 1250, 1500, 1750 or 2000 nmol/kg or other dose deemed appropriate by the treating physician. In other embodiments, the CNP variants are administered at a dose of about 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950 or 1000 ug/kg, or about 1, 1.25, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5 or 10 mg/kg, or other dose deemed appropriate by the treating physician. The doses of CNP variants described herein can be administered according to the dosing frequency/frequency of administration described herein, including without limitation daily, 2 or 3 times per week, weekly, every 2 weeks, every 3 weeks, monthly, etc.

The frequency of dosing/administration of a CNP variant for a particular subject may vary depending upon various factors, including the disorder being treated and the condition and response of the subject to the therapy. The CNP variant can be administered in a single dose or in multiple doses per dosing. In certain embodiments, the CNP variant is administered, in a single dose or in multiple doses, daily, every other day, every 3 days, 2 times per week, 3 times per week, weekly, bi-weekly, every 3 weeks, monthly, every 6 weeks, every 2 months, every 3 months, or as deemed appropriate by the treating physician.

In some embodiments, a CNP variant is administered so as to allow for periods of growth (e.g., chondrogenesis), followed by a recovery period (e.g., osteogenesis). For example, the CNP variant may be administered intravenously, subcutaneously or by another mode daily or multiple times per week for a period of time, followed by a period of no treatment, then the cycle is repeated. In some embodiments, the initial period of treatment (e.g., administration of the CNP variant daily or multiple times per week) is for 3 days, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks or 12 weeks. In a related embodiment, the period of no treatment lasts for 3 days, 1 week, 2 weeks, 3 weeks or 4 weeks. In certain embodiments, the dosing regimen of the CNP variant is daily for 3 days followed by 3 days off; or daily or multiple times per week for 1 week followed by 3 days or 1 week off; or daily or multiple times per week for 2 weeks followed by 1 or 2 weeks off; or daily or multiple times per week for 3 weeks followed by 1, 2 or 3 weeks off; or daily or multiple times per week for 4, 5, 6, 7, 8, 9, 10, 11 or 12 weeks followed by 1, 2, 3 or 4 weeks off.

Modes of Administration

The CNP variants, or pharmaceutical compositions comprising them, can be administered to subjects in various ways such as, e.g., by injection subcutaneously, intravenously, intra-arterially, intraperitoneally, intramuscularly, intradermally, or intrathecally. In an embodiment, the CNP variants are administered by a single subcutaneous, intravenous, intra-arterial, intraperitoneal, intramuscular, intradermal or intrathecal injection once a day.

The CNP variants can also be administered by direct injection at or near the site of disease. Further, the CNP variants can be administered by implantation of a depot at the target site of action (e.g., an abnormal or dysplasic bone). Alternatively, the CNP variants can be administered sublingually under the tongue (e.g., sublingual tablet) or by inhalation into the lungs (e.g., inhaler or aerosol spray), by delivery into the nasal cavity (e.g., intranasal spray), by delivery into the eye (e.g., eye drop), or by transdermal delivery (e.g., by means of a patch on the skin). The CNP variants may also be administered orally in the form of microspheres, microcapsules, liposomes (uncharged or charged (e.g., cationic)), polymeric microparticles (e.g., polyamides, polylactide, polyglycolide, poly(lactide-glycolide)), microemulsions, and the like.

A further method of administration is by osmotic pump (e.g., an Alzet pump) or mini-pump (e.g., an Alzet mini-osmotic pump), which allows for controlled, continuous and/or slow-release delivery of the CNP variant or pharmaceutical composition over a pre-determined period. The osmotic pump or mini-pump can be implanted subcutaneously, or near the target site (e.g., the long bones of limbs, the epiphyses, etc.).

As explained above, the CNP variants can be used to treat conditions or diseases caused by the abnormal growth of vascular smooth muscle cells, including but not limited to restenosis and arteriosclerosis. For local delivery of a CNP variant to the diseased bodily vessel (e.g., blood vessel), the CNP variant can be delivered by means of a medical device (e.g., a stent) implanted at the diseased site. In one embodiment, the CNP variant is impregnated in a polymeric matrix or polymeric coating disposed over a stent. In another embodiment, the CNP variant is contained in reservoirs or channels formed in the body of a stent and covered by a porous polymeric membrane or layer through which the CNP variant can diffuse. The polymeric matrix, coating, membrane or layer can comprise at least one biodegradable (e.g., hydrophilic) polymer, as is known in the art. In a further embodiment, the CNP variant can be contained in micropores in the body of a stent. The CNP variant can be delivered from a stent by burst release, pulse release, controlled release or sustained release, or a combination thereof. For example, the stent can locally deliver the CNP variant to the diseased site in a burst release followed by a sustained release. Sustained release can be over a period up to about 2 weeks, 1 month, 2 months, 3 months, 6 months or 1 year.

It will be apparent to one skilled in the art that the CNP variants or compositions thereof can also be administered by other modes. Determination of the most effective mode of administration of the CNP variants or compositions thereof is within the skill of the skilled artisan.

The CNP variants can be administered as pharmaceutical formulations suitable for, e.g., oral (including buccal and sub-lingual), rectal, nasal, topical, pulmonary, vaginal or parenteral (including intramuscular, intraarterial, intrathecal, subcutaneous and intravenous) administration, or in a form suitable for administration by inhalation or insufflation. Depending on the intended mode of administration, the pharmaceutical formulations can be in the form of solid, semi-solid or liquid dosage forms, such as tablets, suppositories, pills, capsules, powders, liquids, suspensions, emulsions, creams, ointments, lotions, and the like. The formulations can be provided in unit dosage form suitable for single administration of a precise dosage. The formulations comprise an effective amount of a CNP variant, and one or more pharmaceutically acceptable excipients, carriers and/or diluents, and optionally one or more other biologically active agents.

Combination Therapy

In one embodiment, a CNP variant can be used in combination with one or more other active agents useful for treating, ameliorating or preventing CNP-responsive conditions or disorders such as, e.g., bone-related disorders (e.g., skeletal dysplasias) and vascular smooth muscle disorders. The other active agent(s) can enhance the effects of the CNP variant and/or exert other pharmacological effects in addition to those of the CNP variant. Non-limiting examples of active agents that can be used in combination with the CNP variants described herein are other natriuretic peptides (e.g., BNP) and inhibitors (e.g., antagonists) of peptidases and proteases (e.g., NEP and furin), NPR-C and tyrosine kinases (e.g., FGFR-3). By preventing NEP cleavage of the CNP variant, an NEP inhibitor can prolong the half-life of the variant. Examples of NEP inhibitors include, without limitation, thiorphan and candoxatril. Co-use of an NPR-C inhibitor can also prolong the half-life of the CNP variant via inhibition of the variant's clearance by NPR-C. A non-limiting example of an NPR-C inhibitor is the fragment FGIPMDRIGRNPR (SEQ ID NO: 82), which would be released at the target site (e.g., bone growth plate) upon proteolytic cleavage of the FGIPMDRI-GRNPR-CNP22 chimera (Analog CZ) (SEQ ID NO: 82) or similar chimeras comprising variants of CNP22 (e.g., those containing amino acid substitution(s), addition(s), and/or deletion(s) relative to CNP22). Co-use of a tyrosine kinase inhibitor can accentuate the effects of a CNP therapy by inhibiting the tyrosine kinase receptor FGFR-3, a negative regulator of chondrocyte and bone growth. Non-limiting examples of tyrosine kinase inhibitors include those disclosed in U.S. Pat. Nos. 6,329,375 and 6,344,459.

To achieve the appropriate therapeutic outcome in the combination therapies, one would generally administer to the subject the CNP composition and other therapeutic(s) in a combined amount effective to produce the desired therapeutic outcome (e.g., restored bone growth). This process may involve administering the CNP composition and other therapeutic agent(s) at the same time. Simultaneous administration can be achieved by administering a single composition or pharmacological protein formulation that includes both the CNP variant and other therapeutic agent(s). Alternatively, the other therapeutic agent(s) can be taken separately at about the same time as a pharmacological formulation (e.g., tablet, injection or drink) of the CNP variant. The CNP variant can also be formulated into a foodstuff such as brownies, pancakes, or cake, suitable for ingestion.

In other alternatives, administration of the CNP variant can precede or follow administration of the other therapeutic agent(s) by intervals ranging from minutes to hours. In embodiments where the other therapeutic agent(s) and the CNP composition are administered separately, one would generally ensure that the CNP variant and the other therapeutic agent(s) are administered within an appropriate time of one another so that both the CNP variant and the other therapeutic agent(s) can exert, synergistically or additively, a beneficial effect on the patient. For example, one can administer the CNP composition within about 0.5-6 hours (before or after) of the other therapeutic agent(s). In one embodiment, the CNP composition is administered within about 1 hour (before or after) of the other therapeutic agent(s).

Identifying and Monitoring Patient Populations

Protocols can be established to identify subjects suitable for CNP therapy and to determine whether a given patient is responsive to CNP therapy. For example, for treatment of bone-related disorders, indicators of growth can be measured, such as long bone growth measurements in utero and neonatal and measurements of bone growth biomarkers such as CNP, cGMP, Collagen II, osteocalcin, and Proliferating Cell Nuclear Antigen (PCNA).

One CNP signaling marker is cGMP (guanosine 3',5' cyclic monophosphate). The level of this intracellular signaling molecule increases after CNP binds to and activates its cognate receptor NPR-B. Elevated levels of cGMP can be measured from cell culture extracts (in vitro) after CNP exposure, conditioned media from bone ex-plant studies (ex vivo) after CNP exposure, and in the plasma (in vivo) within minutes of CNP administration subcutaneously, intravenously, or via other routes of administration known in the art.

Cartilage and bone-specific analytes (or cartilage- and bone-associated markers) can also be measured to assess CNP efficacy. For example, fragments of cleaved collagen type II are a cartilage-specific marker for cartilage turnover.

Type II collagen is the major organic constituent of cartilage and fragments of type II collagen (cleaved collagen) are released into circulation, and subsequently secreted into the urine, following cartilage turnover. Cartilage turnover precedes new bone formation.

A bone-specific biomarker for bone formation which can be measured is N-terminal propeptides of type I procollagen (PINP). The synthesis of type I collagen is an important step in bone formation, as type I collagen is the major organic component in bone matrix. During collagen synthesis, propeptides are released from the procollagen molecule and can be detected in serum. In addition, fragments of collagen type I can be measured as a marker for bone resorption.

Other potential biomarkers for cartilage and bone formation and growth include aggrecan chondroitin sulfate (cartilage-specific marker for cartilage turnover), propeptides of type TT collagen (cartilage-specific marker for cartilage formation), alkaline phosphatase (bone-specific) and osteocalcin (bone-specific marker for bone formation). Cartilage- and bone-associated biomarkers can be measured, e.g., in serum from efficacy/pharmacodynamic in vivo studies and from the conditioned media of ex vivo studies, using commercially available kits.

In one embodiment, the level of at least one bone- or cartilage-associated biomarker is assayed or measured in a subject that has been administered a CNP variant in order to monitor the effects of the CNP variant on bone and cartilage formation and growth in vivo. For example, an increase in the level of at least one bone- or cartilage-associated biomarker may indicate that administration of a CNP variant has a positive effect on bone growth and is a useful treatment for skeletal dysplasias and other bone- or cartilage-related diseases or disorders associated with decreased CNP activity. Exemplary bone- or cartilage-associated biomarkers include, but are not limited to, CNP (e.g, endogenous levels of CNP), cGMP, propeptides of collagen type II and fragments thereof, collagen type II and fragments thereof, osteocalcin, proliferating cell nuclear antigen (PCNA), propeptides of type I procollagen (PINP) and fragments thereof, collagen type I and fragments thereof, aggrecan chondroitin sulfate, and alkaline phosphatase.

In an embodiment, biomarkers are measured by obtaining a biological sample from a subject who will be administered, is being administered or has been administered a CNP variant. Biomarkers can be measured using techniques known in the art, including, but not limited to, Western Blot, enzyme linked immunosorbant assay (ELISA), and enzymatic activity assay. The biological sample can be blood, serum, urine, or other biological fluids.

Additional aspects and details of the disclosure will be apparent from the following examples, which are intended to be illustrative rather than limiting.

F. Examples

Example 1

Synthesis of CNP Variants

CNP variants were prepared using the methods described herein. Substitutions with natural or unnatural amino acids or peptidomimetics were made, as indicated in Tables 1-3 (shown in Example 3), at the respective amino acid residues in the wild-type sequence of CNP22. In certain variants, additional amino acids were added to the N-terminal and/or C-terminal ends of the whole or a portion of the wild-type CNP22 sequence (see Table 3).

Also prepared were CNP variants in which a PEG (or PEO) moiety was conjugated to the N-terminus of CNP22 or variants thereof (see Table 4, shown in Example 3). PEGylation reagents can be obtained from the commercial sources shown in Table 5.

TABLE 5

Vendor	Product Name	Name	MW (Da)	PEGylation Reagent
NOF	Sunbright ME-200CS	mPEG20K	20,000	CH3(CH2CH2O)450-(CH2)5COO—NHS
NOF	Sunbright ME-050CS	mPEG5K	5,000	CH3(CH2CH2O)110-(CH2)5COO—NHS
Pierce	(Methyl-PEG12)3-PEG4-NHS Ester	(mPEG12)3-PEG4	2,400	[CH3(CH2CH2O)12]3-(CH2CH2O)4-NHCO(CH2)3-COO—NHS
NOF	Sunbright ME-020HS	mPEG2K	2,000	CH3(CH2CH2O)45-(CH2)5COO—NHS
NOF	Sunbright ME-020CS	mPEG2K	2,000	CH3(CH2CH2O)45-(CH2)5COO—NHS
NOF	Sunbright ME-010HS	mPEG1K	1,000	CH3(CH2CH2O)23-CO(CH2)2COO—NHS
Pierce	Methyl PEG24-NHS Ester	MS(PEG)24	1,200	CH3(CH2CH2O)24-(CH2)2COO—NHS
Pierce	EZ-Link NHS-PEG12-Biotin	PEO12-Biotin	940	Biotin-(CH2CH2O)12-(CH2)2COO—NHS
Pierce	Methyl PEG12-NHS Ester	MS(PEG)12	690	CH3(CH2CH2O)12-(CH2)2COO—NHS
Pierce	EZ-Link NHS-PEG4-Biotin	PEO4-biotin	590	Biotin-(CH2CH2O)4-(CH2)2COO—NHS
Pierce	Mono(lactosylamido) mono(succinimidyl)suberate	LSS	590	
Pierce	EZ-link NHS-LC-LC-Biotin	LC-LC-Biotin	570	
Pierce	EZ-link NHS-LC-Biotin	LC-Biotin (LC = long chain)	450	
Pierce	EZ-link NHS-Biotin	Biotin	340	

The PEG (also called PEO) polymers purchased from Pierce Biotechnology (Rockford, Ill.) are monodispersed—i.e., they contain a single discrete polymer of a particular molecular weight. By contrast, the PEG polymers purchased from NOF (Nippon Oil and Fat) are polydispersed—i.e., they

contain a mixture of polymers having a distribution of molecular weights.

To PEGylate CNP22 or variants thereof, reaction and purification conditions are optimized for each PEG-CNP conjugate. According to a general PEGylation procedure, reaction mixtures contain about 1 mM CNP22 or a variant thereof, and about 1 to 5 mM NHS-activated PEG in potassium phosphate buffer, pH between about 5.0 and 6.5. To mono-PEGylate selectively at the peptide N-terminus and minimize PEGylation at an internal site (e.g., Lys4 of CNP22), the PEGylation reaction can be conducted under more acidic conditions (e.g., at a pH between about 5.5 and 6.5) to protonate selectively and hence deactivate the more basic primary amino group on the lysine side chain. After about 1 to 3 hours of incubation at room temperature, the PEGylation reaction is quenched by addition of aqueous glycine buffer. Reaction products are then separated by reverse-phase HPLC, optimized for each PEG-CNP conjugate. Fractionation samples are speedvacced to dryness, and reconstituted/formulated in 1 mM HCl. Identification and purity of each PEG-CNP product are determined by liquid chromatography-mass spectrometry (LC/MS).

Example 2A

Recombinant Production of CNP Variants

CNP variants can be produced using recombinant technology. In certain embodiments, the CNP variants are produced as fusion proteins comprising a cleavable peptide, carrier protein or tag. Exemplary methods for recombinantly producing CNP fusion proteins are disclosed below.

Materials and Methods

Cloning of CNP Fusion Proteins into Expression Vectors

CNP DNA fragments were amplified using polymerase chain reaction (PCR) and the amplified PCR fragments were digested with Nde I and BamHI and cloned into pET21a vector (Novagen, Gibbstown, N.J.). CNP fusion protein DNA was synthesized by DNA2.0 and cloned into different expression vectors (Table 6).

TABLE 6

Construct	Vector	Product	Chemical cleavage	Final product	Expression *E. coli* strain
pJexpress-TAF-CNP	pJexpress401	TAF-CNP inclusion bodies	Formic acid (Asp-Pro)	Pro-CNP	BL21; BL21(DE3)
pJexpress-KSI-CNP(M/N)	pJexpress404	KSI-CNP(M/N) inclusion bodies	CNBr (Met-X)	CNP(M/N)	BL21
pET-31b-KSI-CNP	pET-31b	KSI-CNP inclusion bodies	Formic acid (Asp-Pro)	Pro-CNP	BL21(DE3)
pET-32a-Trx-CNP	pET-32a	Trx-CNP fusion protein (soluble)	Formic acid (Asp-Pro)	Pro-CNP	BL21(DE3)
pMAL-CNP	pMAL-c2X	MBP-CNP fusion protein (soluble)	Formic acid (Asp-Pro)	Pro-CNP	BL21(DE3)

CNP: GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC [Gly-CNP37 (SEQ ID NO: 75);
TAF: human transcription factor TAF12;
KSI: ketosteroid isomerase;
MBP: maltose-binding protein;
Trx: thioredoxin

Expression of CNP Fusion Proteins in *E. coli*

CNP fusion protein expression plasmids were transformed into *E. coli* BL21 or BL21(DE3). Transformed cells were plated on LB plates containing 100 ug/ml carbeniciline or 50 ug/ml kanamycin and incubated overnight at 37° C. One single colony was picked and cultured in 4 ml LB medium containing 100 ug/ml of carbeniciline or 50 ug/ml kanamycin at 37° C. with shaking. When an OD_{600} of bacterial culture reached 0.6, 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) was added to the cell media and the media was incubated at 37° C. for 3 hours with shaking. For cell harvest, bacterial cells were centrifuged at 4000 rpm for 10 minutes and the cell pellets were stored at −80° C. Cell pellets were lysed with B-PER II Bacterial Extraction Reagent (PIERCE, 0.4 ml per 4 ml of bacterial culture) and Benzonase Nuclease (Novagen, 0.025 U/ml) at room temperature for 10 minutes. Bacterial crude extract was reserved and centrifuged to obtain supernatant. Supernatant and crude extract were assayed for CNP fusion protein expression and solubility by SDS-PAGE and Western Blot.

Detection of CNP Fusion Protein Expression with SDS-PAGE and Western Blot

Ten uL of cell lysates or soluble supernatants was run on sodium dodecyl sulfate-polyacrylimide gel electrophoresis (SDS-PAGE) (Invitrogen, Carlsbad, Calif., NuPAGE 4-12% Bis-tris Gel, MES SDS buffer). The gel was stained using 20 ml Imperial Protein Stain (Thermo Fisher, Rockford, Ill.) at room temperature for 1 hour and de-stained with water. For Western blot, the protein was transferred to membrane with Gel blot (Invitrogen). The membrane was blocked in TBS buffer with 5% milk at room temperature for 1 hour. Rabbit anti-CNP22 antibody (1:2500 dilution) (Bachem, Torrance, Calif.) was added to the membrane, which was then incubated at room temperature with shaking for 2 hours, and then the membrane was washed 3 times with TBS buffer.

Alkaline phosphate (AP) conjugated anti-rabbit IgG (1:5000 dilution) was added to the membrane, which was then incubated at room temperature with shaking for 1 hour, and then the membrane was washed 3 times with TBS buffer. Ten ml WESTERN BLUE®Stabilized Substrate (Promega, Madison, Wis.) was added to the membrane, which was then incubated at room temperature with shaking for 1 to 5 min, and then the membrane was washed with TBS buffer to remove excess stain.

Expression of TAF-CNP Fusion Protein in *E. coli* BL21

Cells (*E. coli* strain BL21) expressing TAF-CNP fusion protein were obtained from glycerol stock stored at −80° C. and were grown in 4 ml LB medium containing 50 ug/ml of kanamycin at 37° C. overnight with shaking (250 rpm). Four ml of overnight grown cell culture was transferred to 200 ml

LB medium containing 50 ug/ml of kanamycin and was grown at 37° C. with shaking (250 rpm). When the OD_{600} reached 0.6, IPTG was then added to a final concentration of 1 mM to induce protein expression at 37° C. with shaking (250 rpm) for 3 hours. Cells were then spun down at 3000 rpm for 10 minutes and the resulting cell pellet was frozen at −80° C.

Purification of TAF-CNP Inclusion Bodies and Formic Acid Cleavage

The cell pellet (from 200 ml culture) was resuspended in 25 ml of B-PER II buffer (PIERCE), the pellet was sonicated for 10 minutes (50%, 1 second, pause 2 seconds) on ice, centrifuged at 12000 rpm for 20 minutes at 4° C., and then the pellet was resuspended in 25 ml 20× diluted B-PER II buffer. This was repeated until the supernatant became clear (3-5 times). One mL of resuspended TAF-CNP inclusion bodies was transferred to a 1.5 ml tube and centrifuged at 14000 rpm for 15 min. The supernatant was discarded and the pellet was dissolved with 10 ul of 88% formic acid, and then 490 ul of Millipore filtered water was added immediately. The pellet was mixed well by vortex and incubated at 55° C. for 20 to 24 hours (70° C./6 hours are alternative conditions). The products of the formic acid cleavage were assayed by SDS-PAGE and LC/MS (C4RP).

LC/MS Sample Preparation

Inclusion bodies were isolated from about 8 mL of culture (about 1.5 OD) and the pellet was solubilized in 10 uL nt formate. Resolubilized pellet was immediately diluted to 2% or 10% final formate concentration (0.5 mL) and incubated at 55° C. for 21 hours (pH 2) (cloudiness was more evident in the 2% formate sample the next day). Both samples were centrifuged at 15000 rpm for 2 minutes. Twenty uL of supernatant was injected into an LC/MS (C4 RP) apparatus.

Results

CNP Fusion Proteins were Expressed in *E. coli*

All CNP fusion proteins were expressed in *E. coli* induced with 1 mM IPTG at 37° C. for 3 hours (FIG. **1**). The constructs pJexpress-TAF-CNP, pJexpress-KSI-CNP(M/N) and pET-31b-KSI-CNP were expressed as inclusion bodies, while the constructs pET-32a-Trx-CNP and pMAL-CNP were expressed as soluble fusion proteins. Western blot using anti-CNP22 antibody confirmed the expression of the CNP fusion proteins (FIG. **1**).

CNP was Produced from TAF-CNP Inclusion Bodies by Formic Acid Cleavage

TAF-CNP inclusion bodies were partially purified and treated with 2% formic acid at 55° C. for 20 to 24 hours (70° C./6 hours are alternative conditions). The majority of TAF-CNP was cleaved and one extra band having similar size as Gly-CNP37 peptide appeared on SDS-PAGE (FIG. **2**). The cleaved sample was further analyzed by LC/MS (C4 RP). The LC/MS results showed that CNP was released in soluble form from TAF-CNP inclusion bodies after formic acid cleavage. LC/MS analysis indicated that formic acid cleavage of the CNP fusion proteins resulted in formation of cyclized Pro-Gly-CNP37 (MW=4102). Calculation of protein amounts based on analysis suggested that about 60 ug of formic acid-generated CNP was produced from 8 mL of very low OD culture. From a small scale (e.g., about 8 mL) of low OD (e.g., 1.2 OD) cell culture, approximately 8 ug/ml CNP was produced, while fermentation of a larger scale (e.g., about 8 L) of higher OD (e.g., 380D) cell culture can produce approximately 1 mg/ml CNP.

Conclusion

Five expression constructs were generated to express CNP fusion proteins. Expression of all five constructs produced soluble (Trx and MBP) or insoluble (TAF and KSI) CNP fusion proteins. Approximately 1 mg/ml soluble CNP can be produced from TAF-CNP inclusion bodies by a simple formic acid cleavage procedure.

Example 2B

Production of Additional CNP Variants in *E. coli*

Recombinant production of CNP variants was carried out as described in Example 2A. In this example, additional CNP constructs were generated with a QuikChange II XL site-directed mutagenesis kit (Stratagene) or were synthesized by DNA2.0. Additional CNP constructs and expression vectors are listed in Table 7.

TABLE 7

Construct	Vector	Product	Chemical cleavage	Final product	Expression *E. coli* strain
pJexpress-TAF(C/A)-Pro-CNP38	pJexpress401	TAF(C/A)-Pro-CNP38 inclusion bodies	Formic acid (Asp-Pro)	Pro-CNP38	BL21(DE3)
pJexpress-TAF(4D/4E)-Pro-CNP38	pJexpress401	TAF(4D/4E)-Pro-CNP38 inclusion bodies	Formic acid (Asp-Pro)	Pro-CNP38	BL21(DE3)
pJexpress-TAF(C/A&4D/4E)-Pro-CNP38	pJexpress401	TAF(C/A&4D/4E)-Pro-CNP38 inclusion bodies	Formic acid (Asp-Pro)	Pro-CNP38	BL21(DE3)
pJexpress-TAF(C/A&10D/10E)-Pro-CNP38	pJexpress401	TAF(C/A&10D/10E)-Pro-CNP38 inclusion bodies	Formic acid (Asp-Pro)	Pro-CNP38	BL21(DE3)
pJexpress-TAF-NL-(C/A & 6D/6E)-Pro-CNP38	pJexpress401	TAF-NL-(C/A & 6D/6E)-Pro-CNP38 inclusion bodies	Formic acid (Asp-Pro)	Pro-CNP38	BL21(DE3)
pJexpress-BMP-Pro-CNP38	pJexpress401	BMP-Pro-CNP38 inclusion bodies	Formic acid (Asp-Pro)	Pro-CNP38	BL21; BL21(DE3)

TABLE 7-continued

Construct	Vector	Product	Chemical cleavage	Final product	Expression *E. coli* strain
pJexpress-TAF-Pro-CNP37	pJexpress401	TAF-Pro-CNP37 inclusion bodies	Formic acid (Asp-Pro)	Pro-CNP37	BL21; BL21(DE3)
pJexpress-BMP-Pro-CNP37	pJexpress401	BMP-Pro-CNP37 inclusion bodies	Formic acid (Asp-Pro)	Pro-CNP37	BL21; BL21(DE3)
pJexpress-TAF Pro-HSA-CNP	pJexpress401	TAF-Pro-HSA-CNP inclusion bodies	Formic acid (Asp-Pro)	Pro-HSA-CNP	BL21
pJexpress-TAF	pJexpress401	TAF inclusion bodies	N/A	N/A	BL21(DE3)
pJexpress-BMP	pJexpress401	BMP inclusion bodies	N/A	N/A	BL21(DE3)
pJexpress-TAF(C/A)	pJexpress401	TAF(C/A) inclusion bodies	N/A	N/A	BL21(DE3)
pJexpress-TAF(4D/4E)	pJexpress401	TAF(4D/4E) inclusion bodies	N/A	N/A	BL21(DE3)
pJexpress-TAF (C/A&4D/4E)	pJexpress401	TAF(C/A&4D/4E) inclusion bodies	N/A	N/A	BL21(DE3)
pJexpress-TAF (C/A&10D/10E)	pJexpress401	TAF(C/A&10D/10E) inclusion bodies	N/A	N/A	BL21(DE3)
pJexpress-TAF-NL-(C/A & 6D/6E)	pJexpress401	TAF-NL-(C/A&6D/6E) inclusion bodies	N/A	N/A	BL21(DE3)
pJexpress-TAF-Pro-CNP53	pJexpress401	TAF-Pro-CNP53 inclusion bodies	Formic acid (Asp-Pro)	Pro-CNP53	BL21(DE3)
pJexpress-TAF-CNP34	pJexpress401	TAF-CNP34 inclusion bodies	Formic acid (Asp-Pro)	CNP34	BL21(DE3)

CNP38: GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC [Gly-CNP37 (SEQ ID NO: 75)];
Pro-CNP38: PGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC [Pro-Gly-CNP37] (SEQ ID NO: 145);
CNP37: QEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (SEQ ID NO: 60);
HSA-CNP: GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC [HSA-CNP27 (SEQ ID NO: 144)];
Pro-CNP53: PDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIG-SMSGLGC (SEQ ID NO: 185);
CNP34: PNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (SEQ ID NO: 163);
TAF-Pro-CNP38: MVLTKKKLQDLVREVCPNEQLDEDVEEMLLQIADDFIESVVTAA-CQLARHRKSSTLEVKDVQLHLERQWNM-WIMGSSHHHHHHSSGLVPRGSHT-GDDDDKHMDPGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (SEQ ID NO: 196);
TAF: human transcription factor TAF12 histone fold domain (HFD) and linker from pET-15b vector;
TAF-NL: TAF12 HFD without a linker;
TAF12 HFD: VLTKKKLQDLVREVCPNEQLDEDVEEMLLQIADDFIESVVTAACQLA-RHRKSSTLEVKDVQLHLERQWNMWI (SEQ ID NO: 197);
pET-15b linker: MGSSHHHHHHSSGLVPRGSHTGDDDDKHMD (SEQ ID NO: 195);
TAF(C/A): cysteine in TAF is changed to alanine;
TAF(D/E): aspartic acid in TAF is changed to glutamic acid (number indicates which amino acid residue is changed);
TAF(C/A & D/E): cysteine and aspartic acid in TAF are changed to alanine and glutamic acid, respectively;
BMP: bone morphogenetic protein 2 with seven C/A (cysteine to alanine) mutations;
KSI: ketosteroid isomerase;
MBP: maltose-binding protein;
TRX: thioredoxin

Results

All CNP fusion proteins were expressed in *E. coli* induced with 1 mM IPTG at 37° C. for 3 hours. Constructs pJexpress-BMP-Pro-CNP38, pJexpress-TAF-Pro-CNP37, pJexpress-BMP-Pro-CNP37, pJexpress-Pro-HSA-CNP, pJexpress-TAF and pJexpress-BMP were expressed as inclusion bodies. A Western Blot with anti-CNP antibody was used to confirm the expression (FIG. **3**).

Pro-Gly-CNP37 ("Pro-CNP38") was Produced from TAF-Pro-CNP38 Inclusion Bodies by Formic Acid Cleavage

Formic acid is used as a denaturant for inclusion body proteins and can specifically cleave the peptide bond between Asp and Pro under optimized conditions. TAF-Pro-CNP inclusion bodies were partially purified as described above and treated with 50% formic acid at 25° C., 37° C., 42° C. and 55° C. for 24 hours. Most of the TAF-Pro-CNP38 was cleaved and one extra band with a similar size as the Gly-CNP37 ("CNP38") peptide showed on SDS-PAGE from the 37° C., 42° C. and 55° C. cleavages (FIG. **4**A). The cleavage reactions at 37° C. and 55° C. were neutralized with 10 M NaOH and centrifuged at 14,000 rpm for 15 minutes. The un-cleaved TAF-Pro-CNP38, TAF and other inclusion bodies precipitated in the pellets. The supernatants contained soluble Pro-CNP38 and were further analyzed by LC/MS. The LC/MS

result showed the supernatants contained a mixture of non-specific cleaved peptides generated by excess acidic hydrolysis.

When TAF-Pro-CNP inclusion bodies were treated with 2% and 10% formic acid at 55° C. for 20 hours, the majority of TAF-Pro-CNP38 was cleaved and one extra band having a similar size as Gly-CNP37 was observed on SDS-PAGE (FIG. **4**B). The cleaved sample was further analyzed by LC/MS. The LC/MS analysis showed that correct Pro-CNP38 was released in soluble form from TAF-Pro-CNP38 inclusion bodies after formic acid cleavage. The yields of Pro-CNP38 from 2% and 10% formic acid cleavages were similar (FIG. **4**C).

Formic Acid Cleavage and Neutralization for Pro-Gly-CNP37 ("Pro-CNP38") Production and Purification

Formic acid can dissolve and cleave TAF-Pro-CNP38, BMP-Pro-CNP38 and other inclusion bodies. The pH neutralization results in precipitation of insoluble contaminating proteins/peptides (un-cleaved TAF-Pro-CNP38 and BMP-Pro-CNP38, TAF, BMP and others). The soluble Pro-CNP38 stays in the supernatant after centrifugation. TAF-Pro-CNP38 and BMP-Pro-CNP38 were cleaved in 2% formic acid at 55° C. or 70° C. for 24 hours. The cleavage reactions were neutralized with 1:1 ratio of 0.5 M Tris buffer and centrifuged at 14,000 rpm for 15 minutes. Results showed that the supernatants contained almost pure peptides and there was no observed recovery loss of Pro-CNP38 upon neutralization. This is a simple and efficient step for Pro-CNP38 purification.

Analysis of Varying Conditions for Formic Acid Cleavage of TAF-Pro-CNP38 Inclusion Bodies for Pro-Gly-CNP37 ("Pro-CNP38") Production

Formic acid can specifically cleave the peptide bond between Asp and Pro under optimized conditions. Non-specific cleavage of peptide bonds between Asp and any other amino acids or even non-specific cleavage of any peptide bond may occur if the formic acid cleavage conditions are not optimized. TAF-Pro-CNP38 inclusion bodies were cleaved with 2% formic acid at 42° C., 55° C. or 70° C. for 6, 24 or 48 hours. FIG. **5**A shows that TAF-Pro-CNP38 was cleaved completely at 70° C. for 24 hours or at 55° C. for 48 hours. The 70° C. cleavage could be completed within 17 hours (FIG. **5**B). Although the 70° C./24 h cleavage gave the highest yield, the non-specific cleavage products (for example, the peptide with molecular weight of 3142 generated from Pro-CNP38 by cleaving between peptide bond Asp and Arg) increased dramatically (FIG. **5**C).

The yield and purity of Pro-CNP38 production were improved when TAF-Pro-CNP38 inclusion bodies were purified or treated with B-PER II buffer before formic acid cleavage. Because B-PER II buffer contains detergent octylthioglucoside and is relatively expensive, other commonly used detergents or buffers were tested for large-scale Pro-CNP38 production. TAF-Pro-CNP38 inclusion bodies were re-suspended in different detergents (Octylsucrose; Triton x-100; Tween-20; NP-40; CA-630) or buffers (B-PER II; B-PER II 1/20 dilution; B-PER; B-PER phosphate buffer; 25 mM tris, 150 mM NaCl, pH 7.9; 25 mM tris, pH 7.5; filtered water; PBS) and incubated at room temperature (RT) for 24 hours. All detergents were 1% in 25 mM tris buffer, pH 7.5. After incubation in detergent or buffer, TAF-Pro-CNP38 inclusion bodies were cleaved by 2% formic acid at 55° C. for 22 hours. Results showed that BPER II continued to exhibit good yield, and positive results were also obtained with CA630 and Triton X-100.

Pro-Gly-CNP37 ("Pro-CNP38") Proteolytic Cleavage Products

One unidentified protease, possibly a membrane associated protease, cleaved the Pro-CNP38 peptide (produced from BL21 strain, MW 4102) into two peptides during Pro-CNP38 purification, resulting in peptides PGQEHPNAR (MW 1004) (SEQ ID NO: 198) and KYKGANKKGLSKGCFGLKLDRIGSMSGLGC (MW 3115) (SEQ ID NO: 199). Not to be bound by theory, one possible reason why detergents may improve the yield and purity of Pro-CNP38 is that detergents can remove most, but possibly not all, of the unidentified protease. High temperature, basic pH and EDTA were tested to identify if these agents could inhibit the protease cleavage. TAF-Pro-CNP38 inclusion bodies were incubated at RT or 120° C. for 2 hrs and centrifuged at 14000 rpm for 15 min. The pellets were re-suspended in 2% formic acid and incubated at 55° C. or 70° C. for 18 hours. A 1:1 ratio of 0.5 M Tris was added to neutralize the cleavage. The neutralized samples were centrifuged at 14000 rpm for 5 min and the supernatants were left at RT for 6 hrs or 22 hrs with or without 10 mM EDTA, pH 10. The proteolytic cleavage was assayed by LC/MS (Table 8).

TABLE 8

LC/MS result of Pro-CNP38 proteolytic cleavage

Sample		A210 Peak Area	Protein Conc. (mg/mL)		Percent MW4102	Percent MW3115
1	H 55 C.	942	0.04	*	91.8	8.2
2	H 70 0	2878	0.11	*	79.2	20.7
3	H 70 6H	2675	0.10	*	80.6	19.4
4	H 70 24	2741	0.11	*	79.2	20.7
5	H 70 EDTA	2385	0.09	*	80.8	19.2
6	H 70 pH 10	1917	0.07	*	81.2	18.8
7	55 C.	1291	0.05		61.1	38.9
8	70 C. 0	4533	0.18		96.8	3.2
9	70 C. 6H	4120	0.16		97.5	2.5
10	70 C. 24	4108	0.16		96.5	3.5
11	70 C. EDTA	4336	0.17		96.9	3.1
12	70 C. pH 10	3425	0.13		97.5	2.4

All samples (8-12) cleaved at 70° C. showed limited proteolytic cleavage (less than 4% cleavage of Pro-CNP38). Almost 40% of Pro-CNP38 was cleaved by protease when cleavage was carried out at 55° C. (sample 7). Basic pH and EDTA did not influence the non-specific proteolytic cleavage. High temperature (120° C. for 2 hrs) non-specifically cleaved Pro-CNP38.

It should be noted that the BL21(DE3) strain from Stratagene does not have the unidentified protease.

Production of Pro-Gly-CNP37 ("Pro-CNP38") and Other CNP Variants from Different Constructs:

Pro-CNP38 can be produced in large scale in *E. coli* by means of overexpression of a TAF-Pro-CNP38 fusion protein as inclusion bodies followed by formic acid cleavage of the fusion protein. Following the methods described herein, other TAF-CNP fusion proteins (TAF-CNP34 and TAF-Pro-CNP53) were expressed as inclusion bodies and then cleaved with formic acid to generate the CNP variants CNP34 and Pro-CNP53. FIG. **6** depicts the expression of TAF-CNP34, FIG. **7** the expression of TAF-Pro-CNP53, FIG. **8** the products of formic acid cleavage of TAF-CNP34 and TAF-Pro-CNP53, FIG. **9** the peak for CNP-34 in an LC/MS chromatogram, and FIG. **10** the peak for Pro-CNP53 in an LC/MS chromatogram.

Use of formic acid may result in unspecific cleavage(s) at peptide bond(s) other than the targeted Asp-Pro bond. To

improve the purity and overall titer of the desired formic acid cleavage product, different residues of aspartic acid in TAF12 or fragments thereof were changed to glutamic acid. Moreover, one or more cysteine residues in TAF12 or fragments thereof were changed to alanine to prevent unspecific disulfide bond formation. All TAF-Pro-CNP38 fusion proteins having such mutations in TAF12 were expressed as inclusion bodies and cleaved with formic acid to produce Pro-CNP38. FIG. **7** shows the expression of TAF-NL-(C/A & 6D/6E)-Pro-CNP38 and TAF(C/A & 10D/10E)-Pro-CNP38, FIG. **11** the expression of TAF(C/A & 4D/4E)-Pro-CNP38 and TAF(4D/4E)-Pro-CNP38, FIG. **12** the products of formic acid cleavage of TAF(4D/4E)-Pro-CNP38 and TAF(C/A & 4D/4E)-Pro-CNP38, and FIG. **13** the products of formic acid cleavage of TAF-NL-(C/A & 6D/6E)-Pro-CNP38 and TAF(C/A & 10D/10E)-Pro-CNP38. Table 9 summarizes the purity (prior to purification) and titer of Pro-CNP38 obtained from the various TAF-Pro-CNP38 constructs.

TABLE 9

Construct	Purity	Titer (ug/mL)
pJexpress-TAF-Pro-CNP38	32%	44
pJexpress-TAF(C/A)-Pro-CNP38	41%	50
pJexpress-TAF(4D/4E)-Pro-CNP38	36%	52
pJexpress-TAF(C/A & 4D/4E)-Pro-CNP38	42%	58
pJexpress-TAF(C/A & 10D/10E)-Pro-CNP38*	32%	26
pJexpress-TAF-NL-(C/A & 6D/6E)-Pro-CNP38	50%	55

*Cells with pJexpress-TAF(C/A&10D/10E)-Pro-CNP38 growed more slowly and the final cell density (OD_{600}) was lower compared to other TAF-Pro-CNP38 constructs.

Large-scale Production of Pro-Gly-CNP37 ("Pro-CNP38") by Fermentation and Formic Acid Cleavage

BL21(DE3) cells comprising the pJexpress-TAF-CNP construct were grown in a 10 liter fermenter at 37° C. for about 16-17 hours until OD_{600} reached 64. The cells were then grown/cultured in the presence of 1 mM IPTG at about 35-37° C., to induce expression of the TAF-Pro-CNP38 fusion protein, for about 7-8 hours until OD_{600} reached 160. The fermentation produced a titer of 9 g/L TAF-Pro-CNP38. FIG. **14** displays a Western blot of the TAF-Pro-CNP38 fusion protein produced in the fermentation.

A cell pellet recovered from 750 mL cell culture from the 10 L fermentation was resuspended in phosphate-buffered saline, pH 7.4 (PBS) and lysed by three passes through a pressure homogenizer (10,000 bar). The resulting lysate was centrifuged at 6,500 g for 10 minutes and the supernatant was discarded. The pellet fraction containing insoluble TAF-Pro-CNP38 fusion protein inclusion bodies was resuspended in 500 mL PBS with a rotostator. The suspension was centrifuged at 6,500 g for 10 minutes and the supernatant was discarded. The resulting inclusion body pellet was resuspended in 500 mL water with a rotostator and incubated at 55° C. for 30 minutes. 250 mL 6% formic acid was added to the warmed inclusion body suspension for a final concentration of 2% formic acid and incubated at 55° C. for 20-24 hours. 50 mL of 400 mM Na_2HPO_4 was added after 20-24 hours to begin the neutralization of the formic acid cleavage reaction, and the resulting mixture was titrated with 50% w/v NaOH to pH 6.9-7.4 and left at room temperature for 30 minutes. Upon neutralization, a heavy precipitate formed and was removed by centrifugation at 6,500 g for 10 minutes. The supernatant was retained and contained on average 1.3 g/L culture of 80% pure Pro-CNP38. The majority of the *E. coli* and TAF-related proteins and peptides remained in the pellet.

The soluble Pro-CNP38 resulting from the formic acid cleavage and neutralized supernatant was 80% pure and contained a mixture of linear and cyclized Pro-CNP38 peptides along with other product-related impurities. The pH neutral supernatant containing Pro-CNP38 was sterile-filtered. Further purification by anion-exchange chromatography using a Fractogel TMAE Hi-CAP column (EMD Biosciences) to remove DNA, endotoxins, and peptide contaminants, which should bind to the column, was performed at pH 7-7.4. The flow-through fraction contained partially cyclized Pro-CNP38. Cupric sulfate was added to the flow-through fraction to a final concentration of 10 uM and incubated at room temperature for 1 hour. The addition of Cu^{++} at neutral pH catalyzed the oxidation of free cysteine sulfhydryl groups on the peptide to form an intramolecular disulfide bond, resulting in 100% cyclized Pro-CNP38 and no detectable linear peptide. The conductivity of the solution was adjusted to <15 mS/cm by the addition of water. Cation-exchange chromatography using an SP-Sepharose column (GE Healthcare) and a sodium phosphate buffer (pH 7) was then performed to remove any remaining DNA and endotoxins in the flow-through and further purify Pro-CNP38 to about 95-96% purity with <0.5% non-product related impurities. FIG. **15** is an SDS-PAGE of eluate fractions from the SP-Sepharose column; the highest concentrations of Pro-CNP38 were found in fractions 22 to 30. Reverse-phase HPLC/MS analysis of non-pooled fraction 24 indicated the presence of 90% Pro-CNP38, 5% Pro-CNP38 with an oxidized methionine residue, 3% Pro-CNP38 cleaved at the Gly-Cys bond to form CNP-17, and 1.6% Pro-CNP38 cleaved at the Asp-Arg bond in the cyclic domain. The yield of pure Pro-CNP38 peptide upon final purification was 0.9 g/L cell culture (36% total recovery).

Pro-CNP38 collected from five separate purifications was pooled for formulation. The pooled product contained 93.5% Pro-CNP38, 3.3% Pro-CNP38 with an oxidized methionine residue, 1.3% deamidated Pro-CNP38, and 1% Pro-CNP38 cleaved at the Gly-Cys bond to form CNP-17. Samples were diluted with 50 mM sodium phosphate (pH 7) to a conductivity of 10 mS/cm and loaded onto a CM-Sepharose column (GE-Healthcare) for concentration and buffer exchange. The weak cation-exchange property of the CM-Sepharose resin allows peptides to dissociate from the column with weak acid solutions rather than typical salt gradients. Acid concentrations required for dissociation of Pro-CNP38 from the CM-Sepharose column depended upon column loading. At 50 mg Pro-CNP38 per mL of resin, 10 mM HCl was sufficient for Pro-CNP38 elution. At 9 mg Pro-CNP38 per mL of resin, 50 mM HCl was required for elution. When the loading was 9 mg Pro-CNP38 per mL of resin, Pro-CNP38 eluted in less than one column volume, which significantly concentrated the peptide. The eluate fraction contained 20.3 mg/mL of 95% pure Pro-CNP38, along with 3% Pro-CNP38 with an oxidized methionine residue, 1% deamidated Pro-CNP38, and <1% Pro-CNP38 cleaved at the Gly-Cys bond to form CNP-17. The concentrated solution of Pro-CNP38 in weak acid is suitable for dilution into appropriate buffers for either liquid or lyophilized formulations.

Example 3

Cleavage of CNP Variants by Neutral Endopeptidase In Vitro

To determine the effects of amino acid substitutions, amino acid extensions, backbone modifications, side chain modifications and PEGylation on the susceptibility of CNP variants to neutral endopeptidase (NEP) cleavage, peptide cleavage assays were carried out using an in vitro assay that monitored disappearance of the non-cleaved CNP variant.

Recombinant human NEP (1 ug/mL final concentration) was added to 100 uM CNP variant diluted in 0.1 M Tris, pH 7. The reaction mixture was incubated at 37° C. for various periods of time, and the reaction was quenched with EDTA (10 mM final) followed by heat denaturation. The reaction mixture was reduced and then the reaction products were analyzed using HPLC and mass spectroscopy. The half-life of the CNP variant was calculated based on the disappearance of intact CNP variant over time. The results for digested CNP variants were compared to a parallel wtCNP22 digestion and normalized to the results for 100 uM CNP22 digested by 1 mg/mL NEP ($t_{1/2}$=80 min).

Table 1 lists the half-lives, based on the in vitro NEP cleavage assay, of various CNP variants having backbone or side chain modifications. Removal of three of the six NEP cleavage sites in Analog L nevertheless resulted in a substantially shorter half-life. Of tested CNP variants, the greatest resistance to NEP cleavage was exhibited by Analog N, which contains the D-enantiomer of all 22 amino acids of CNP22, and by Analog M, which has an N-methylated amide bond at both Leu9 and Leu11. However, both Analogs N and M failed to stimulate production of cGMP (see below).

Comparing the half-lives of Analogs A, B, E, F, G and H to one another, half-lives were determined to be about 1.5- to about 2.5-fold longer for Analogs E and G compared to those for Analogs A, B, F and H. All of these six analogs showed resistance or improved resistance to cleavage at the Cys6-Phe7 bond relative to wtCNP22 (data not shown). The rank order of analog resistance to NEP at 1 ug/ml, based on half-life, is Analog G (3-Cl-Phe)≥Analog E (D-Phe)>Analog H ("beta-2 Phe"), Analog B (N-Me-Phe), and Analog F (t-Bu-Gly)=wtCNP22>Analog A (Cys-CH_2—NH). Analogs E and G have about 1.5 times longer half-life in comparison to wtCNP22. Besides resistance to cleavage of the Cys6-Phe7 bond, Analogs B, E, F, G and H also exhibited resistance to cleavage of the Gly8-Leu9 bond in the presence of 1 ug/mL NEP (data not shown). These results indicate that CNP variants having backbone or side chain modifications between Cys6 and Gly8 can be resistant to NEP cleavage of the Cys6-Phe7 bond and/or Gly8-Leu9 bond, but do not necessarily have improved overall resistance to NEP or a longer half-life than CNP22. The results seem to be contrary to reports in the literature that NEP first cleaves at the Cys6-Phe7 bond of CNP22 and then elsewhere.

TABLE 1

Analog	Backbone and Side Chain Modifications / Natriuretic Peptide	cGMP Response rel. to 1 uM CNP22[1] 10 nM	cGMP Response rel. to 1 uM CNP22[1] 1 uM	NEP Cleavage ($t_{1/2}$, min)
	CNP22 (SEQ ID NO: 1)	46 ± 10	100 ± 13	80[2]
N	D-CNP22 (all D-amino acids) (SEQ ID NO: 115)	2	1	>>160
A	CNP22, C6—CH2—NH (reduced carbonyl) (SEQ ID NO: 56)	6	66	55
B	CNP22, N-methyl-F7 (methylated amide) (SEQ ID NO: 57)	2	38	80
BD	CNP22, N-methyl-L9 (SEQ ID NO: 116)	2	8	ND
BN	CNP22, N-methyl-L11 (SEQ ID NO: 117)	10	51	ND
BE	CNP22, N-methyl-L20 (SEQ ID NO: 118)	2	5	ND
M	CNP22, N-methyl-L9, N-methyl-L11 (SEQ ID NO: 94)	1	11	>>160
K	CNP22, N-methyl-L9, N-methyl-L20 (SEQ ID NO: 92)	1	1	80
L	CNP22, N-methyl-L9, N-methyl-L11, N-methyl-L20 (SEQ ID NO: 93)	18	10	30
J	CNP22, C6—CH2—NH, N-methyl-L9, N-methyl-L20 (SEQ ID NO: 91)	ND	ND	50
E	CNP22, D-F7 (D-Phe) (SEQ ID NO: 136)	2	6	130
H	CNP22, Beta-2-F7 (3-amino-2-phenylpropionyl) (SEQ ID NO: 57)	2	2	80
G	CNP22, 3-chloro-F7 (SEQ ID NO: 137)	17	93	135
F	CNP22, t-butyl-G8 (SEQ ID NO: 58)	2	18	80
V	CNP22, K4G, 3,4-dichloro-F7 (SEQ ID NO: 119)	ND	ND	68
X	CNP22, K4G, 3-methyl-F7 (SEQ ID NO: 120)	ND	ND	68
	ANP	10	23	ND

[1]Stimulation of cGMP production in NIH3T3 cells by natriuretic peptide relative to cGMP production in the presence of 1 uM CNP22

[2]CNP22 NEP resistance $t_{1/2}$ averaged 80 min. Due to variations in NEP catalytic activity between experiments, all CNP22 $t_{1/2}$ digestions were normalized to 80 min and the difference coefficient was used to calculate analog $t_{1/2}$ in each experiment to obtain an adjusted $t_{1/2}$.

ND = Not Determined

Table 2 lists the half-lives, based on the in vitro NEP cleavage assay, of various CNP variants having substitutions with natural and/or unnatural amino acids. Of tested variants, the greatest resistance to NEP cleavage was shown by Analog BK, which has K4R and G15S substitutions, and Analog BJ, which has K4R and G15N substitutions.

TABLE 2

Analog	Specificity Mutations Natriuretic Peptide	cGMP Response relative to 1 uM CNP22[1] 10 nM	1uM	NEP Cleavage ($t_{1/2}$, min)
	CNP22 (SEQ ID NO: 1)	46 ± 10	100 ± 13	80
AH	CNP22, K4R (SEQ ID NO: 35)	59	121	80
BP	CNP22, K4R, G5S (SEQ ID NO: 121)	45	ND	ND
BO	CNP22, K4R, G5R (SEQ ID NO: 122)	18	80	ND
P	CNP22, K4G (SEQ ID NO: 123)	ND	ND	68
Z	CNP22, K4R, F7Y (SEQ ID NO: 95)	2	18	ND
AB	CNP22, K4R, G8S (SEQ ID NO: 97)	26 ± 26	86 ± 17	ND
AA	CNP22, K4R, G8V (SEQ ID NO: 96)	3	25	ND
AC	CNP22, K4R, G8T (SEQ ID NO: 98)	11 ± 2	66 ± 16	80
AD	CNP22, K4R, L9T (SEQ ID NO: 99)	4	68	ND
BH	CNP22, K4R, K10R (SEQ ID NO: 112)	12	80	ND
BF	CNP22, K4R, K10Cit (SEQ ID NO: 110)	6	33	ND
BG	CNP22, K4R, K10Q (SEQ ID NO: 111)	9	45	ND
BY	CNP22, K4R, K10S (SEQ ID NO: 124)	16	53	ND
BK	CNP22, K4R, G15S (SEQ ID NO: 114)	13 ± 1	71 ± 11	≥160
BJ	CNP22, K4R, G15N (SEQ ID NO: 113)	4	41	150
AE	CNP22, K4R, G15R (SEQ ID NO: 100)	0.3	0.3	ND
AF	CNP22, K4R, G15Cit (SEQ ID NO: 101)	1.4	2	ND
BZ	CNP22, K4R, S16Q (SEQ ID NO: 125)	42	116	ND
BX	CNP22, K4R, M17N (SEQ ID NO: 126)	40 ± 2	103 ± 17	ND
AG	CNP22, K4R, M17V (SEQ ID NO: 102)	10	65	ND
BQ	CNP22, K4R, G19S (SEQ ID NO: 127)	21	63	ND
BR	CNP22, K4R, G19R (SEQ ID NO: 128)	22 ± 6	84 ± 10	ND
AJ	CNP22, K4R, L20V (SEQ ID NO: 103)	0.2	8	ND
AK	CNP22, K4R, L20t-butyl-Ala (SEQ ID NO: 104)	1	21	ND
AT	CNP22, G1E, K4E (SEQ ID NO: 105)	11	54	60
BS	CNP22, K4R, L20R (SEQ ID NO: 129)	11	8	ND
BT	CNP22, K4R, G21S (SEQ ID NO: 130)	7	39	ND
BU	CNP22, K4R, G21T (SEQ ID NO: 131)	6	21	ND
BW	CNP22, K4R, G21R (SEQ ID NO: 132)	20	21	ND
	ANP	10	23	ND

[1]Stimulation of cGMP production in NIH3T3 cells by natriuretic peptide relative to cGMP production in the presence of 1 uM CNP22
[2]CNP22 NEP resistance $t_{1/2}$ averaged 80 min. Due to variations in NEP catalytic activity between experiments, all CNP22 $t_{1/2}$ digestions were normalized to 80 min and the difference coefficient was used to calculate analog $t_{1/2}$ in each experiment to obtain an adjusted $t_{1/2}$.
ND = Not Determined

Table 3 lists the half-lives, based on the in vitro NEP cleavage assay, of CNP variants having N-terminal and/or C-terminal modifications, including amino acid extensions. Of the analogs tested, Analogs AZ, CC, CF, BL, CS, CK and CL, Pro-Gly-CNP37 and HSA-CNP27 were most resistant to NEP degradation.

TABLE 3

	N- and C-Terminal Modifications Natriuretic Peptide	cGMP Response rel. to 1 uM CNP22[1] 10 nM	1 uM	NEP Cleavage ($t_{1/2}$, min)
	CNP22	46 ± 10	100 ± 13	80
BC	Pentanoic acid (N-term.)-CNP22, G1E (SEQ ID NO: 109)	ND	ND	ND
BB	Heptanoic acid (N-term.)-CNP22, G1E (SEQ ID NO: 108)	32 ± 4	84 ± 19	45-65
AV	Pentanoic acid (N-term.)-CNP22, G1E, K4E (SEQ ID NO: 106)	ND	ND	120
AW	Heptanoic acid (N-term.)-CNP22, G1E, K4E (SEQ ID NO: 107)	ND	ND	<20
AX	CNP17 (delta N-term) (SEQ ID NO: 2)	18	69	ND
	R-CNP22 (SEQ ID NO: 40)	ND	ND	ND
AZ	R-CNP22, K4R (SEQ ID NO: 41)	54 ± 11	106 ± 15	≥160
	ER-CNP22 (SEQ ID NO: 38)	ND	ND	ND

TABLE 3-continued

	N- and C-Terminal Modifications	cGMP Response rel. to 1 uM CNP22[1]		NEP Cleavage
	Natriuretic Peptide	10 nM	1 uM	($t_{1/2}$, min)
BA	ER-CNP22, K4R (SEQ ID NO: 39)	38 ± 10	113 ± 10	90
	GANRR-CNP22 (SEQ ID NO: 65)	ND	ND	ND
AY	GANRR-CNP22, K4R (SEQ ID NO: 36)	59 ± 9	105 ± 20	65
	GANQQ-CNP22 (SEQ ID NO: 64)	ND	ND	ND
CH	GANQQ-CNP22, K4R (SEQ ID NO: 69)	44 ± 8	95 ± 11	ND
	GANPR-CNP22 (SEQ ID NO: 66)	ND	ND	ND
CI	GANPR-CNP22, K4R (SEQ ID NO: 37)	50 ± 1	105 ± 12	ND
	GANSS-CNP22 (SEQ ID NO: 67)	ND	ND	ND
CG	GANSS-CNP22, K4R (SEQ ID NO: 70)	27 ± 1	88 ± 1	95
CA	AAWARLLQEHPNA-CNP22 (SEQ ID NO: 61)	24	76	ND
CB	AAWARLLQEHPNAR-CNP22 (SEQ ID NO: 62)	36	84	ND
CC	DLRVDTKSRAAWAR-CNP22 (SEQ ID NO: 63)	34	101	>160
CF	GQPREPQVYTLPPS-CNP22 (IgG1(Fc) fragment) (SEQ ID NO: 79)	23 ± 9	72 ± 19	>160
	PNARKYKGANKK-CNP22 (CNP34)	ND	ND	ND
BL	QEHPNARKYKGANKK-CNP22 (CNP37) (SEQ ID NO: 60)	43 ± 15	97 ± 27	>>160
	PQEHPNARKYKGANKK-CNP22 (Pro-CNP37)	ND	ND	ND
CE	GERAFKAWAVARLSQ-CNP22 (HSA fragment) (SEQ ID NO: 81)	15	87	ND
CY	GQHKDDNPNLPRGANPR-CNP22 (HSA fragment) (SEQ ID NO: 80)	ND	ND	ND
CQ	GHHSHEQHPHGANQQ-CNP22 (HRGP fragment) (SEQ ID NO: 76)	16	95	ND
CX	GHHSHEQHPHGANPR-CNP22 (HRGP fragment) (SEQ ID NO: 78)	ND	ND	ND
CS	GQEHPNARKYKGANPK-CNP22 (modified CNP37) (SEQ ID NO: 129)	19	61	>>160
CT	GQEHPNARKYKGANQK-CNP22 (modified CNP37) (SEQ ID NO: 130)	60	121	ND
CU	GQEHPNARKYKGANQQ-CNP22 (modified CNP37) (SEQ ID NO: 131)	9	57	ND
DB	GQEHPNARKYKGANKK-CNP22 (Gly-CNP37) (SEQ ID NO: 75)	50 ± 14	98 ± 17	>>160
	PGQEHPNARKYKGANKK-CNP22 (Pro-Gly-CNP37)	49 ± 6	103 ± 17	>>160
CW	GQEHPNARKYKGANKP-CNP22 (modified CNP37) (SEQ ID NO: 74)	ND	ND	ND
CR	GAHHPHEHDTHGANQQ-CNP22 (HRGP fragment) (SEQ ID NO: 128)	14 ± 5	77 ± 12	ND
CZ	FGIPMDRIGRNPR-CNP22 (osteocrin "NPR-C inhibitor") (SEQ ID NO: 82)	ND	ND	ND
DA	GKRTGQYKLGSKTGPGPK-CNP22 (FGF2 "heparin-binding domain" fragment) (SEQ ID NO: 83)	ND	ND	ND
CK	GQPREPQVYTGANQQ-CNP22, K4R (IgG1(Fc) fragment) (SEQ ID NO: 84)	2	32	≥160
CL	GVPQVSTSTGANQQ-CNP22, K4R (HSA fragment) (SEQ ID NO: 85)	3	35	>160

TABLE 3-continued

	N- and C-Terminal Modifications	cGMP Response rel. to 1 uM CNP22[1]		NEP Cleavage
	Natriuretic Peptide	10 nM	1 uM	($t_{1/2}$, min)
	GHKSEVAHRFKGANKK-CNP22 (HSA-CNP27) (SEQ ID NO: 144)	51 ± 9	109 ± 15	>>160
	PGHKSEVAHRFKGANKK-CNP22 (Pro-HSA-CNP27)	32	107	ND
CN	GQTHSSGTQSGANQQ-CNP22, K4R (fibrinogen) (SEQ ID NO: 87)	12	115	ND
CM	GQPSSSSQSTGANQQ-CNP22, K4R (fibronectin) (SEQ ID NO: 86)	ND	ND	ND
CO	GSTGQWHSESGANQQ-CNP22, K4R (fibrinogen) (SEQ ID NO: 88)	2	33	ND
CP	GSSSSSSSSSGANQQ-CNP22, K4R (zinc finger) (SEQ ID NO: 89)	ND	ND	ND
CD	SPKMVOGSG-CNP17-KVLRRH ("BNP tails") (SEQ ID NO: 68)	25	102	ND
CJ	RSSCFGGRIDRIGAC ("C-ANP4-23", ANP-derived) (SEQ ID NO: 133)	ND	ND	ND
	CNP22, K4R, K10R, N.-term.--N-term. dimer/ disuccinimidyl glutarate (SEQ ID NO: 134)	19	44	ND
	CNP22, K4R, K10R, N-term.--N.-term. dimer/Bis-PEO5 (SEQ ID NO: 135)	19	41	ND
BM	CNP53 (SEQ ID NO: 4)	61	101	>>160
	ANP	10	23	ND

[1]Stimulation of cGMP production in N1H3T3 cells by natriuretic peptide relative to cGMP production in the presence of 1 uM CNP22
[2]CNP22 NEP resistance $t_{1/2}$ averaged 80 min. Due to variations in NEP catalytic activity between experiments, all CNP22 $t_{1/2}$ digestions were normalized to 80 min and the difference coefficient was used to calculate analog $t_{1/2}$ in each experiment to obtain an adjusted $t_{1/2}$.
ND = Not Determined

Table 4 lists the half-lives, based on the in vitro NEP cleavage assay, of CNP variants conjugated to PEG (or PEO) polymers at the N-terminus. All the PEGylated CNP variants tested and shown in Table 4 displayed resistance or enhanced resistance to NEP cleavage except for PEO12-GANPR-CNP22(K4R), which had the same half-life as wtCNP22. N-terminal PEGylation of CNP22 having a K4G substitution does not seem to confer substantial improvement in NEP resistance. For example, PEG2K-CNP22(K4G) was only slightly more resistant to NEP cleavage than CNP22 (data not shown), whereas PEG2K-CNP22 had a much longer half-life in vitro than CNP22.

TABLE 4

N-Terminal PEGylation		cGMP Response rel. to 1 uM CNP22[1]		NEP Cleavage
Natriuretic Peptide	PEG	10 nM	1uM	($t_{1/2}$, min)
CNP22		46 ± 10	100 ± 13	80
CNP22	PEG20K	0	15	>>160
CNP22	PEG5K	8 ± 1	20 ± 7	>>160
CNP22	PEG2K	6 ± 2	32 ± 4	>>160
CNP22	PEO4-$(PEO12)_3$ (branched)	17 ± 1	52 ± 6	>>160
CNP22	PEO24 (1.2 kDa)	8 ± 5	46 ± 10	>>160
CNP22	PEG1K	15 ± 3	68 ± 17	>160
CNP22	PEO12 (0.6 kDa)	12 ± 7	57 ± 18	160
CNP22	(PEO12)-Biotin	19	81	140
CNP22, K4G	(PEO12)-Biotin	10	27	100
CNP22, K4R	PEO24	15	56	ND
CNP22, K4R	PEO12	13	44	ND
CNP-17	PEG2K	5	50	>160
R-CNP22, K4R (SEQ ID NO: 41)	PEO24	15 ± 2	75 ± 12	ND
R-CNP22, K4R (SEQ ID NO: 41)	PEO12	23 ± 2	93 ± 19	≥160
ER-CNP22, K4R (SEQ ID NO: 39)	PEO24	6 ± 2	60 ± 10	ND
ER-CNP22, K4R (SEQ ID NO: 39)	PEO12	20 ± 1	92 ± 25	ND

TABLE 4-continued

N-Terminal PEGylation		cGMP Response rel. to 1 uM CNP22[1]		NEP Cleavage
Natriuretic Peptide	PEG	10 nM	1uM	($t_{1/2}$, min)
GANRR-CNP22, K4R (SEQ ID NO: 36)	PEG2K	15 ± 2	45 ± 18	ND
GANRR-CNP22, K4R (SEQ ID NO: 36)	PEO24	28 ± 9	82 ± 18	>>160
GANRR-CNP22, K4R (SEQ ID NO: 36)	PEG1K	15 ± 0.4	56 ± 23	>160
GANRR-CNP22, K4R (SEQ ID NO: 36)	PEO12	40 ± 2	99 ± 13	>160
GANQQ-CNP22, K4R (SEQ ID NO: 69)	PEO24	16 ± 13	73 ± 30	ND
GANQQ-CNP22, K4R (SEQ ID NO: 69)	PEO12	30	78	ND
GANPR-CNP22, K4R (SEQ ID NO: 37)	PEO24	ND	ND	ND
GANPR-CNP22, K4R (SEQ ID NO: 37)	PEO12	ND	ND	80
GANSS-CNP22, K4R (SEQ ID NO: 70)	PEO24	8 ± 5	46 ± 21	ND
GANSS-CNP22, K4R (SEQ ID NO: 70)	PEO12	8 ± 0.3	52 ± 13	ND

[1]Stimulation of cGMP production in NIH3T3 cells by natriuretic peptide relative to cGMP production in the presence of 1 uM CNP22
[2]CNP22 NEP resistance $t_{1/2}$ averaged 80 min. Due to variations in NEP catalytic activity between experiments, all CNP22 $t_{1/2}$ digestions were normalized to 80 min and the difference coefficient was used to calculate analog $t_{1/2}$ in each experiment to obtain an adjusted $t_{1/2}$.
ND = Not Determined

FIG. **16** shows the NEP resistance profile of five N-terminal PEGylated conjugates of CNP22. The CNP22 peptides conjugated to PEG (or PEO) polymers of increasing mass exhibited increasing resistance to NEP degradation. In particular, PEO24-CNP22, PEG2K-CNP22 and PEG5K-CNP22 were resistant to NEP degradation over the assay period of 160 minutes.

FIG. **17** displays the NEP resistance profile of CNP variants CNP37 (Analog BL), CNP53 and GANRR-CNP22 (K4R) (SEQ ID NO: 36) having an N-terminal amino acid extension. As can be clearly seen, both CNP37 and CNP53 were resistant to NEP degradation in this in vitro assay, whereas GANRR-CNP22(K4R) (SEQ ID NO: 36) had the same lability to NEP hydrolysis as CNP22.

FIG. **18** depicts the NEP resistance profile of CNP17 and GANRR-CNP22(K4R) (SEQ ID NO: 36) conjugated to a PEG (or PEO) moiety at the N-terminus. PEGylation of GANRR-CNP22(K4R) (SEQ ID NO: 36) greatly improved the NEP resistance of this CNP variant, with PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) being completely resistant to NEP cleavage over the assay period of 160 minutes. Increasing the mass of the PEO moiety from about 0.6 kDa (PEO12) to about 1.2 kDa (PEO24) improved the NEP resistance of PEGylated GANRR-CNP22(K4R) (SEQ ID NO: 36). PEGylation of GANRR-CNP22(K4R) (SEQ ID NO: 36) to a monodispersed PEO24 moiety rather than a polydispersed PEG1K moiety also improved NEP resistance. Finally, although both PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) and PEG2K-CNP17 have a similar total mass (keeping in mind that PEG2K is polydispersed), the former displayed substantially better NEP resistance.

NEP resistance assays were also performed on wtCNP22 and CNP variants G-CNP37, GHKSEVAHRFK-wtCNP27 ("CNP27-HSA", SEQ ID NO: 144) and PEO12-GANRR-CNP22(K4R) ("CNP27-PEO12") (SEQ ID NO: 36). FIG. **19** shows that G-CNP37 and CNP27-HSA were completely resistant to NEP cleavage, and CNP27-PEO12 exhibited much greater stability to NEP degradation compared to wtCNP22.

Example 4

CNP Variant Stimulation of cGMP Production in NIH3T3 Cells

To determine the functional activity of CNP variants, the production of cGMP was measured in NIH3T3 cells exposed to the CNP variants. Murine NIH3T3 cells express endogenously the CNP signaling receptor, NPR-B, which shares 98% protein sequence identity with human NPR-B. NIH3T3 cells were cultured in high glucose Dulbecco's Modified Eagle Medium supplemented with 10% fetal calf serum and antibiotics at 37° C. with 5% CO). Twenty four to 48 hours prior to signaling, cells were passaged to 12-well plates with a density of $2–5\times10^5$ cells per well at the time of the assay. CNP variants were resuspended in 1 mM HCl to a stock concentration of 1 mg/mL (455 uM for wtCNP22) and subsequently diluted to a 30 uM working stock solution with phosphate-buffered saline (PBS). Ten-fold serial dilutions were prepared in phosphate-buffered saline. Culture medium was removed from the cells and replaced with 0.4 mL PBS/Dulbecco's modified Eagle medium (50/50, v/v) containing 0.75 mM isobutylmethylxanine. Plates were incubated at 37° C., 5% CO_2 for 15 minutes before addition of 0.2 mL CNP variant in PBS and continued incubation at 37° C. for 15 minutes. Reactions were stopped by the addition of 0.2 mL lysis buffer supplied with the CatchPoint cGMP assay kit (Molecular Devices), and cGMP production was determined with the CatchPoint cGMP Assay (Molecular Devices). All stimulation experiments were performed in duplicate.

Tables 1-4 summarize the ability of CNP variants having backbone or side chain modifications, amino acid substitutions, N-terminal amino acid extensions, and/or N-terminal PEGylation, respectively, to stimulate cGMP production in NIH3T3 cells. In all four tables, the values for cGMP production in NIH3T3 cells exposed to 10 nM or 1 uM CNP variant are normalized to cell number and cGMP production in the presence of 1 uM wtCNP22.

Regarding the results in Table 1, only Analog G having 3-Cl-Phe at position 7 displayed substantially the same NPR-B stimulation activity at 1 uM as wtCNP22. With respect to Table 2, various CNP variants with amino acid substitutions, including Analogs AH, BO, AB, BH, BZ, BX and BR, showed substantially similar NPR-B stimulation activity as wtCNP22.

Considering the results in Table 3, many CNP variants having N-terminal and/or C-terminal modifications, including amino acid extensions, exhibited comparable NPR-B stimulation activity as wtCNP22. The functional CNP variants include Analog BB, which is CNP22(G1E) attached to heptanoic acid at the N-terminus, and Analog CD, which is the cyclic domain of CNP22 ("CNP17" retaining the Cys6 to Cys22 sequence) conjugated to the N-terminal and C-terminal "tails" of BNP. FIG. **20** illustrates that GANRR-CNP22

(K4R) (SEQ ID NO: 36), CNP37 (Analog BL) and CNP53 all had similar NPR-B stimulation activity as wtCNP22 in the in vitro assay.

Of note from Table 3 is that among the CNP variants assayed for both CNP functionality and NEP resistance, Analog AZ (R-CNP22(K4R)), Analog CC, Analog CF, Analog BL (CNP37), Analog DB (Gly-CNP37) and GHKSEVAHRFK-CNP27 (HSA-CNP27) (SEQ ID NO: 144) all had substantially similar NPR-B stimulation activity as CNP22 while being substantially more resistant to NEP cleavage than CNP22.

With regard to the results in Table 4, nine N-terminal PEGylated CNP variants at 1 uM stimulated cGMP production to at least about 70% of the level achieved by wtCNP22. Several noteworthy aspects appear in Table 4. First, N-terminal PEGylation of GANRR-CNP22(K4R) (SEQ ID NO: 36) with a monodispersed PEO polymer (PEO12 is about 0.6 kDa, PEO24 about 1.2 kDa) resulted in better NPR-B functionality than that with a polydispersed PEG polymer (PEG1K has a polymer number average molecular weight (M_n) of around 1 kDa, PEG2K around 2 kDa) (see also FIG. **21**). Second, N-terminal PEGylation of wtCNP22 with a polydispersed PEG polymer of increasing M_n (PEG1K, PEG2K, PEG5K and PEG20K) or with a monodispersed PEO polymer of greater mass (PEO12 and PEO24) correspondingly decreased the NPR-B activation ability of the CNP variants (see also FIG. **22**). Third, PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36), having the N-terminal GANRR (SEQ ID NO: 8) extension, stimulated greater cGMP production than PEO24-CNP22 and PEO24-CNP22 (K4R). Also of note is that among the N-terminal PEGylated CNP variants assayed for both CNP functionality and NEP resistance, PEO12-R-CNP22(K4R), PEO12-GANRR-CNP22(K4R) (SEQ ID NO: 36) and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) all had substantially similar NPR-B stimulation activity as CNP22 while being much more resistant to NEP degradation than CNP22.

Among the CNP variants listed in Tables 1-4 and assayed for both CNP functionality and NEP resistance, Analogs G, BK, AZ, CC, CF, BL and DB, Pro-Gly-CNP37, HSA-CNP27 (GHKSEVAHRFK-CNP27) (SEQ ID NO: 144), PEG1K-CNP22, (PEO12)-biotin-CNP22, PEO12-R-CNP22(K4R), PEO12-GANRR-CNP22(K4R) (SEQ ID NO: 36), and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) all had substantially similar NPR-B stimulation activity as wtCNP22 while being substantially more resistant to NEP cleavage than wtCNP22.

cGMP production assays were also carried out on wtCNP22 and CNP variants G-CNP37, GHKSEVAHRFK-wtCNP27 ("CNP27-HSA", SEQ ID NO: 144), wtCNP29 and PEO12-GANRR-CNP22(K4R) ("CNP27-PEO12") (SEQ ID NO: 36). FIG. **23** shows that CNP22 and all the CNP variants assayed induced production of similar cGMP levels at either low or high dose of CNP.

Example 5

Binding Specificity for NPR-A, NPR-B and NPR-C

Signaling Competition Assay

To determine the binding specificity of CNP variants for the clearance receptor NPR-C, a signaling competition assay is carried out. Expression plasmids for human NPR-B or NPR-C (each purchased from OriGene) are transiently transfected and receptors are expressed in HEK293T cells. Forty hours after transfection, NPR-B, NPR-C and native HEK293T cells are harvested, counted and plated at a ratio of 1:1 (NPR-B cells:competing cells (either NPR-C or native HEK293T cells)) in 12-well or 96-well plates. Twenty hours after plating, cells are processed for the NPR-B/cGMP stimulation assay described in Example 4. If present, the natriuretic clearance receptor, NPR-C, is expected to bind and internalize CNP, thereby reducing the overall CNP concentration available for signaling through NPR-B, resulting in decreased cGMP production and a shift in the dose-response curve to the right. A rightward shift in the dose-response curve has been verified for wtCNP22. CNP variants having reduced affinity for NPR-C are not expected to induce a shift, or are expected to induce a smaller shift, in the dose-response curve to the right. This signaling competition assay is similar to that previously described by Cunningham (U.S. Pat. No. 5,846,932; B. Cunningham, EMBO J. 13(11): 2508-2515 (1994); H. Jin et al., J. Clin. Invest. 98(4): 969-976 (1996)).

The cGMP stimulation activity of wtCNP-22, Pro-Gly-wtCNP37 and ANP through NPR-B and NPR-A, and their selectivity for NPR-B vs. NPR-C and for NPR-A vs. NPR-C, were evaluated in signaling competition assays. NPR-A, NPR-B and NPR-C individually were transiently tranfected into HEK293T cells. Thirty hours after transfection, the cells were plated into 96-well plates: (A) 20,000 NPR-B cells+20,000 mock transfected cells; (B) 20,000 NPR-B cells+20,000 NPR-C cells; (C) 20,000 NPR-A cells+20,000 mock transfected cells; and (D) 20,000 NPR-A cells+20,000 NPR-C cells. Twenty hours after plating, the culture media was removed and replaced with serum-free media:PBS (1:1)+ 0.75 uM IBMX for 15 minutes. For cGMP signaling through NPR-B, dose series for ANP, CNP-22 and Pro-Gly-CNP37 were added and incubated at 37° C. for 12 minutes before the assay was stopped by cell lysis. For cGMP signaling through NPR-A, dose series for CNP-22 and Pro-Gly-CNP37 were incubated at 37° C. for 12 minutes, while dose series for ANP were incubated at 37° C. for 6 minutes (because NPR-A appeared to be a "faster" guanylyl cyclase than NPR-B, the incubation time for ANP was shortened in order not to max out (use up all cellular GTP) too soon when signaling with ANP). FIGS. **24**A and B show that CNP-22 and Pro-Gly-CNP37 ("Pro-CNP38") stimulated cGMP production through NPR-B with similar dose-response curves, and to a much greater extent than through NPR-A, and exhibited a similar profile for NPR-B vs. NPR-C selectivity in the signaling competition assays.

Determination of Binding Affinities (K_i) for NPR-A, NPR-B and NPR-C

The binding affinities (K_i) of CNP variants for NPR-A, NPR-B and NPR-C are determined in a heterologous competition binding assay (U.S. Pat. No. 5,846,932; B. Cunningham, EMBO J. 13(11): 2508-2515 (1994); H. Jin et al., J. Clin. Invest. 98(4): 969-976 (1996)). Membranes from HEK293 cells, or another suitably transfectable cell line (e.g., HeLa cells), expressing human NPR-A, NPR-B or NPR-C are prepared for radio-labeled ligand binding assays. Membrane preparations are diluted in an appropriate buffer and varying concentrations of wtCNP22 or CNP variant (competitor) are added with I^{125}-labeled wtCNP22 (Bachem). Samples are incubated at room temperature to allow for ligand/receptor equilibration and bound peptide is separated from free peptide by filtration through PVDF filter membranes. Filters are washed before the addition of scintillant and counting by a scintillation counter. Binding is measured in duplicate for each concentration of competitor peptide. CNP variant affinity (K_i, equilibrium dissociation constant) and B_{max}, (receptor number) are calculated by non-linear regression analysis and/or the Cheng-Prusoff equation.

CNP variants exhibiting reduced affinity to NPR-C are expected to have reduced susceptibility to clearance by NPR-C and thus a longer plasma or serum half-life. Increased half-life of the CNP variants in circulation would increase the availability of the variants for therapeutic activity.

Example 6

Effect of CNP Variants on the Growth of Rat Chondrosarcoma (RCS) Cells and cGMP Production in RCS Cells

To assess the ability of CNP variants to stimulate bone growth, skeletal dysplasia is simulated in cell culture by treating rat chondrosarcoma (RCS) cells with fibroblast growth factor 2 (FGF-2), which activates fibroblast growth factor receptor 3 (FGFR-3) and induces growth arrest (Krejci et al., J. Cell Sci., 118(21):5089-5100 (2005)).

Optimal CNP treatment parameters are determined by varying CNP concentration (0.05, 0.1, 0.2 and 0.5 uM), and treatment duration and interval (continuous; 2.5 min, 10 min, 30 min, 1 hr, 2 hr, 4 hr and 8 hr once a day; 2.5 min, 10 min, 30 min, 1 hr, 2 hr and 4 hr twice a day). After 72 hours, cells are counted using an automated cell counter, and the amount of extracellular matrix is estimated using alcian blue staining.

RCS cells are then treated with a CNP variant using the optimal conditions determined from the growth experiments with wtCNP22. The concentration of cGMP is measured by competitive ELISA for untreated RCS cells, RCS cells treated with CNP, and RCS cells treated with the CNP variant. Cell growth and matrix synthesis resulting from treatment with the CNP variant are also monitored and compared to those resulting from CNP treatment.

To assess the effect of CNP variants in a human cell culture system, primary re-differentiated human chondrocytes in alignate beads are treated with wtCNP22 and CNP variants, and cGMP concentration is determined by competitive ELISA as a measure of effective CNP signaling.

The methods described herein can be employed to assess the ability of CNP variants to stimulate cGMP production in and growth of rat chondrosarcoma cells in vitro.

Example 7

Dose Response Study in Rat Chondrosarcoma Cells

The tyrosine kinase receptor fibroblast growth factor receptor 3 (FGFR-3), a negative regulator of chondrocyte growth, is contitutively on in achondroplasia subjects. Stimulation of the FGFR-3 receptor with FGF-2 causes growth arrest by prolonged activation of Erk MAPK, and causes decreased matrix synthesis and loss of matrix, as well as a change in cell shape. Continuous exposure of rat chondrosarcoma (RCS) cells to fibroblast growth factor 2 (FGF-2) simulates achondroplasia in cell culture by activating FGFR-3 and inducing growth arrest (Krejci et al., J. Cell Sci., 118(21): 5089-5100 (2005)). To determine the dose of CNP variant and frequency of dosing that stimulate sufficient growth of bone cells, a dose response study was performed using the RCS cell assay as described in Example 6.

RCS cells were seeded at 10×10^3 cells per well in 24-well plates, grown for 24 hr, treated for 72 hr, and then counted. RCS cells were continuously exposed to FGF-2 (5 ng/mL) to simulate a constitutively active FGFR-3, which induced cell growth arrest (see bar #5 in FIG. **25**). Wild-type CNP22 (0.2 uM) was cultured continuously (72 hr), 1 hr daily or 2 hr daily. All stimulants were changed daily Continuous exposure of RCS cells to 0.2 uM CNP22 in the presence of 5.0 ng/mL FGF-2 partially reversed FGF2-induced growth arrest, leading to the growth of approximately 200×10^3 cells per well (bar #6 in FIG. **25**), compared to approximately 100×10^3 cells per well in the absence of CNP22 (bar #5 in FIG. **25**).

Both 1 hr exposure to CNP22 (0.2 uM) once a day and 2 hr exposure to CNP22 (0.2 uM) once a day achieved about 84% of the effect of continuous CNP22 (0.2 uM) exposure on chondrocyte growth (bars #7 and 8 in FIG. **25**). These results demonstrate that continuous exposure of growth-arrested chondrocytes to CNP22 is not required for reversal of cell growth arrest. Additionally, dose response studies demonstrate that lower doses of CNP22 are capable of reversing growth arrest (FIG. **26**A).

Furthermore, histological and cell morphological analysis of the extracellular matrix showed that CNP22 treatment antagonized FGF2-mediated loss of chondrosarcoma extracellular matrix and increased matrix synthesis. Exposure to FGF-2 decreased matrix synthesis and increased degradation, while addition of CNP22 to the FGF-2 cell culture increased matrix synthesis and partially inhibited FGF-2, as assessed by ^{35}S-sulfate and ^{3}H-Pro incorporation into, or decrease from, matrix (FIGS. **27**A-D). Analysis of aggrecan and fibronectin production (mRNA and protein) in RCS cells cultured with FGF-2 and CNP22 shows that FGF-2 decreased aggrecan level and increased fibronectin level, which was inhibited by addition of CNP22 (FIGS. **28**A-C). FGF-2 induces and activates matrix-processing molecules predominantly via Erk, and addition of CNP22 shows some effect on this activation.

Additional high-throughput assays for measuring growth arrest, such as crystal violet staining, are useful for measuring the effects of CNP22 and variants thereof on RCS cells.

Similar dose response studies can be conducted with the CNP variants described herein to determine their effective dose for reversing FGF2-induced growth arrest of RCS cells.

Example 8A

Ex Vivo Stimulation of Growth of Tibia and Femur from Mice with Mild Achondroplasia

Mouse tibial organ culture model has been used to demonstrate the efficacy of wild-type CNP22 in stimulating longitudinal bone growth. Treatment of wild-type tibiae with CNP22 at 10^{-8}, 10^{-7} or 10^{-6} M for 6 days increased longitudinal growth by 31%, 40% and 42%, respectively. Histological evaluation also showed expansion of the hypertrophic zone, e.g., an increase in the number and size of hypertrophic chondrocytes in the growth plate (Agoston et al., BMC Dev. Biol. 7:18 (2007)). Similar findings were observed in tibiae isolated from FGFR3^{Ach} mice (Yasoda et al., Nat. Med. 10: 80-86 (2004)).

To determine the efficacy of CNP variants in stimulating longitudinal bone growth, CNP variants were tested in a mouse organ culture model of endochondral bone growth in wild-type mice and transgenic mice having a G380R mutation in the human FGFR-3 gene (FGFR3$^{wt/Ach}$ heterozygote) which represent a mouse model of mild achondroplasia. In brief, the pharmacological activity of wild-type CNP22 and CNP variants was compared in an organ culture model of embryonic or neonatal mouse tibiae, isolated from wild-type and FGFR3$^{wt/Ach}$ littermates. Overall bone growth and histological changes within the growth plate were assessed. Conditioned culture medium is also assessed for biomarkers of intracellular signaling (cGMP), cartilage metabolism (type II collagen, other collagens, aggrecan chondroitin sulfate), bone metabolism (bone alkaline phosphatase, osteocalcin, type I collagen [C-telopeptide, N-telopeptide]), and inflammation (interleukin-1, interleukin 6, interleukin-11).

Effective CNP variants are identified by their ability, e.g., to stimulate production of cGMP, and bone growth as measured by increased longitudinal bone length and expansion of the cells in the hypertrophic zone of the growth plate.

Measurement of Bone Growth

The efficacy of wtCNP22, CNP37 and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) in stimulating longitudinal femoral growth was evaluated in the mouse organ culture model. For these experiments femora were isolated from 2-3 day old wild-type mice and cultured in alphaMEM supple-

mented with 0.2% BSA, 0.5 mM L-glutamine, 40 units penicillin/mL and 40 ug streptomycin/mL, for 8 days in the presence of vehicle, CNP22 or CNP variants. The treatment commenced at day 0 and was repeated every two days thereafter, as the medium was changed. Bones were measured prior to treatment and every two days thereafter, using a dissection microscope fitted with a 1 cm eye-piece reticule. Conditioned medium was used for biomarker analysis. At day 8 bones were fixed in 4% paraformaldehyde for 24 hr, decalcified in 5% formic acid for 24 hrs, dehydrated and embedded in paraffin. Bones were sectioned at 5 um (microns), which were then deparaffinized, rehydrated, and stained with Alcian Blue for 30 min (pH 2.5; MasterTech). Alcian Blue stains cartilage blue. Stained sections were visualized and photographed by brightfield microscopy. The thickness of the hypertrophic region of the growth plate cartilage was determined by image analysis.

FIG. **29** illustrates the effect of wtCNP22, CNP37 and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) on longitudinal growth of 3-day old wild-type mouse femurs treated with the CNP peptides every two days. The results were normalized to measurements prior to treatment (day 0). The studies were performed in triplicate (vehicle) or quadruplicate (CNP peptides). As shown in FIG. **29**, CNP37 and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36), as well as CNP22, were effective in stimulating longitudinal femoral growth, with the N-terminal PEGylated CNP variant being the most effective.

The growth of wild-type and FGFR3^{ach} mouse femur and tibia in response to CNP22, CNP37 and PEO24-GANRR-CNP22(K4R) ("CNP27-PEO24") (SEQ ID NO: 36) was also assessed. Culture of either wild-type or achondroplastic (FGFR3^{ach}) mouse tibia showed that CNP37 and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) both increased the longitudinal growth of the tibia compared to vehicle or CNP22 (FIGS. **30** and **31**). CNP22, CNP37 and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) also stimulated the growth of wild-type mouse femur (FIG. **32**). Moreover, each of CNP22, PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) and CNP37 increased the longitudinal growth of FGFR3^{ach} mouse femur compared to vehicle (FIG. **33**).

Furthermore, ex vivo distribution of CNP37 in the growth plate of FGFR3^{ach} mouse tibia was evaluated. Bone samples were prepared as described above. Paraffin sections were cut and heat fixed for 1 hour at 60° C. Antigen retrieval with 1% hyaluronidase at 37° C. (30 minutes) was followed by a 1 hour serum block (10% Normal Goat Serum). CNP22 antibody (1:500 dilution; Peninsula Laboratories Inc., San Carlos, Calif.) was applied overnight at 4° C. For immunodetection, Vectastain ELITE ABC kit (Vector, Burlingame, Calif.) was used according to the manufacturer's recommendations. Specific bound peroxidase was visualized by incubation with DAB substrate kit (Vector) and the reaction was developed for 3 minutes. Slides were then dehydrated and mounted and photographed using a brightfield microscope. Staining for CNP in the growth plate of FGFR3^{ach} mouse tibia showed that CNP immunoreactivity was increased in the regions of articular and hypertrophic chondrocytes (FIG. **34**), indicating that CNP37 was delivered to the chondrocytes.

In addition to the distribution of CNP variants in the bone growth plate, the ex vivo effects of CNP37, CNP22 and vehicle on cells in the FGFR3^{ach} and wild-type growth plate, e.g., hypertrophic cell size and cellularity of proliferating zone, were also evaluated. Bone samples were prepared, including Alcian Blue staining, as described above. Images of the entire proximal growth plate were taken at 4× magnitude. The growth plate is divided into three zones, starting from the epiphyseal side of cartilage: the resting zone (individual small chondrocytes), proliferating zone (columns of stacked chondrocytes parallel to the long axis of the bone), and hypertrophic zone (large chondrocytes and thin septa between the chondrocytes). In these regions, measurements were made by ImageJ software, including the number of proliferating chondrocytes per column and the density of hypertrophic chondrocytes. A test square (4×4 mm^2) at five different regions of the hypertrophic zone was used to determine the density of hypertrophic chondrocytes. The cell size of hypertrophic chondrocytes was calculated by 1 over determined cell density. Cellularity of proliferating columns was increased by CNP37 and CNP22 in both wild-type and FGFR3^{ach} mice (FIGS. **35**B and C). Chondrocyte hypertrophy in FGFR3^{ach} mice was also increased as a result of culture with CNP22 or CNP37 (FIGS. **36**B and C).

Ex vivo studies of cultures of mouse bones indicated that CNP37 was delivered to the growth plate and was able to increase chondrocyte cellularity and hypertrophy, which are associated with growth plate expansion and longitudinal bone growth. To assess the biodistribution of CNP37 in the bone growth plate in vivo and the in vivo effects of CNP37 on the growth plate (including total growth plate thickness, hypertrophic zone thickness, and cellularity of proliferating zone), bone samples were obtained from FGFR3^{ach} mice treated with vehicle or CNP37 as described above. For biodistribution and in vivo effects studies, tibias were fixed and stored in 70% ethanol. For immunohistochemistry, the samples were decalcified in 5% formic acid for 2 days, dehydrated and embedded in paraffin. Bones were sectioned at 5 um (microns), which were then deparaffinized, rehydrated, and used for CNP immunohistochemistry as described above. For cellular image analysis, bones were sectioned at 5 (microns), and then deparaffinized, rehydrated, and stained with Alcian Blue for 30 minutes (pH 2.5; MasterTech) and Hematoxylin & Eosin for 30 seconds. Stained sections were visualized and photographed by brightfield microscopy. Thicknesses of the growth plate and the proliferating and hypertrophic zones were measured using ImageJ software.

In vivo biodistribution studies demonstrated that, similar to the ex vivo studies, CNP immunoreactivity was increased in the regions of articular and hypertrophic chondrocytes in the tibia growth plate of FGFR3^{ach} mice treated with CNP37, indicating that CNP37 was delivered in vivo to the growth plate of FGFR3^{ach} mouse tibia (FIG. **37**). Furthermore, CNP37 treatment significantly increased the total growth plate thickness, proliferating zone thickness and hypertrophic zone thickness of FGFR3^{ach} mouse tibia in vivo (FIGS. **38**A-C).

These results demonstrate that CNP variants of the disclosure penetrate into the growth plate of wild-type and achondroplastic animals, increase the number and size of chondrocytes, increase the thickness of the proliferating zone and the hypertrophic zone of the growth plate, and increase longitudinal bone growth in treated wild-type and achondroplasic animals. Therefore, the CNP variants are useful for stimulating bone growth in achondroplastic subjects.

Measurement of Biomarkers

In addition to measurement of bone growth in response to CNP variants, assay of the levels of biomarkers for cartilage and bone formation and growth induced in response to CNP variants is useful for evaluating the effect of CNP variants on bone growth.

Femurs and tibias were isolated from wild-type and FGFR3^{ach} mice as described above. Bones were cultured with CNP22 or a variant thereof for eight days with the replacement of media every two days. On the eighth day, media was

collected and analyzed for the biomarkers cGMP (cyclic guanosine 3', 5' cyclic monophosphate) and fragments of cleaved collagen type II, a cartilage-specific marker for cartilage turnover. Both markers were measured using commercially available enzyme-linked immunosorbent assays (ELISA) for cGMP (Cayman Chemical Co., Ann Arbor, Mich.) and cleaved collagen type II (Cartilaps) (Immunodiagnostic Systems, Fountain Hills, Ariz.), following the manufacturer's protocol.

Levels of cGMP and collagen type II fragments were measured from cell culture extracts after exposure to CNP22, CNP37 or PEO24-GANRR-CNP22(K4R) ("CNP27-PEO24") (SEQ ID NO: 36). FIGS. **39-42** show a large increase ($p<0.01$) in the levels of cGMP in the media after exposure of ex-planted wild-type and $FGFR3^{ach}$ mouse femurs and tibias to CNP22, CNP37 or PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36). In addition, exposure of wild-type and $FGFR3^{ach}$ mouse femurs to CNP22, CNP37 or PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) increased the levels of cleaved collagen type II, with the treated $FGFR3^{ach}$ mouse femurs showing significantly increased ($p<0.05$) levels of collagen type II fragments (FIG. **43**). Elevated levels of collagen type II fragments indicate a turnover of the cartilage matrix, and cartilage turnover typically precedes new bone formation in growing bones.

Example 8B

Ex Vivo Stimulation of Growth of Femur from Mice with Severe Achondroplasia

The effect of a CNP variant on the growth of bones from mice with severe achondroplasia was evaluated ex vivo. Used in the study were transgenic mice expressing a human FGFR-3gene having a Y367C mutation ($FGFR3^{Y367C}$) [S. Pannier et al., Biochim. Biophys. Acta, 1792(2): 140-147 (2009)], which represent a mouse model of severe achondroplasia. Femurs were isolated at embryonic day 16.5 and cultured for 6 days in the presence of 1 uM Pro-Gly-CNP37. Bone lengths were measured at Day 1 and Day 7. The bones were then paraffin-embedded, sectioned and stained with hematoxylin and eosin to assess histological changes and cellular morphology. Treatment of bone explants isolated from $FGFR3^{Y367C}$ mice with Pro-Gly-CNP37 ("ProCNP38") resulted in increase in bone growth and expansion in the growth plate (FIG. **44**). Femurs from $FGFR3^{Y367C}$ mice treated with vehicle for 6 days showed an 18% deficiency in growth length-wise compared to wild-type femurs treated with vehicle. Treatment of femurs from $FGFR3^{Y367c}$ mice with 1 uM Pro-Gly-CNP37 for 6 days reduced the growth deficiency to just 11%, i.e., reduced the growth defect by around 40%.

Example 9

Serum/Plasma Stability of CNP Variants In Vitro

In preparation for pharmacokinetics (PK) studies, the stability of CNP variants in serum and/or plasma is evaluated.

Briefly, the analyte is isolated by the removal of serum or plasma proteins by either a 2% trichloroacetic acid precipitation or a 1:3 serum:acetonitrile precipitation. The precipitation mixture is vortexed at 14,000 rpm for five minutes, and a portion of the supernatant is removed and diluted with water prior to transfer to a silanized autosampler vial for analysis. Serum extracts are then analyzed by reverse-phase high performance liquid chromatography (RP-HPLC) with electrospray ionization mass spectrometry (ESI-MS). A single mass (m/z), shown to be specific for the CNP variant, is monitored for quantitation purposes.

Initially, analytical stability and recovery is determined. Analytical (RP-HPLC and ESI-MS) parameters are optimized through the analysis of matrix standards (serum extracts fortified with analyte post-precipitation). After optimization, analytical recovery is determined by spiking serum samples at known concentrations and comparison of the analyte response to that of matrix standards prepared at similar concentrations. Analyte stability in serum extracts is also determined to assure no significant losses occur after serum precipitation and prior to actual analysis. To test the effect of freezing on serum stability, a two-cycle freeze/thaw study is also performed. In this study a serum sample is spiked with CNP variant and analyzed prior to freezing overnight at −20° C. The sample is then thawed at room temperature and re-analyzed. The process is repeated for a second freeze/thaw cycle.

Serum stability of CNP variant is determined by spiking of serum/plasma samples with CNP variant at a concentration of 10 ug/mL. The sample is placed in a 37° C. water bath for a period of three hours. At 30 minute intervals duplicate aliquots of serum are removed and analyzed. If rapid losses of analyte are evident (>50% in 30 minutes), the study may be repeated with 10 minute timepoints.

In an exemplary method for determining the stability of CNP variants in murine plasma, a mixture of CNP variant (10 uL of a stock solution of about 2.5-5.0 mg/mL), heparinized murine plasma (50 uL, Bioreclamation, CD-1 Lith Hep 62231), and 5 M NaCl (10 uL) is incubated at 37° C. and 5% CO_2 for 0-5 hr, and then quenched with 10× protease inhibitor cocktail (15 uL, Sigma P2714). For extraction, 150 uL of MeOH/0.1% FA is added to 85 uL of the reaction mixture, and the resulting mixture is vortexed for 1 min and then centrifuged at 15° C. for 15 min. 75 uL of the supernatant is added to 300 uL of aqueous 0.1% FA. A small portion of the resulting mixture is subjected to analysis by LC/MS.

Example 10

Pharmacokinetics and cGMP Production in Rats and Mice

Studies were conducted in normal rats to evaluate the pharmacokinetics (PK) profile of CNP22 and certain CNP variants and the time courses of plasma cGMP concentration after single intravenous (i.v.) or subcutaneous (s.c.) administration of the CNP peptides. Plasma CNP immunoreactivity was determined by using a competitive radioimmunoassay (RIA) with an anti-CNP rabbit polyclonal antibody. Plasma cGMP concentration was determined by RIA using a commercially available kit (YAMASA cyclic GMP Assay kit, YAMASA Corporation).

Normal male rats, 7-8 weeks of age, were used. Recombinant wild-type CNP22, CNP37 and PEO24-GANRR-CNP22 (K4R) (SEQ ID NO: 36) were evaluated. A dosage of 20 nmol/kg of each CNP peptide as a solution in 5% mannitol was intravenously injected once into the tail, or a dosage of 50 nmol/kg of each CNP peptide as a solution in 0.03 mol/L acetic acid buffer solution, pH 4.0, containing 1% (w/v) benzyl alcohol and 10% (w/v) sucrose, was subcutaneously injected once into the back.

Plasma CNP immunoreactivity was determined by the competitive RIA using anti-CNP rabbit polyclonal antibody. Standard and QC samples were prepared. Fifty uL of the standard, QC and assay samples were added, respectively, to

test tubes containing 50 uL of RIA buffer. Diluted anti-CNP rabbit polyclonal antibody (100 uL) was added to the tubes. All tubes were kept at 4° C. overnight. ^{125}I-[Tyr^{0}]-CNP22 solution (100 uL) and rabbit IgG solution (100 uL) were added and left at approximately 4° C. overnight. One milliliter of anti-rabbit IgG goat serum containing 10% polyethylene glycol was added, vortexed and left at approximately 4° C. for at least 1 hour, and then the insoluble fraction was precipitated by centrifugation. After aspiration of the supernatant, the amount of radiation (gamma line) in the sediment was measured by a gamma-counter. Each sample was measured in duplicate, and the mean was adopted as the value determined.

Plasma cGMP concentrations in the sample at 5, 30, 60 and 90 minutes after i.v. dosing, or at 5, 30, 60, 120 and 180 minutes after s.c. dosing, were determined by the competitive RIA using anti-cGMP monoclonal antibody. Standard samples were prepared. 100 uL of the assay samples (standard solutions for the calibration curve or the diluted plasma samples for cGMP determination) were transferred to test tubes. Then 100 uL of anti-cGMP monoclonal antibody solution and 100 uL of ^{125}I-labeled succinyl cGMP tyrosine methyl ester solution were added to the tubes, respectively. All tubes were kept at 4° C. overnight. After the addition of 500 uL dextran charcoal solution, the tubes were vortexed and then placed on ice for 10 minutes. The reaction mixture was centrifuged and 500 uL of the supernatant was transferred from each sample to a new test tube. The amount of radiation (gamma line) in the supernatant was measured by a gamma-counter. Each sample was measured in duplicate, and the mean was adopted as the value determined.

Plasma CNP immunoreactivity was employed for pharmacokinetics (PK) analysis. PK analysis was performed using WINNONLIN® Professional (Pharsight Corporation). The PK profiles of CNP22, CNP37 and PEO24-GANRR-CNP22 (K4R) (SEQ ID NO: 36) after i.v. administration were calculated using PK parameters such as concentration at 0 hour (C_0: extrapolation, pmol/mL), total body clearance (CL_{tot}: mL/min/kg), distribution volume at steady state (V_{dss}: mL/kg), area under the plasma concentration-time curve (AUC: pmol·min/mL), mean residence time (MRT: min), and half-life ($T_{1/2}$: min). The PK profiles of the CNP peptides after s.c. administration were calculated using PK parameters such as maximum plasma concentration (C_{max}: pmol/mL), time to reach C_{max} (T_{max}: min), area under the plasma concentration-time curve (AUC: pmol·min/mL), mean residence time (MRT: min), and half-life ($T_{1/2}$: min).

In plasma spike recovery experiments, the RIA detected CNP22, CNP37 and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) similarly (data not shown).

Procedures similar to those described above are employed to study in mice the PK profiles of CNP22 and variants thereof and their ability to stimulate cGMP production.

The PK profiles of CNP22, CNP37 and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) after i.v. administration in three rats are illustrated in FIG. **45**. As shown by FIG. **45**, CNP37 and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) had a much longer half-life and a much greater bioavailability than CNP22. The half-life, $T_{1/2}$ (min), was 1.42 (±0.45) for CNP22, 22.3 (±1.5) for PEO24-GANRR-CNP22 (K4R) (SEQ ID NO: 36), and 49.5 (±28.0) for CNP37. The area under the curve, AUC (pmol·min/mL), was 320 (±54) for CNP22, 1559 (±568) for CNP37, and 2084 (±424) for PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36).

The PK profiles of the three CNP peptides after s.c. administration in three rats are depicted in FIG. **46**. Compared to CNP22, PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) had a much longer half-life (78.1 min (±16.4) vs. 10.0 (±5.0)) and a much greater bioavailability (60% (±6%) vs. 19% (±9%)).

The time courses of plasma cGMP concentrations after i.v. administration of the three CNP peptides in three rats are displayed in FIG. **47**. FIG. **47** clearly demonstrates that i.v. administration of CNP37 and PEO24-GANRR-CNP22 (K4R) (SEQ ID NO: 36) resulted in much higher plasma levels of cGMP at 30, 60 and 90 minutes than i.v. administration of CNP22.

The time profiles of plasma cGMP concentrations after s.c. administration of the three CNP peptides in three rats are shown in FIG. **48**. Subcutaneous administration of PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) and CNP37 also resulted in substantially higher plasma concentrations of cGMP than s.c. administration of CNP22, with the difference relative to CNP22 increasing over time for PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36), but decreasing over time for CNP37.

The rat studies indicate that compared to wtCNP22, the CNP variants CNP37 and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) had a substantially longer half-life in vivo, had a substantially greater bioavailability in vivo, and stimulated substantially higher levels of cGMP production in vivo for an extended period of time. The resistance of CNP37 and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) to NEP degradation correlates to a longer plasma half-life in vivo, which in turn correlates to prolonged NPR-B/cGMP signaling in vivo. These results show that compared to CNP22, the CNP variants of the disclosure, administered by i.v. or s.c. injection (e.g., once daily), can be more effective in treating CNP-responsive conditions or disorders, such as bone-related disorders and vascular smooth muscle disorders.

Example 11

Pharmacokinetics Study in Mice

To determine CNP variants having increased NEP resistance, for efficacy study in FGFR3^{ach} mice (see Example 13), a pharmacokinetics (PK) study is carried out that compares the pharmacokinetics properties of CNP variants to wild-type CNP22. The FGFR3^{ach} mouse is a mutant mouse model of mild achondroplasia, containing a single transgene on a background of FVB mice.

Wild-type CNP22 or variant thereof is administered as a single intravenous (i.v.) dose in 6-week old wild-type FVB mice. Exemplary PK studies were conducted using wtCNP22. Six-week old FVB/N mice were intravenously administered wtCNP22 in a single dose at 100 nmol/kg. Mean plasma levels of CNP22 were calculated, and the estimated half-life of CNP22 was determined to be from 0.76 min to 1.03 min.

CNP variants displaying greater resistance to NEP degradation are expected to exhibit increased serum concentrations over time and a longer half-life in vivo.

Example 12

Efficacy of CNP Variants in Wild-Type Mice

The in vivo effects of CNP variants on bone growth were assessed in wild-type mice. Three week old FVB wild-type male mice received daily subcutaneous (s.c.) injection of either vehicle, G-CNP37 (200 nmol/kg) or PEO12-GANRR-

CNP22(K4R) ("CNP27-PEO12") (SEQ ID NO: 36) (200 nmol/kg) for 5 weeks. Body weight was measured at least once weekly. Tail length was measured at least once weekly using digital caliper readings, and body length (naso-anal length), bone length (tibia, femur, ulna and humerus), skull length (anterior to posterior cranial segment) and lumbar vertebrae 5 (LV5) length were measured after 5 weeks of treatment using a caliper. X-rays were taken at baseline and after 5 weeks of treatment.

Five weeks of treatment of wild-type mice with G-CNP37 resulted in significant body weight gain, with increased body weight being observed beginning at Day 9 ($p<0.05$) (FIG. **49**). Treatment with G-CNP37 also resulted in significantly increased tail length, beginning at the second week after treatment ($p<0.01$) (FIG. **50**).

Table 10 shows the percentage change in tail length, body (naso-anal) length, skull (anterior to posterior cranial segment) length, bone (femur, tibia, humerus and ulna) lengths, and lumbar vertebrae 5 (LV5) length in wild-type mice injected s.c. once daily with 200 nmol/kg of either G-CNP37 or PEO12-GANRR-CNP22(K4R) ("CNP27-PEO12") (SEQ ID NO: 36) for 5 weeks, relative to a value of 100% for wild-type mice treated with vehicle only.

TABLE 10

	Tail	Naso-Anal	Anterior to Posterior (Skull)	Femur	Tibia	Humerus	Ulna	LV5
G-CNP37	119%**	114%**	104%*	109%**	105%**	104%**	101%	108%*
CNP27-PEO12	101%	104%**	101%	102%*	100%	102%*	96%*	103%

**p < 0.01,

*p < 0.05

Treatment with G-CNP37 resulted in significantly increased tail length, body (naso-anal) length, skull length, proximal bone (femur and humerus) length, distal bone (tibia) length, and vertebral (lumbar vertebrae 5) length compared to treatment with vehicle.

Lower doses of Pro-Gly-CNP37 dosed daily s.c. at 5 nmol/kg, 20 nmol/kg or 70 nmol/kg for five weeks resulted in a dose-dependent increase in tail length, body (naso-anal) length, and bone lengths compared to vehicle. Table 11 shows the percentage change in tail length, body (naso-anal) length, and bone (femur, tibia, humerus and ulna) lengths in wild-type mice injected s.c. once daily with 5 nmol/kg, 20 nmol/kg or 70 nmol/kg Pro-Gly-CNP37 for 5 weeks, relative to a value of 100% for wild-type mice treated with vehicle only.

TABLE 11

	Tail	Naso-Anal	Femur	Tibia	Humerus	Ulna
5 nmol/kg	107.6%**	102.7%**	103.3%**	101.7%**	101.1%	100.9%*
20 nmol/kg	107.8%**	107.6%**	107.1%**	103.4%**	102.3%**	102.5%**
70 nmol/kg	113.6%**	112.5%**	109.3%**	105.9%**	103.6%**	104.1%**

**p < 0.01,

*p < 0.05

In another study, various dosing regimens of Pro-Gly-CNP37 were administered for nine weeks followed by one week of recovery. Wild-type FVB mice were dosed s.c. with:

(1) vehicle daily for nine weeks, followed by one week of recovery;

(2) 20 nmol/kg Pro-Gly-CNP37 daily for one week, followed by three doses per week for eight weeks and one week of recovery;

(3) 20 nmol/kg Pro-Gly-CNP37 on alternating weeks; or

(4) 5 nmol/kg Pro-Gly-CNP37 daily for nine weeks followed by one week of recovery.

Increased growth in tail length, body length, and bone lengths was observed in all treatment groups (Groups 2, 3 and 4) at the end of the study. Table 12 shows the percentage change in tail length, body (naso-anal) length, and bone (femur, tibia, humerus and ulna) lengths in wild-type mice administered Pro-Gly-CNP37 ("Pro-CNP38") under the dose regimens described above, relative to a value of 100% for wt mice treated with vehicle only.

TABLE 12

	Total Pro-CNP38 received (nmol/kg)	Tail	Naso-Anal	Femur	Tibia	Humerus	Ulna
Group 2	620	104.8%**	106.0%**	105.3%**	100.8%	102.5%	101.7%
Group 3	700	106.7%**	106.5%**	104.1%**	102.5%**	102.6%*	102.8%*
Group 4	350	104.9%**	105.1%**	107.3%**	102.7%**	102.3%**	103.6%**

**p < 0.01,
*p < 0.05

While all dose regimens of Pro-Gly-CNP37 increased axial and appendicular growth parameters measured, daily dosing of Pro-Gly-CNP37 promoted appendicular growth (femur, tibia, humerus, and ulna) at a lower total dose level (Group 4) compared to regimens with less frequent dosing (Groups 2 and 3).

Example 13

Efficacy in Mouse Model of Mild Achondroplasia

The efficacy of CNP variants in enhancing growth and correcting achondroplasia was tested in a mouse model of mild achondroplasia, using a strain of transgenic mice expressing a human FGFR-3 gene having a G380R mutation ($FGFR3^{ach}$) (Wang et al., Proc. Natl. Acad. Sci. USA, 96(8): 4455-4460 (1999); Naski et al., Development USA, 125: 4977-4988 (1998); U.S. Pat. Nos. 6,265,632 and 6,136,040).

At 3 weeks of age, $FGFR3^{ach}$ mice and their wild-type littermates were anesthetized to have lateral whole-body X-ray images taken by Faxitron, and randomized by body weight into the following treatment groups (n=8/group): (1) wild-type/vehicle, (2) $FGFR3^{ach}$/vehicle, (3) $FGFR3^{ach}$/CNP37, and (4) $FGFR3^{ach}$/PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36). The mice received once daily subcutaneous (s.c.) administration of designated test article (vehicle or 200 nmol/kg CNP variant) for 5 weeks. Satellite groups (n=3) were used to confirm exposure of each test article following a single subcutaneous administration on Day 1. Wild-type male FVB mice that received daily s.c. injection of vehicle for 5 weeks were used as a control for normal growth.

X-ray measurements at baseline and at the end of the study were performed to determine change in the head length, area of the skull, and the external auditory meatus (EAM, the ear canal running from the outer ear to the middle ear). Body weight and tail length were measured at least once weekly, using a digital caliper to measure tail length, and body (naso-anal) length was measured after 5 weeks of treatment. Bone (tibia, femur, ulna and humerus) lengths were measured using a digital caliper at necropsy.

On Day 37, all mice were sacrificed by terminal anesthesia and whole-animal photographs and X-ray images by Faxitron were taken. Left and right tibia, femur, humerus, and ulna were collected and measured using a digital caliper. The left portions of each bone were processed for histology, and the right portions were snap-frozen for archival. Samples obtained from the bones were used to evaluate the effects of CNP variants on endochondral bone growth.

Treatment of $FGFR3^{ach}$ mice with CNP37 by once daily s.c. injection for 5 weeks resulted in significantly increased body length (FIG. **51**), tail length (FIG. **52**), distal bone (ulna and tibia) lengths (FIGS. **53**A and B), and proximal bone (humerus and femur) lengths (FIGS. **54**A and B). Moreover, treatment with CNP37 increased the head length (FIG. **56**), area of the external auditory meatus (FIG. **57**), and spine length (FIG. **58**) via extension of the vertebral bodies. Additionally, treatment with CNP37 corrected rhizomelia (disproportion of the length of the proximal limbs) of $FGFR3^{ach}$ mice, i.e., restored proportional growth of proximal bones, as assessed by the femur:tibia ratio (FIG. **55**). Table 13 summarizes the percentage change in tail length, body (naso-anal) length, and bone (femur, tibia, humerus and ulna) lengths in $FGFR3^{ach}$ mice injected s.c. once daily with 200 nmol/kg of either CNP37 or PEO24-GANRR-CNP22(K4R) ("CNP27-PEO24") (SEQ ID NO: 36) for 5 weeks, relative to a value of 100% for $FGFR3^{ach}$ mice treated with vehicle only.

TABLE 13

	Tail	Naso-Anal	Femur	Tibia	Humerus	Ulna
CNP37	115%**	109%**	112%**	107%**	105%*	106%**
CNP27-PEO24	100%	99%	100%	102%	100%	101%

**p < 0.01,
*p < 0.05

The results of these studies show that CNP37 can stimulate spinal and long bone growth, help to correct rhizomelia by preferentially increasing the length of the femur over the tibia, and help to restore craniofacial proportions in $FGFR3^{ach}$ mice. These results indicate that CNP37 and potentially other CNP variants may be effective in correcting the symptoms of achondroplasia and treating subjects having defects in bone growth or in need of increased bone growth.

Example 14

Measurement of Biomarkers and Evaluation of Immunogenicity in Wild-Type and Achondroplastic Mice

Measurement of Biomarkers After CNP Administration

The levels of bone growth biomarkers were measured in wild-type and achondroplastic ($FGFR3^{ach}$) mice.

Transgenic FVB $FGFR3^{ach}$ mice (a mouse model of mild achondroplasia) were treated daily for 5 weeks by subcutaneous injection with either vehicle (30 mM acetic acid/acetate buffer, 1% benzyl alcohol, 10% sucrose, pH 4.0), CNP22, CNP37 or PEO24-GANRR-CNP22(K4R) ("CNP27-PEO24") (SEQ ID NO: 36) at 200 nmol/kg for each CNP compound, as described above. Plasma and serum were collected during the study. Harvested plasma was stored with 10× protease inhibitor at −80° C. until analysis. cGMP levels were measured 15 minutes post-injection on Day 36 from K2-ETDA plasma collection tubes. Fragments of cleaved collagen type II (cartilage-associated biomarkers), osteocalcin (bone-associated biomarker), and IgG (relating to immunogenicity) were measured from terminal bleed serum at the

end of the study (Day 37). cGMP was measured using a commercially available ELISA kit (Cayman Chemical Co., Cat. No. 581021.1), cleaved collagen type II (Cartilaps) was measured using a commercially available kit from Immunodiagnostic Systems (Cat. No. 3CAL4000), and osteocalcin was measured using a commercially available kit from Biomedical Technologies Inc. (Stoughton, Mass.).

FIG. **59** shows an increase in cGMP plasma levels in FGFR3^{ach} mice treated with CNP37 or PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) compared to vehicle. FIGS. **60** and **61** show that administration of CNP37 resulted in the greatest elevation of serum levels of cleaved collagen type II and osteocalcin. These results indicate that administration of the CNP peptides, in particular CNP37, to FGFR3^{ach} mice led to increased levels of bone growth markers, suggesting increased bone formation and growth in CNP peptide-treated FGFR3^{ach} mice.

For evaluation of biomarkers in wild-type mice, wild-type FVB mice were treated daily for 5 weeks by subcutaneous injection with either vehicle (30 mM acetic acid/acetate buffer, 1% benzyl alcohol, 10% sucrose, pH 4.0), 200 nmol/kg G-CNP37, 200 nmol/kg PEO12-GANRR-CNP22(K4R) ("CNP27-PEO12") (SEQ ID NO: 36), or 20 nmol/kg or 70 nmol/kg Pro-Gly-CNP37. Plasma and serum were collected during the study. Harvested plasma was stored with 10× protease inhibitor at −80° C. until analysis. cGMP levels were measured 15 minutes post-injection on Day 36 from K2-ETDA plasma collection tubes. The levels of cleaved collagen type II, bone-specific alkaline phosphatase, and IgG (relating to immunogenicity) were measured from terminal bleed serum at the end of the study (Day 37). cGMP and cleaved collagen type II (Cartilaps) were measured as described above. Bone-specific alkaline phosphatase was measured using a commercially available kit (Cusabio, Cat. No. CSB-E11914m).

Administration of G-CNP37 significantly increased ($p<0.05$) the level of cGMP (FIG. **62**) and particularly the level of fragments of cleaved collagen type II (FIG. **63**) in wild-type mice compared to vehicle. The significantly higher level of collagen type II fragments resulting from administration of G-CNP37 indicates turnover of the cartilage matrix, suggesting that G-CNP37 stimulated new bone formation in growing bones in wild-type mice.

Both doses of Pro-Gly-CNP37 ("Pro-CNP38"), 20 and 70 nmol/kg, significantly increased ($p<0.05$) plasma cGMP level 15 minutes after administration compared to wild-type mice treated with vehicle (FIG. **64**). Administration of the higher dose (70 nmol/kg) of Pro-Gly-CNP37 also significantly increased ($p<0.05$) the level of cleaved collagen type II compared to vehicle-treated mice (FIG. **65**), suggesting that the higher dose of Pro-Gly-CNP37 stimulated cartilage matrix turnover prior to new bone formation in wild-type mice. Furthermore, administration of the higher dose (70 nmol/kg) of Pro-Gly-CNP37 increased ($p<0.05$) the level of bone-specific alkaline phosphatase compared to vehicle-treated mice (FIG. **66**), suggesting that the higher dose of Pro-Gly-CNP37 increased bone remodeling in wild-type mice.

Evaluation of Immunogenicity of CNP Variants

Because the CNP variants are peptide derivatives, it is possible that administration of the peptides may lead to an immunogenic response in vivo. To assess whether an immune response occurred after successive administrations of CNP variant, measurement of serum antibody levels was performed.

An IgG assay was performed to assess whether an IgG immune response was triggered by 5 week exposure of achondroplastic FGFR3^{ach} mice to CNP22, CNP37 or PEO24-GANRR-CNP22(K4R) ("CNP27-PEO24") (SEQ ID NO: 36). IgG is the most predominant immunoglobulin in mouse and human serum and is produced as part of the secondary immune response to an antigen. The IgG response to administration of the CNP peptides was determined as follows. 96-well plates were coated with 100 ng/mL CNP22, CNP37 or PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) in BupH PBS buffer (Pierce/Thermo, Cat. No. 28372, Rockford, Ill.). After overnight incubation, plates were blocked with Casein-PBS blocking buffer (Pierce/Thermo, Cat. No. 37528) for two hours at room temperature with shaking at 300 rpm. After washing with wash buffer (1×PBS with 0.05% Tween), diluted serum samples from a terminal bleed (diluted 1:25) were added to the plate. Positive and negative controls were also loaded onto the plate. Positive control was 1:25 diluted serum (pooled from 6 individual FVB mice) with anti-CNP22 antibody added (Bachem rabbit anti-CNP22 IgG, Cat. No. T-4222) at 1:1000 dilution. Negative control was the diluted pooled serum. After two hours of incubation, the plates were washed and secondary antibody diluted in blocking buffer was added to the wells. For the mouse serum samples, anti-mouse IgG Fcγ (peroxidase-conjugated affinipure goat anti-mouse IgG, Fcγ fragment, Cat. No. 115-035-071, Jackson Immunoresearch, West Grove, Pa.) was added at a 1:10,000 dilution. For the positive and negative controls, anti-rabbit IgG-HRP (Santa Cruz Biotechnology, Cat. No. SC-2004, Santa Cruz, Calif.) was added to blocking buffer. After two hours of incubation at room temperature with shaking at 300 rpm, plates were washed with wash buffer. 100 uL of TMB (One-step TMB, Pierce/Thermo, Cat. No. 34022) was added to all wells. Plates were incubated at room temperature for 15 minutes with shaking at 300 rpm. Colorimetric reactions were stopped by the addition of 100 uL of 2 N H_2SO_4. Plates were read at 450 nm (Spectramax, Molecular Devices, Sunnyvale, Calif.) and data was analyzed using SoftMax Pro software (Molecular Devices).

Serum samples from FGFR3^{ach} mice treated with CNP22 or PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) indicated no positive IgG immune response in any of the mice. Only one out of nine CNP37-treated FGFR3^{ach} mice showed a slightly positive IgG response.

The immunogenic response of wild-type mice administered G-CNP37, PEO12-GANRR-CNP22(K4R) (SEQ ID NO: 36) or Pro-Gly-CNP37, as measured by an increase in serum IgG level, was also assessed by examining terminal bleed serum samples as described above. None of the wild-type mice treated with PEO12-GANRR-CNP22(K4R) (SEQ ID NO: 36) or Pro-Gly-CNP37 (20 or 70 nmol/kg) showed a positive IgG immune response, and only one out of six wild-type mice administered G-CNP37 showed a slightly positive IgG response.

Measurement of Biomarkers Under Different CNP Dosing Regimens

Wild-type FVB mice were treated daily for 9 weeks by subcutaneous (s.c.) injection of vehicle (30 mM acetic acid/acetate buffer, 1% benzyl alcohol, 10% sucrose, pH 4.0) or Pro-Gly-CNP37 ("Pro-CNP38") under different dosing regimens. Group 1 included vehicle-treated mice injected s.c. daily for 9 weeks followed by 1 week of no treatment. Group 2 included mice treated with Pro-Gly-CNP37 (20 nmol/kg) once daily for 1 week, followed by three doses per week for 8 weeks, followed by 1 week of no treatment. Group 3 contained mice treated with Pro-Gly-CNP37 (20 nmol/kg) once daily in alternate weeks (weeks 1, 3, 5, 7 and 9), with 1 week of no treatment following each week of treatment. Group 4 contained mice treated with Pro-Gly-CNP37 (5 nmol/kg)

once daily for 9 weeks followed by 1 week of no treatment. Finally, Group 5 included mice treated with Pro-Gly-CNP37 (5 nmol/kg) once daily for 5 weeks without any week of no treatment. Terminal bleed serum was collected from the mice, and cleaved collagen type II, total alkaline phosphatase and total antibody levels were measured therefrom. Cleaved collagen type II levels were measured as described above. Total alkaline phosphatase levels, predominantly from the liver and bones, were measured with the assistance of a veterinary diagnostic laboratory testing facility (Antech).

A total antibody assay was developed to evaluate potential immune response. The platform used for the total antibody assay was an electrochemiluminescent assay (ECLA). The ECLA platform utilizes a biotin-labeled drug (here, biotin-Pro-Gly-CNP37) and a ruthenium-labeled drug (here, Ru—Pro-Gly-CNP37). The biotin-labeled drug binds to a streptavidin-coated plate that contains electrodes, and the ruthenium-labeled drug functions as the detection component of the assay, as ruthenium can be electrochemically stimulated. A drug-specific antibody (here, a CNP-specific antibody) binds to the biotin-labeled drug and the ruthenium-labeled drug and "bridges" the two labeled drugs. Among the advantages of the ECLA platform, any isotype of antibody (IgG, IgM, etc.) can be detected, and the ECLA assay is species-independent.

The total antibody assay was performed as follows. Pro-Gly-CNP37 was labeled with biotin in a 4:1 challenge ratio, and Pro-Gly-CNP37 was also labeled separately with ruthenium in a 10:1 challenge ratio. Both separate labeling reactions were quenched by the addition of glycine, and the samples from both reactions were buffer-exchanged to PBS. Low and high QCs were prepared by using commercially available anti-CNP22 antibody (Bachem rabbit anti-CNP22 IgG, Cat. No. T-4222) added at low and high concentrations to 5% diluted FVB mouse serum. Mouse serum samples were diluted to 5% using assay diluent (5% BSA in PBS, MSD Cat. No. R93AA-1). A working solution was prepared by adding biotin-labeled Pro-Gly-CNP37 to assay diluent, then adding ruthenium-labeled Pro-Gly-CNP37 to assay diluent, and then combining the two solutions together. Twenty-five uL of low and high QCs were loaded onto the plate as controls. Then 25 uL of sample was added to a non-binding 96-well plate, followed by the addition of 50 uL of working solution to all wells. Samples and QCs were incubated with the working solution at room temperature for 2 hours with shaking at 350 rpm. In the meantime, an MSD streptavidin plate (MSD Cat. No. L13SA-1) was blocked with blocking buffer (MSD Cat. No. R93AA-1) at room temperature for 2 hours with shaking at 350 rpm. At the end of the two hour incubation, the MSD streptavidin plate was washed, and then 50 uL of sample or QC was transferred to the MSD plate. The plate was then incubated at room temperature for 1 hour with shaking at 350 rpm. At the end of the 1 hour incubation, the MSD plate was washed and 150 uL of 2× read buffer (MSD Cat. No. R92TC-2) was added to the plate. The plate was read using an MSD PR400 machine.

All five groups of mice exhibited substantially comparable levels of cleaved collagen type II (FIG. **67**). Treatment of the mice in Group 5 with 5 nmol/kg Pro-Gly-CNP37 once daily for 5 weeks significantly increased ($p<0.001$) total alkaline phosphatase level (FIG. **68**), which is indicative of bone remodeling. Serum samples from each of the four groups of mice treated with Pro-Gly-CNP37 did not show a positive antibody response in the total antibody assay.

Not to be bound by any theory, there are possible explanations as to why the four groups of CNP-treated mice did not exhibit statistically significant difference in cleaved collagen type II level compared to vehicle-treated mice, and why only the CNP-treated mice in Group 5 exhibited a statistically significant increase in total alkaline phosphatase level compared to vehicle-treated mice. It is possible that because the vehicle-treated mice in the study were also growing, cleaved collagen type II and alkaline phosphatase, which are biomarkers of growth, were also produced in the vehicle-treated mice. In addition, there was a one week period of no treatment for the mice in each of Groups 1 to 4, which possibly diluted out any changes in the cleaved collagen type II and total alkaline phosphatase levels between the CNP-treated mice in Groups 1-3 and the vehicle-treated mice. There was no period of no treatment for the CNP-treated mice in Group 5, and those mice exhibited significantly ($p<0.001$) greater total alkaline phosphatase level compared to vehicle-treated mice.

Example 15

Dose Responses of CNP Variants in Mice

The effects of varying doses of CNP variants were assessed in wild type FVB mice.

For the dose study, in two separate studies (S1 and S2), groups of 10 mice were administered Pro-Gly-wtCNP37 ("Pro-CNP38") at 20 and 70 nmol/kg subcutaneously once daily for 36 injections. Tail length and body weight were measured over the course of treatment. Animals were sacrificed at the end of the experiment and bone length assessed.

Tail length of vehicle treated animals was approximately 8 cm at day 36, whereas animals treated with 20 nmol/kg Pro-CNP38 exhibited tail length of approximately 8.75 cm and 70 nmol/kg Pro-CNP38 increased tail length to approximately 9.5 cm. Administration of Pro-CNP38 in either dose induces a significant ($p<0.05$) relative increase in total body length compared to control treated animals, demonstrating approximately a 130% increase in growth velocity at 20 nmol/kg and approximately a 160% increase in growth velocity at 70 nmol/kg Pro-CNP38 (FIG. **69**).

Treatment with Pro-CNP 38 also significantly increased bone length in most of the long bones assessed as well as total naso-anal (body length) of the animal. Table 14 is representative of the relative % increase in bone length in treated animals in comparison to vehicle treated animals.

TABLE 14

Pro-CNP38	Tail	Naso-Anal	LV4-6	Femur	Tibia	Humerus	Ulna
20 nmol/kg	8%*	7%*	10%*	7%*	4%*	3%*	3%
70 nmol/kg	16%*	13%*	13%*	10%*	7%*	4%*	5%*

Relative % increase,
*$p < 0.05$ ANNOVA (Dunnett's) v vehicle

Bone mineral density (BMD) and bone mineral content (BMC) were also evaluated after administration of Pro-CNP38 at different doses. Results (FIG. **70**) showed that administration of Pro-CNP38 at 70 nmol/kg significantly decreased bone mineral density (FIG. **70**A) and increased bone mineral content (FIG. **70**B), suggesting there is a delay in bone mineralization in treated animals, but the mineralization process itself is not adversely affected by treatment with CNP.

There were no significant changes in organ weight between Pro-CNP38 or vehicle treated animals.

Bioanalytical Studies of Dose Response in Mice

Bioanalytical studies were carried out to measure markers of CNP activity after in vivo administration of varying doses

of Pro-CNP38. Plasma cGMP levels, serum levels of collagen type II and serum levels of alkaline phosphatase from in vivo samples were analyzed. Wild type mice were administered 20 and 70 nmol/kg Gly-wtCNP37 ("CNP38"), 20 and 70 nmol/kg Pro-Gly-wtCNP37 ("Pro-CNP38") and 70 and 200 nmol/kg of GHKSEVAHRFK-wtCNP27 ("HSA-CNP27") (SEQ ID NO: 144) subcutaneously once daily for 36 days. Plasma was collected 15 minutes after the last injection on day 36 and mice were sacrified 24 hr later. At sacrifice, terminal bleed serum was collected and used for biomarker analysis as described previously.

FIG. **71** shows that 20 nmol/kg CNP38 and 70 nmol/kg HSA-CNP27 signficantly increased plasma cGMP ($p<0.01$), raising plasma cGMP levels to approximately 300 pmol and 400 pmol, respectively. Adminsitration of 70 nmol/kg CNP38 increased cGMP to approximately 500 pmol ($p<0.01$), while administration of 70 nmol/kg Pro-CNP38 increased cGMP to approximately 575 pmol ($p<0.001$). Administration of 200 nmol/kg HSA-CNP27 increased cGMP to approximately 675 pmol ($p<0.001$).

CNP variants also significantly increased serum levels of cleaved collagen type II (FIG. **72**). CNP38 at 20 nmol/kg increased collagen to approximately 9 pg/ml ($p<0.05$), CNP38 at 70 nmol/kg increased collagen to approximately 8 pg/ml ($p<0.05$), Pro-CNP38 at 20 nmol/kg increased collagen to approximately 12 pg/ml ($p<0.05$), Pro-CNP38 at 70 nmol/kg increased collagen to approximately 16 pg/ml ($p<0.05$), HSA-CNP27 at 70 nmol/kg increased collagen to approximately 10 pg/ml, and HSA-CNP27 at 200 nmol/kg increased collagen to approximately 10 pg/ml ($p<0.05$).

Serum alkaline phosphatase (AP) levels also increased after administration of CNP variants (FIG. **73**). CNP38 at 20 nmol/kg increased AP to approximately 130 IU/L, CNP38 at 70 nmol/kg increased AP to approximately 160 IU/L ($p<0.001$), Pro-CNP38 at 20 nmol/kg increased AP to approximately 155 IU/L ($p<0.001$), Pro-CNP38 at 70 nmol/kg increased AP to approximately 180 IU/L ($p<0.001$), HSA-CNP27 at 70 nmol/kg increased AP to approximately 120 IU/L, and HSA-CNP27 at 200 nmol/kg increased AP to approximately 140 IU/L ($p<0.01$). Table 15 illustrates the percent of total AP that is bone specific.

TABLE 15

	Total AP IU/L	Bone-specific AP IU/L	% of Total Alk Phos
Vehicle	109.8	24.7	22.5
CNP38 (20 nmol/kg)	135.9	47.9	35.2
CNP38 (70 nmol/kg)	166.3	63.5	38.2
Pro-CNP38 (20 nmol/kg)	159.9	65.9	41.2
Pro-CNP38 (70 nmol/kg)	183.6	78.4	42.7
HSA-CNP27 (20 nmol/kg)	121.4	51.7	42.6
HSA-CNP27 (70 nmol/kg)	136.1	76.5	56.2

Analysis of anti-CNP antibodies showed that only HSA-CNP27 elicited an IgG antibody response in mice.

The results above illustrate that administration of CNP variants increases the concentration of collagen type II and alkaline phosphatase in serum, indicating that CNP increases factors relevant for increased bone growth, and suggest that administration of CNP variants at doses as low as 20 nmol/kg are effective in increasing bone growth in vivo.

cGMP Response After Different Dose Regimens

Bioanalytical analysis was also assessed at different times after administration of Pro-Gly-wtCNP37 ("Pro-CNP38") to wild type CD-1 mice, 8-10 weeks old, (n=3 per treatment group). Pro-CNP38 was given in a single subcutaneous dose of 200 nmol/kg and levels of cGMP measured at 15 minutes, 3 hours, 1 day, 2 days and 3 days post injection in the plasma, epiphysis, cortical bone (marrow removed), lung and brain. Blood was collected on K2EDTA. Tibial and femoral epiphysis and cortical bone, ear pinna, brains, kidneys and lungs were harvested, placed in boiling water for 5 minutes, then frozen to −70° C. Both plasma and tissue were assayed for cGMP (Cayman Chemical cyclic GMP ELISA kit).

Results showed that cGMP levels increased at 15 minutes post injection in plasma (approximately 1300 pmol/ml) and epiphysis (approximately 2.5 pmol/ml/mg). By 3 hours, plasma levels had decreased approximately to control levels, while levels in the epiphysis were approximately 3 fold lower than cGMP levels at 15 minutes post injection, but higher than control levels. Levels of cortical bone increased to approximately 0.5 pmol/ml/mg at 15 minutes and remained at this level at 3 hours. Levels of cGMP at all timepoints were back to control levels by 1 day after injection. Little to no cGMP was detected in lung or brain at any timepoint.

cGMP levels were also measured in mice administered multiple injections of Pro-CNP38. Groups of mice (n=3) were given Pro-CNP38 as follows: 20 nmol/kg single dose, subcutaneously; 200 nmol/kg single dose, subcutaneously; 20 nmol/kg subcutaneously on days 0 and 1; 200 nmol/kg subcutaneously on days 0 and 1; 20 nmol/kg subcutaneously on days 0 and 3; 200 nmol/kg subcutaneously on days 0 and 3. Mice were sacrificed 15 minutes after the final dose of Pro-CNP38 and plasma levels of cGMP analyzed. There does not appear to be a modulation of the plasma cGMP signal due to the different dose regimens. cGMP responses in the cartilage are also investigated.

Evaluation of Potential Desensitization of NPR-B Receptor

Histological analysis shows that CNP, when administered daily at 200 nmol/kg, accumulates in the growth plate of animals, based on increased CNP immunoreactivity. It is possible that this accumulation, or daily stimulation of the CNP receptor, in the growth plate could desensitize the CNP receptor.

To determine if multiple dosing desensitizes the NPR-B receptor in vitro, normal human articular chondrocytes were cultured with Pro-Gly-wtCNP37 ("Pro-CNP38") for varying times and cGMP secretion measured.

Primary normal human chondrocytes isolated from articular cartilage were cultured as recommended by the supplier (Lonza). At 60-80% confluence the chondrocytes were treated with 1 uM Pro-CNP38 twice, with increasing amounts of time between treatments (first treatment at time 0, then at 15 min, 30 min, 60 min, 2 hrs, 3 hrs, 4 hrs, 6 hrs after the initial treatment). In the following experiment the treatments were either applied twice, with increasing amounts of time between treatment (first treatment at time 0, then at 6, 16, 24, 48 hrs after initial treatment) or only once, in parallel to the second treatment (only at 6, 16, 24 and 48 hrs-naïve responses). Treatments were either applied for 15 min only (acute treatment), or for the duration of the experiment (chronic treatment when CNP was left in the medium). Cell lysates and conditioned medium were collected and analyzed for total cGMP secretion (Molecular Devices ELISA).

In a short term experiment, cells were stimulated with Gly-wtCNP37 ("CNP38") 1 uM two times for 15 minutes (acute treatment), or two times with CNP38 throughout culture (chronic treatment). The periods between treatments were 15 min, 30 min, 60 min, 2 hrs, 3 hrs, 4 hrs, and 6 hrs. Peak cGMP stimulation was obtained by treating cells once only, at the last time point of the experiment, (6 hours; >0.1 pmol/well in the acute experiment and 0.2 pmol/well in the chronic experiment). In the acute experiment, when the cells

are treated twice, the response decreases to 0.1 pmol/well if the cells are treated at time 0 and then again at 15 minutes. In the acute experiment, when the cells are treated twice, the response decreases to approximately 0.5 pmol/well if the cells are treated at time 0 and again at 30 min, 60 min, 2, 3, and 4 hours. In the chronic experiment, when the cells are treated twice, the response decreases to approximately 0.16 pmol/well if the cells are treated at time 0 and at 15 minutes. In the chronic experiment, when the cells are treated twice, the response decreases to approximately 0.6 pmol/well if the cells are treated at time 0 and again at 30 min, 60 min, or 2 hours. In the chronic experiment, when the cells are treated twice, the response decreases to <0.5 pmol/well if the cells are treated at time 0 and again at 2, 4 or 6 hours.

Long term studies were also carried out. For acute treatment, 1 uM CNP38 was added to cell culture as described above. For chronic treatment, 1 uM CNP38 was added to cell culture throughout the experiment duration as described above. Results indicate that the NPR-B receptor can desensitize after repeated, daily CNP administration in vitro, and 48 hours between doses is sufficient to recover to 60% of the maximial NPR-B response to CNP38, if the CNP is removed after the first dose, and to <40% if the CNP is incubated throughout the experiment (FIG. **74**).

To evaluate whether treatment with a CNP variant desensitizes the NPR-B receptor in vivo, experiments were conducted with wild-type mice. CD-1 male mice 8-10 weeks of age were injected subcutaneously with vehicle control or Pro-Gly-wtCNP37 ("Pro-CNP38") at 200 nmol/kg on the appropriate days. The mice were either injected with Pro-CNP38 daily for up to 8 days, or were injected with Pro-CNP38 on the first day, the first and second days, or the first and third days of the study. Fifteen minutes following the final injection, the mice (n=3 per treatment group) were deeply anesthetized and ex-sanguinated via thoracotomy and aortic cannulation. Circulating blood was flushed from the body with PBS via an aortic cannula. The kidneys and right tibias, right femurs and left femurs were collected, boiled in water for 5 minutes and/or snap frozen in liquid nitrogen and stored on dry ice or in a −70° C. freezer until cGMP assay. For estimation of cGMP production in cartilage, distal femurs and/or proximal tibias were dissected, weighed and pulverized using Covaris Cryoprep CP02. Powdered samples were homogenized using a Covaris E210 sonicator in 5% perchloric acid, and neutralized in 60% KOH. Samples were then centrifuged at 10,000 rpm at 4° C. for 5 minutes, and the supernatant was used for the cGMP assay (Cyclic GMP Enzyme Immunoassay Kit, Cayman, Mich.). Additionally, left tibias were collected, fixed in 10% normal buffered formalin, and saved for further immunohistochemical analysis.

FIG. **75**A shows that repeated daily treatment of wild-type mice with 200 nmol/kg of Pro-CNP38 for 1, 4, 6, 7 and 8 days did not result in desensitization of the cGMP response. To the contrary, potentiation of the cGMP response was observed after 4 days of daily treatment. Further daily treatment resulted in a plateau of the cGMP reponse, up to 8 days. The results indicate that daily treatment of wild-type mice with 200 nmol/kg of Pro-CNP38 for up to 8 days do not desensitize the cGMP response.

The kinetics of the cGMP response in distal femoral cartilage after treatment of the wild-type mice with Pro-CNP38 was also investigated. Treatment of the mice once a day for two days potentiated the cGMP response to Pro-CNP38, compared with the cGMP response to a single treatment (FIG. **75**B). When the mice were treated on the first and third days, but not on the first and second days, the cGMP response after the second treatment (on the third day) was substantially similar to the cGMP response observed after a single treatment on day one (FIG. **75**B). The results suggest that dosing on consecutive days is beneficial for potentiating the cGMP response to Pro-CNP38 in this mouse study.

Activation of NPR-B in Different Tissues

To evaluate potential activation of NPR-B in different tissues by a CNP variant, wild-type male CD-1 mice were injected with 200 nmol/kg of Gly-CNP37, and the cGMP response was measured in certain tissues at different time points. Two mice were used for each treatment group. Each mouse received one subcutaneous injection of Gly-CNP37 or vehicle control. Fifteen, 30 or 60 minutes or 3 hours following the injection, the mice were deeply anesthetized and ex-sanguinated via thoracotomy and aortic cannulation. Circulating blood was removed by a PBS flush with an aortic cannula. Heart, liver, lung, kidney, ear pinna, aorta and brain were collected. All tissues were boiled in water for 5 minutes, finely dissected (including flushing of marrow from femoral cortices with PBS), weighed, cooled in liquid nitrogen, and pulverized in a BioPulverizer. The resulting powdered samples were homogenized with a Polytron in 6% pre-chilled perchloric acid and neutralized with 60% KOH. Samples were then centrifuged at 10,000 rpm and 4° C. for 5 minutes, and the supernatant was used for the assay of cGMP (Cyclic GMP Enzyme Immunoassay Kit, Cayman Chemical Company, Ann Arbor, Mich.). The results were normalized for tissue weight.

Secretion of cGMP in response to treatment with Gly-CNP37 ("CNP" in FIG. **76**) was detectable in distal femurs (cartilage and bone), femoral cortices (bone), ear pinna (cartilage), and kidney (FIGS. **76**A-D). Maximal cGMP responses in those tissues were observed 15 min after treatment. Liver, heart, lung and brain tissues did not exhibit appreciable cGMP secretion in response to Gly-CNP37 relative to vehicle control at the studied time points (FIGS. **76**E-H). The results indicate that treatment with 200 nmol/kg of Gly-CNP37 stimulated cGMP secretion in cartilage, bone and renal tissues.

Example 16

Dose Responses of CNP Variants in Monkeys

The effects of the CNP variant Pro-Gly-CNP37 on bone growth and the levels of bone growth-related biomarkers are evaluated in cynomolgus monkeys. Eight normal juvenile cynomolgus monkeys (about 2.5 years of age at the start of the on-going study) are subcutaneously injected daily with 10 or 36 μg/kg/day of Pro-Gly-CNP37 (n=4 per dose group). Four such monkeys are administered vehicle as control. The total length of treatment is 6 months. Various measures of growth plate expansion and bone growth are made by digital X-ray and magnetic resonance imaging, and by measurement of limb and body lengths externally. Blood and urine samples are collected periodically for clinical pathology and measurement of levels of Pro-Gly-CNP37 and biomarkers. Following termination of the study, gross pathology is performed and tissue samples are evaluated histologically for assessment of efficacy and safety.

Data obtained thus far in the on-going study show that both doses of Pro-Gly-CNP37 have increased growth plate width by digital X-ray (FIG. **77**), increased right and left tibia lengths by digital X-ray (FIGS. **78**A and B), increased leg length by external measurement (FIG. **79**), increased arm length by external measurement (FIG. **80**), increased body length by external measurement (FIG. **81**), and increased the serum level of alkaline phosphatase, a biomarker for bone

formation (FIG. **82**). The data demonstrate that Pro-Gly-CNP37 can stimulate bone growth in normal juvenile cynomolgus monkeys at hemodynamically acceptable doses.

Example 17

Effects of CNP Variants on Cardiovascular System in Mice

Natriuretic peptides such as CNP have been reported to affect the cardiovascular system. Wang et al. (Eur J Heart Fail. 9:548-57. 2007) describe that CNP has been shown to have a cardioprotective effect in preventing myocardial ischaemia/reperfusion injury and improving cardiac remodelling after myocardial infarction in rats. Wang demonstrated that mice overexpressing CNP have reduced incidence of cardiac hypertrophy caused by myocardial infarction. Additionally, CNP has been shown to cause endothelium-independent vasodilation (M. Honing et al., Hypertension, 37:1179-1183 (2001)) and therefore may transiently decrease blood pressure in vivo.

To assess the effects of CNP variants on the cardiovascular system, the blood pressure and heart rate in anesthetized wild-type FVB mice is studied following subcutaneous injection of the variants.

After a pilot study to define a broad dose range of cardiovascular activity, a dose-response study is conducted to examine the effects of three different dose levels of each CNP variant. Three male FVB mice aged 8 weeks comprise each treatment group. Doses are administered subcutaneously to anesthetized mice, and systolic, diastolic and mean arterial pressure (MAP), as well as heart rate, are monitored via implanted intraarterial pressure transducers.

Example 18

Formulation of CNP Variants

CNP preformulation studies were carried out to assess the stability of CNP variant Gly-wtCNP37 ("CNP38") at different pH's (pH 3, 4, 5, 6, 7 and 8) and temperatures (5° C., 25° C., and 40° C.) over time. CNP38 exhibited greater stability at pH 4-6 than at the other pH's in the studies. CNP38 was stable at 5° C. at pH 4-6, with ≥about 95% of CNP38 remaining after 15 weeks. When the temperature was raised to 25° C., at pH 4 about 85% of CNP38 remained after 15 weeks, at pH 5 about 85% remained after 15 weeks, and at pH 6 about 80% remained after 15 weeks. When the temperature was raised to 40° C., at pH 4 about 55-60% of CNP38 remained after 15 weeks, at pH 5 about 65% remained after 15 weeks, and at pH 6 about 40% remained after 15 weeks. FIG. **83** illustrates the observed plot of pseudo-first order degradation rate constant (K_{obs}) vs. pH from pH 3 to 8 and at 5° C., 25° C. and 40° C. The stability data for CNP38 in the preformulation studies suggests CNP formulations having a pH in the range from about 4 to about 6. An acidic pH (e.g., pH≤about 6) can promote the stability of a CNP variant by, e.g., minimizing or avoiding deamidation of asparagine and/or glutamine residue(s), isomerization of aspartic acid residue(s), or degradation of the CNP variant by other pathways.

CNP variants can be formulated in pharmaceutical carriers for administration to subjects affected by, e.g., bone growth conditions. In some embodiments, liquid formulations of CNP variants are formulated according to any combinations of the ingredients and their amounts or concentrations in Table 16.

TABLE 16

Ingredient Class	Ingredient	Concentration Range
Active ingredient	CNP variant	10 mg/mL ± 9.9 mg/mL
Buffering agent	Acetic acid/acetate	10 mM ± 5 mM, or pH 5 ± 1
Buffering agent	Citric acid/citrate	10 mM ± 5 mM, or pH 5 ± 1
Isotonicity-adjusting agent	NaCl	140 mM ± 20 mM
Isotonicity-adjusting agent	Sucrose	10% ± 5%
Preservative	m-Cresol	0.4% ± 0.1% or 0.2%
Preservative/anti-adsorbent	Benzyl alcohol	1.5% ± 0.5%
Stabilizer	Glycerin (glycerol)	5%-100% (neat)[1]
Stabilizer	Methionine	0.01%-0.2%
Stabilizer	Ascorbic acid/ ascorbate salt	0.1%-1%
Stabilizer	Thioglycerol	0.1%-1%
Anti-adsorbent	Polysorbate 20	0.001%-0.5%
	Polysorbate 80	0.001%-0.5%
	Benzyl alcohol	0.5%-1.5%

[1]Glycerin is used to minimize or prevent water-driven hydrolysis, deamidation, isomerization or cleavage of CNP variants. For lyophilized formulations, 4-6% or 6-20% mannitol or sucrose can be substituted for NaCl.

In certain embodiments, lyophilized formulations of CNP variants are prepared from formulations formulated according to any combinations of the ingredients and their amounts or concentrations in Table 17.

TABLE 17

Ingredient Class	Ingredient	Concentration Range
Active ingredient	CNP variant	10 mg/mL ± 9.9 mg/mL
Buffering agent	Acetic acid/acetate	10 mM ± 5 mM, or pH 5 ± 1
Buffering agent	Citric acid/citrate	10 mM ± 5 mM, or pH 5 ± 1
Isotonicity-adjusting agent/bulking agent	Sorbitol	5% ± 3%
Isotonicity-adjusting agent/bulking agent	Mannitol	5% ± 3%
Isotonicity-adjusting agent/bulking agent	Sucrose	10% ± 5%
Preservative	m-Cresol	0.4% ± 0.2%
Preservative/anti-adsorbent	Benzyl alcohol	1.5% ± 0.5%
Stabilizer	Glycerin (glycerol)	5%-100% (neat)[1]
Stabilizer	Methionine	0.01%-0.2%
Stabilizer	Ascorbic acid/ ascorbate salt	0.1%-1%
Stabilizer	Thioglycerol	0.1%-1%
Anti-adsorbent	Polysorbate 20	0.001%-0.5%
	Polysorbate 80	0.001%-0.5%
	Benzyl alcohol	0.5%-1.5%

[1]Glycerin is used to minimize or prevent water-driven hydrolysis, deamidation, isomerization or cleavage of CNP variants.

In certain embodiments, a formulation comprising a CNP variant has a pH of about 3-7, or about 3-6, or about 3.5-6.5, or about 4-6, or about 4-5, or about 4.5-5.5. In some embodiments, for pH 4-5.5 a suitable buffering agent is acetic acid/acetate (e.g., sodium acetate), and for pH 5.5-6 a suitable buffering agent is citric acid/citrate. Citric acid/citrate (e.g., sodium citrate) is also a suitable buffering agent in the range of pH 3-6 or pH 4-6. In certain embodiments, the buffering agent has a concentration in the formulation of about 2-50 mM, or about 2-40 mM, or about 2-30 mM, or about 5-30 mM, or about 2-20 mM, or about 5-20 mM, or about 5-15 mM.

To minimize or avoid deamidation of a CNP variant, the variant can be formulated in pharmaceutically acceptable organic cosolvents, such as glycerin, ethanol, and propylene

glycol. Because deamidation occurs by hydrolysis, substitution of an organic cosolvent for water minimizes contact of the CNP variant with water. The concentration of one or more organic solvents in an organic-aqueous solvent system can be, e.g., from about 10% to about 99%, or about 100% if water is not used.

Also to minimize or avoid deamidation of a CNP variant, water can be removed from the formulation by lyophilization. In some embodiments, lyophilized formulations contain any combinations of the following components:

- buffer: sodium acetate and acetic acid, or sodium citrate and citric acid;
- isotonicity/bulking agent: mannitol (e.g., 3-10%, 2-8% or 4-6%);
  sucrose (e.g., 6-20%, 5-15% or 8-12%);
- antioxidants: methionine and/or ascorbic acid with molal ratio of each antioxidant to CNP variant from about 0.1:1 to about 1:1, or from about 0.5:1 to about 5:1, or from about 1:1 to about 15:1, or from about 1:1 to about 10:1, or from about 3:1 to about 10:1.

Deamidation can also be minimized or avoided by storing a CNP composition (e.g., a liquid formulation or a lyophilized formulation) at lower temperature, such as at about 5° C., 0° C., −10° C., −20° C., −30° C., −40° C., −50° C., −60° C., −70° C., −80° C., −90° C., or −100° C.

To minimize or avoid oxidation of oxidizable residues (e.g., methionine) in a CNP variant, the variant can be formulated with one or more antioxidants. Exemplary antioxidants include, but are not limited to, methionine, ascorbic acid, and thioglycerol. Oxidation of, e.g., methionine residues can also be minimized or prevented by purging oxygen from a liquid medium (if a liquid formulation) with nitrogen or argon, and/or by purging oxygen from a container or packaging with nitrogen or argon.

In some embodiments, to minimize or prevent adsorption (e.g., adsorption of a CNP variant to plastic or glass), Polysorbate 20, Polysorbate 80 or benzyl alcohol, or a combination thereof, is added to a CNP formulation. In certain embodiments, each of the anti-adsorbent(s) is in a concentration from about 0.001% to about 0.5%, or from about 0.01% to about 0.5%, or from about 0.1% to about 1%, or from about 0.5% to about 1%, or from about 0.5% to about 1.5%, or from about 0.5% to about 2%, or from about 1% to about 2%. Exemplary range(s) of anti-adsorbent(s) in the formulation include without limitation from about 0.001% to about 0.5% of Polysorbate 20, from about 0.001% to about 0.5% of Polysorbate 80, and/or from about 0.5% to about 1.5% of benzyl alcohol.

In certain embodiments, a liquid CNP formulation comprises, or a lyophilized CNP formulation is prepared from a formulation that comprises, (1) an acetic acid/acetate (e.g., sodium acetate) buffer having a concentration of about 30 mM±5 or 10 mM buffering agent and a pH of about 4±0.5 or 1, and (2) benzyl alcohol (e.g., as a preservative and/or anti-adsorbent) at a concentration of about 1%±0.5%, and optionally (3) sucrose at a concentration of about 10%±5%.

Example 19

Clinical Evaluation of CNP Variants

The following example provides guidance on the parameters to be used for the clinical evaluation of compositions comprising CNP22 or variants thereof in the therapeutic methods of the present disclosure. As discussed herein, CNP22 or variants thereof will be used in the treatment of disorders responsive to CNP, including disorders of the bone and vascular smooth muscle. Clinical trials will be conducted which will provide an assessment of doses of CNP22 or variants thereof for safety, pharmacokinetics, and initial response of both surrogate and defined clinical endpoints. The trial will be conducted for a minimum, but not necessarily limited to, 24 weeks to collect sufficient safety information on about 100 evaluable patients. The initial dose for the trials will vary from about 0.001 to about 1.0 mg/kg/week, or any of the doses described herein. In the event that the initial dose in this range does not produce a significant direct clinical benefit, the dose should be increased within this range or beyond this range as necessary, and maintained for an additional minimal period of, but not necessarily limited to, 24 weeks to establish safety and to evaluate efficacy further.

Measurements of safety will include adverse events, allergic reactions, complete clinical chemistry panel (including kidney and liver functions), urinalysis, and CBC with differential. In addition, other parameters relevant to clinical benefit will be monitored. The present example also includes the determination of pharmacokinetic parameters of CNP22 or variants thereof, including absorption, distribution, metabolism, excretion, and half-life and bioavailability in the blood. It is anticipated that such analyses will help relate dose to clinical response.

Methods

Patients will undergo a baseline medical history and physical exam, and a standard set of clinical laboratory tests (including CBC, Panel 20, CH50, and UA). The patients will be followed closely with weekly visits to the clinic. The patients will return to the clinic for a complete evaluation one week after completing the treatment period. Should dose escalation be required, the patients will follow the same schedule outlined above. Safety will be monitored throughout the trial.

Diagnosis and Inclusion Criteria

The patients may be male or female, with a documented diagnosis of a potentially CNP-responsive disorder. A specific example of a potentially CNP-responsive, bone-related disorder is achondroplasia, which may be confirmed by genetic testing and other evidence of an FGFR-3 mutation or dysfunction. The ideal age range of achondroplasia patients will be from infant (<1 year of age) to pre-adolescent (<13 years of age). A patient will be excluded from this study if the patient is pregnant or lactating; has received an investigational drug within 30 days prior to study enrollment; or has a medical condition, serious intercurrent illness, or other extenuating circumstance that may significantly decrease study compliance.

Safety

Therapy with CNP22 or variants thereof will be determined to be safe if no significant acute or chronic drug reactions occur during the course of the study. The longer-term administration of the drug will be determined to be safe if no significant abnormalities are observed in the clinical examinations, clinical labs, or other appropriate studies.

It has been shown that compared to wild-type CNP22, certain CNP variants of the disclosure are much more resistant to NEP degradation in vitro, have a much longer plasma half-life and bioavailability in rats, stimulate a much higher level of cGMP production in rats, and/or induce a significantly greater increase in long bone length and body length in achondroplastic mice. Furthermore, it has been shown that short duration dose regimen treatments with CNP22 are nearly as effective as continuous CNP22 treatment in reversing FGF2-induced arrest of chondrocyte growth in vitro. These results, among others described herein, demonstrate the utility of CNP variants of the disclosure in treating CNP-responsive conditions or disorders such as, e.g., bone-related disorders and vascular smooth muscle disorders.

It is understood that every embodiment of the disclosure described herein may optionally be combined with any one or more of the other embodiments described herein. Every patent literature and every non-patent literature cited herein are incorporated herein by reference in their entirety.

Numerous modifications and variations to the disclosure, as set forth in the embodiments and illustrative examples described herein, are expected to occur to those skilled in the art. Consequently only such limitations as appear in the appended claims should be placed on the disclosure.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 237

<210> SEQ ID NO 1
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<220> FEATURE:
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<223> OTHER INFORMATION: wtCNP-22
<220> FEATURE:
<221> NAME/KEY: DISULFID
<222> LOCATION: (6)..(22)
<223> OTHER INFORMATION: Cysteines in positions 6 and 22 form cyclic domain via disulfide bond

<400> SEQUENCE: 1

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 2
<211> LENGTH: 17
<212> TYPE: PRT
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<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: CNP-17

<400> SEQUENCE: 2

Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly
1 5 10 15

Cys

<210> SEQ ID NO 3
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: CNP-27

<400> SEQUENCE: 3

Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu
1 5 10 15

Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25

<210> SEQ ID NO 4
<211> LENGTH: 53
<212> TYPE: PRT
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<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:

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<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: CNP-53

<400> SEQUENCE: 4

Asp Leu Arg Val Asp Thr Lys Ser Arg Ala Ala Trp Ala Arg Leu Leu
1 5 10 15

Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly
20 25 30

Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met
35 40 45

Ser Gly Leu Gly Cys
50

<210> SEQ ID NO 5
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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
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<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
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<223> OTHER INFORMATION: Gly is optionally modified with an amino acid sequence derived from a natriuretic polypeptide, a non-natriuretic polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (22)..(22)
<223> OTHER INFORMATION: Cys is optionally modified with an amino acid sequence derived from a natriuretic polypeptide, a non-natriuretic polypeptide

<400> SEQUENCE: 5

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 6
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Gly is modified with a synthetic or natural polymeric group or combination thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: Xaa is Lys or a conservative amino acid substitution thereof, any natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amine on a side chain, Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Glu or Ser
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<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is Lys or a conservative amino acid substitution thereof, any natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amine on a side chain, Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Glu or Ser
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (22)..(22)
<223> OTHER INFORMATION: Cys is optionally modified with a synthetic or natural polymeric group or combination thereof

<400> SEQUENCE: 6

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Gly Leu Ser Xaa Gly Cys Phe Gly Leu Xaa Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 7
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 7

Gly Ala Asn Lys Lys
1 5

<210> SEQ ID NO 8
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 8

Gly Ala Asn Arg Arg
1 5

<210> SEQ ID NO 9
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 9

Gly Ala Asn Pro Arg
1 5

<210> SEQ ID NO 10
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 10

Gly Ala Asn Gln Gln
1 5

<210> SEQ ID NO 11
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 11

Gly Ala Asn Ser Ser
1 5

<210> SEQ ID NO 12
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

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<400> SEQUENCE: 12

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1 5

<210> SEQ ID NO 13
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 13

Gly Ala Asn Arg Met
1 5

<210> SEQ ID NO 14
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 14

Gly Ala Asn Arg Thr
1 5

<210> SEQ ID NO 15
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 15

Gly Ala Asn Arg Ser
1 5

<210> SEQ ID NO 16
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 16

Ala Ala Trp Ala Arg Leu Leu Gln Glu His Pro Asn Ala
1 5 10

<210> SEQ ID NO 17
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 17

Ala Ala Trp Ala Arg Leu Leu Gln Glu His Pro Asn Ala Arg
1 5 10

<210> SEQ ID NO 18
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 18

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Asp Leu Arg Val Asp Thr Lys Ser Arg Ala Ala Trp Ala Arg
1 5 10

<210> SEQ ID NO 19
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 19

Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys
1 5 10 15

<210> SEQ ID NO 20
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 20

Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Arg Arg
1 5 10 15

<210> SEQ ID NO 21
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 21

Asp Leu Arg Val Asp Thr Lys Ser Arg Ala Ala Trp Ala Arg Leu Leu
1 5 10 15

Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys
20 25 30

<210> SEQ ID NO 22
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 22

Asp Leu Arg Val Asp Thr Lys Ser Arg Ala Ala Trp Ala Arg Leu Leu
1 5 10 15

Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Arg Arg
20 25 30

<210> SEQ ID NO 23
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 23

Ser Leu Arg Arg Ser Ser
1 5

<210> SEQ ID NO 24
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 24

Asn Ser Phe Arg Tyr
1 5

<210> SEQ ID NO 25
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 25

Ser Pro Lys Met Val Gln Gly Ser Gly
1 5

<210> SEQ ID NO 26
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 26

Met Val Gln Gly Ser Gly
1 5

<210> SEQ ID NO 27
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 27

Lys Val Leu Arg Arg Tyr
1 5

<210> SEQ ID NO 28
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 28

Lys Val Leu Arg Arg His
1 5

<210> SEQ ID NO 29
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 29

Gly Gln His Lys Asp Asp Asn Pro Asn Leu Pro Arg
1 5 10

<210> SEQ ID NO 30
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

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<400> SEQUENCE: 30

Gly Val Pro Gln Val Ser Thr Ser Thr
1 5

<210> SEQ ID NO 31
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 31

Gly Glu Arg Ala Phe Lys Ala Trp Ala Val Ala Arg Leu Ser Gln
1 5 10 15

<210> SEQ ID NO 32
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 32

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
1 5 10

<210> SEQ ID NO 33
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Amino acid extention

<400> SEQUENCE: 33

Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys
1 5 10 15

<210> SEQ ID NO 34
<211> LENGTH: 37
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: CNP-22 with amino acid extention
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (19)..(19)
<223> OTHER INFORMATION: Xaa is Lys or a conservative amino acid
substitution thereof, any natural or unnatural amino acid, Arg,
Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Glu or Ser
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (25)..(25)
<223> OTHER INFORMATION: Xaa is Lys or a conservative amino acid
substitution thereof, any natural or unnatural amino acid, Arg,
Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Glu or Ser
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (37)..(37)
<223> OTHER INFORMATION: Cys is optionally modified with a synthetic,
natural or combination polymeric group

<400> SEQUENCE: 34

Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly
1 5 10 15

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Leu Ser Xaa Gly Cys Phe Gly Leu Xaa Leu Asp Arg Ile Gly Ser Met
20 25 30

Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 35
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: CNP22 (K4R)

<400> SEQUENCE: 35

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 36
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: GANRR-CNP22(K4R) - CNP27(Arg4)

<400> SEQUENCE: 36

Gly Ala Asn Arg Arg Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu
1 5 10 15

Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25

<210> SEQ ID NO 37
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: GANPR-CNP22(K4R) - CNP27(Pro4) - Analog CI

<400> SEQUENCE: 37

Gly Ala Asn Pro Arg Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu
1 5 10 15

Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25

<210> SEQ ID NO 38
<211> LENGTH: 24
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: ER-CNP22

<400> SEQUENCE: 38

Glu Arg Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile
1 5 10 15

Gly Ser Met Ser Gly Leu Gly Cys

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20

<210> SEQ ID NO 39
<211> LENGTH: 24
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BA - ER-CNP22(K4R)

<400> SEQUENCE: 39

Glu Arg Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile
1 5 10 15

Gly Ser Met Ser Gly Leu Gly Cys
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<210> SEQ ID NO 40
<211> LENGTH: 23
<212> TYPE: PRT
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<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: R-CNP22

<400> SEQUENCE: 40

Arg Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly
1 5 10 15

Ser Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 41
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog AZ - R-CNP22(K4R)

<400> SEQUENCE: 41

Arg Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly
1 5 10 15

Ser Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 42
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: CNP22 (K4R)(K10R)

<400> SEQUENCE: 42

Gly Leu Ser Arg Gly Cys Phe Gly Leu Arg Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 43

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<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
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<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: CNP-22 (Cys6-Phe7 variable bond)
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -CH2-NH-
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -C(=O)-N(R)- where R is methyl, ethyl, n-propyl, isopropyl, cyclopropyl, n-butyl, isobutyl, sec-butyl or tert-butyl
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -C(=O)-NH-CH2-
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -CH2-S-
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -CH2-S(O)n where n is 1 or 2
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -CH2-CH2-
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -CH=CH-
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -C(=O)-CH2-
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -CH(CN)-NH-
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -CH(OH)-CH2-
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -O-C(=O)-NH-
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with NHC(=O)NH-

<400> SEQUENCE: 43

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 44

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<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Xaa is 3-amino-2-phenylpropionic acid or D-Phe

<400> SEQUENCE: 44

Gly Leu Ser Lys Gly Cys Xaa Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 45
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: CNP22 - modified Cys6
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(6)
<223> OTHER INFORMATION: Xaa is Cys, homocysteine, penicillamine, 2-mercaptopropionic acid, or 3-mercaptopropionic acid.

<400> SEQUENCE: 45

Gly Leu Ser Lys Gly Xaa Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 46
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Gly is optionally modified with a bone or cartilage targeting compound, a biphosphonate, polyAsp, polyGlu, osteopontin, osteocalcin, or sialoprotein peptides, polymers, PEG, carbohydrates, hydrophobic acids, or amino acids
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: Xaa is Lys or a conservative amino acid substitution thereof, any natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amine on a side chain, Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser, or Glu
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Xaa is Cys with peptide bond isosteres between positions 6 and 7
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Xaa is L-Phe, D-Phe, 3-amino-2-phenylpropionic acid, or N-alkylated derivatives of Phe
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Xaa is Gly, tert-butyl-Gly (tBu-Gly), Thr, Ser, Val or Asn
<220> FEATURE:

-continued

<221> NAME/KEY: MOD_RES
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: Xaa is Leu, Ser, Thr or peptide bond isosteres thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is Lys or a conservative amino acid substitution thereof, any natural or unnatural amino acid, Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (11)..(11)
<223> OTHER INFORMATION: Xaa is Leu or peptide bond isosteres thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (14)..(14)
<223> OTHER INFORMATION: Xaa is Ile, tBu-Gly and peptide bond isosteres thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (17)..(17)
<223> OTHER INFORMATION: Xaa is Met, Val, Asn, beta-Cl-Ala, 2-aminobutyric acid (Abu) or 2-amino-isobutyric acid (Aib)
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Xaa is Leu, norleucine (Nle), homoleucine (Hleu), Val, tert-butyl-Ala (tBu-Ala), Ser, Thr, Arg or peptide bond isosteres thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (22)..(22)
<223> OTHER INFORMATION: Cys is optionally modified with a bone or cartilage targeting compound, a bisphosphonate, polyAsp, polyGlu, osteopontin, osteocalcin or sialoprotein peptides, or amino acid sequences derived from non-CNP polypeptides

<400> SEQUENCE: 46

Gly Leu Ser Xaa Gly Xaa Xaa Xaa Xaa Xaa Xaa Asp Arg Xaa Gly Ser
1 5 10 15

Xaa Ser Gly Xaa Gly Cys
20

<210> SEQ ID NO 47
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Gly is optionally modified with a peptide sequence containing one to five amino acids from a natriuretic or non-natriuretic polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Gly is optionally modified with a peptide sequence containing one to five amino acids from a natriuretic or non-natriuretic polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: Xaa is Lys or a conservative amino acid substitution thereof, any natural or unnatural amino acid or peptidomimetic, Arg, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser, or Glu
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(6)
<223> OTHER INFORMATION: Xaa is Cys or decarboxy Cys
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Peptide bond between positions 6 and 7 may be

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replaced with -CH2-NH-
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Peptide bond between positions 6 and 7 may be replaced with -C(=O)-N(R)- where R is methyl, ethyl, n-propyl, isopropyl, cyclopropyl, n-butyl, isobutyl, sec-butyl or tert-butyl
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Peptide bond between positions 6 and 7 may be replaced with -C(=O)-NH-CH2-
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Peptide bond between positions 6 and 7 may be replaced with -CH2-S-
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Peptide bond between positions 6 and 7 may be replaced with -CH2-S(O)n where n is 1 or 2
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Peptide bond between positions 6 and 7 may be replaced with -CH2-CH2-
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Peptide bond between positions 6 and 7 may be replaced with -CH=CH-
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Peptide bond between positions 6 and 7 may be replaced with -C(=O)-CH2-
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Peptide bond between positions 6 and 7 may be replaced with -CH(CN)-NH-
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Peptide bond between positions 6 and 7 may be replaced with -CH(OH)-CH2-
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Peptide bond between positions 6 and 7 may be replaced with -O-C(=O)-NH-
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Peptide bond between positions 6 and 7 may be replaced with -NHC(=O)NH-
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Xaa is L-Phe, D-Phe, 3-amino-2-phenylpropionic acid, or N-alkylated derivatives of Phe
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Xaa is Gly, tert-butyl-Gly (tBu-Gly), Val, Ser, Thr or Asn
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: Xaa is Leu, Ser, Thr or peptide bond isosteres thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (11)..(11)

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<223> OTHER INFORMATION: Xaa is Leu or peptide isosteres thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (14)..(14)
<223> OTHER INFORMATION: Xaa is Ile, tBu-Gly or peptide isosteres thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (17)..(17)
<223> OTHER INFORMATION: Xaa is Met, Val, Asn, beta-Cl-Ala, 2-aminobutyric acid (Abu) or 2-amino-isobutyric acid (Aib)
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Xaa is Leu, norleucine (Nle), homoleucine (Hleu), Val, tert-butyl-Ala (tBu-Ala), Ser, Thr, Arg, or peptide bond isosteres thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (22)..(22)
<223> OTHER INFORMATION: Cys is optionally modified with a synthetic bone targeting compound, a bisphosphonate, polyAsp, polyGlu, osteopontin, osteocalcin or sialoprotein peptides, or charged PEG molecules

<400> SEQUENCE: 47

Gly Leu Ser Xaa Gly Xaa Xaa Xaa Xaa Xaa Xaa Asp Arg Xaa Gly Ser
1 5 10 15

Xaa Ser Gly Xaa Gly Cys
20

<210> SEQ ID NO 48
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Xaa is a synthetic or natural polymeric group or combination thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is absent or may be one or more amino acids from Gly-Leu-Ser-Lys-Gly and/or substitutions at one or more of these positions
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Xaa is Lys, a conservative amino acid substitution, any natural or unnatural amino acid or peptidomimetic that does have a reactive primary amine on a side chain
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (19)..(19)
<223> OTHER INFORMATION: Cys is optionally modified with a synthetic or natural polymeric group or a combination thereof

<400> SEQUENCE: 48

Xaa Xaa Cys Phe Gly Leu Xaa Leu Asp Arg Ile Gly Ser Met Ser Gly
1 5 10 15

Leu Gly Cys

<210> SEQ ID NO 49
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES

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<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Xaa is absent or may be a peptide fragment or water soluble polymer
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is Cys or peptide bond isosteres thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (3)..(3)
<223> OTHER INFORMATION: Xaa is L-Phe, D-Phe, 3-amino-2-phenylpropionic acid or N-alkylated derivatives of Phe
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: Xaa is Gly, tert-butyl-Gly (tBu-Gly), Val, Ser, Thr or Asn
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (5)..(5)
<223> OTHER INFORMATION: Xaa is Leu, Ser, Thr or N-Me-Leu
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(6)
<223> OTHER INFORMATION: Xaa is Lys or a conservative amino acid substitution thereof, any natural or unnatural amino acid, Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Xaa is Leu and peptide bond isosteres thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is Ile, tBu-Gly or peptide bond isosteres thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (13)..(13)
<223> OTHER INFORMATION: Xaa is Met, Val, Asn, beta-Cl-Ala, 2-aminobutyric acid (Abu) or 2-amino-isobutyric acid (Aib)
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (16)..(16)
<223> OTHER INFORMATION: Xaa is Leu, norleucine (Nle), homoleucine (Hleu), Val, tert-butyl-Ala (tBu-Ala), Ser, Thr, Arg or peptide bond isosteres thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (18)..(18)
<223> OTHER INFORMATION: Cys is optionally modified with amino acid sequences useful in bone/cartilage targeting, polyAsp or polyGlu, osteopontin, osteocalcin, sialoprotein or sequences derived from NPPC or non-CNP polypeptides

<400> SEQUENCE: 49

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asp Arg Xaa Gly Ser Xaa Ser Gly Xaa
1 5 10 15

Gly Cys

<210> SEQ ID NO 50
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Gly is optionally modified with bone targeting compound, moieties to reduce renal clearance, charged PEG molecules or hydrophilic polymers
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: Xaa is Lys or a conservative amino acid substitution thereof, any natural or unnatural amino acid, Arg,

Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(6)
<223> OTHER INFORMATION: Xaa is Cys or peptide isosteres between positions 6 and 7
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Xaa is L-Phe, D-Phe, 3-amino-2-phenylpropionic acid or N-alkylated derivatives of Phe
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Xaa is Gly, tert-butyl-Gly, Thr, Ser, Val or Asn
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: Xaa is Leu, Ser, Thr or peptide bond isosteres thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is Lys or a conservative amino acid substitution thereof, any natural or unnatural amino acid, Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (11)..(11)
<223> OTHER INFORMATION: Xaa is Leu, Asn or peptide bond isosteres thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (14)..(14)
<223> OTHER INFORMATION: Xaa is Ile, tert-butyl-Gly (tBu-Gly), Asn or peptide bond isosteres thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: Xaa is Gly, Arg, Ser or Asn
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (17)..(17)
<223> OTHER INFORMATION: Xaa is Met, Val, Asn, beta-Cl-Ala, 2-aminobutyric acid (Abu) or 2-amino-isobutyric acid (Aib)
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Xaa is Leu, norleucine (Nle), homoleucine (Hleu), Val, tert-butyl-Ala (tBu-Ala), Arg, Thr, Ser or peptide bond isoteres thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (22)..(22)
<223> OTHER INFORMATION: Cys is optionally modified with bone targeting compound, moieties to reduce renal clearance, charged PEG molecules or hydrophilic polymers

<400> SEQUENCE: 50

Gly Leu Ser Xaa Gly Xaa Xaa Xaa Xaa Xaa Xaa Asp Arg Xaa Xaa Ser
1 5 10 15

Xaa Ser Gly Xaa Gly Cys
20

<210> SEQ ID NO 51
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Xaa is Arg or Glu

<400> SEQUENCE: 51

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Xaa Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 52
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (5)..(5)
<223> OTHER INFORMATION: Xaa is Arg, Gln or Ser

<400> SEQUENCE: 52

Gly Leu Ser Lys Xaa Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 53
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: Xaa is Ser, Asn, Arg, or Cit

<400> SEQUENCE: 53

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Xaa Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 54
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (19)..(19)
<223> OTHER INFORMATION: Xaa is Ser, Arg, or Asn

<400> SEQUENCE: 54

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Xaa Leu Gly Cys
20

<210> SEQ ID NO 55
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (21)..(21)
<223> OTHER INFORMATION: Xaa is Ser, Thr, or Arg

<400> SEQUENCE: 55

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Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Xaa Cys
20

<210> SEQ ID NO 56
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog A
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (6)..(6)
<223> OTHER INFORMATION: CH2NH

<400> SEQUENCE: 56

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 57
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analogs B
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Xaa replaced with N-Me-Phe

<400> SEQUENCE: 57

Gly Leu Ser Lys Gly Cys Xaa Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 58
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog F
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: tBu

<400> SEQUENCE: 58

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 59
<211> LENGTH: 22
<212> TYPE: PRT

-continued

<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog H
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Xaa is replaced with NHCH2-CH(Phe)CO

<400> SEQUENCE: 59

Gly Leu Ser Lys Gly Cys Xaa Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 60
<211> LENGTH: 37
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BL - CNP37

<400> SEQUENCE: 60

Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly
1 5 10 15

Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met
20 25 30

Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 61
<211> LENGTH: 35
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CA

<400> SEQUENCE: 61

Ala Ala Trp Ala Arg Leu Leu Gln Glu His Pro Asn Ala Gly Leu Ser
1 5 10 15

Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly
20 25 30

Leu Gly Cys
35

<210> SEQ ID NO 62
<211> LENGTH: 36
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CB

<400> SEQUENCE: 62

Ala Ala Trp Ala Arg Leu Leu Gln Glu His Pro Asn Ala Arg Gly Leu
1 5 10 15

Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser

-continued

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            20                  25                  30

Gly Leu Gly Cys
        35


<210> SEQ ID NO 63
<211> LENGTH: 36
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CC

<400> SEQUENCE: 63

Asp Leu Arg Val Asp Thr Lys Ser Arg Ala Ala Trp Ala Arg Gly Leu
1               5                   10                  15

Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser
            20                  25                  30

Gly Leu Gly Cys
        35


<210> SEQ ID NO 64
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: GANQQ-CNP22

<400> SEQUENCE: 64

Gly Ala Asn Gln Gln Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu
1               5                   10                  15

Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
            20                  25


<210> SEQ ID NO 65
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: GANRR-CNP22

<400> SEQUENCE: 65

Gly Ala Asn Arg Arg Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu
1               5                   10                  15

Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
            20                  25


<210> SEQ ID NO 66
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: GANPR-CNP22

<400> SEQUENCE: 66

Gly Ala Asn Pro Arg Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu
1               5                   10                  15
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Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
            20                  25


<210> SEQ ID NO 67
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: GANSS-CNP22

<400> SEQUENCE: 67

Gly Ala Asn Ser Ser Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu
1               5                   10                  15

Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
            20                  25


<210> SEQ ID NO 68
<211> LENGTH: 32
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequene
<220> FEATURE:
<223> OTHER INFORMATION: Sythetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CD
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: N-terminus BNP tail
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (32)..(32)
<223> OTHER INFORMATION: N-terminus BNP tail

<400> SEQUENCE: 68

Ser Pro Lys Met Val Gln Gly Ser Gly Cys Phe Gly Leu Lys Leu Asp
1               5                   10                  15

Arg Ile Gly Ser Met Ser Gly Leu Gly Cys Lys Val Leu Arg Arg His
            20                  25                  30


<210> SEQ ID NO 69
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: GANQQ-CNP22(K4R) - Analog CH

<400> SEQUENCE: 69

Gly Ala Asn Gln Gln Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu
1               5                   10                  15

Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
            20                  25


<210> SEQ ID NO 70
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: GANSS-CNP22(K4R) - Analog CG
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<400> SEQUENCE: 70

Gly Ala Asn Ser Ser Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu
1 5 10 15

Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25

<210> SEQ ID NO 71
<211> LENGTH: 38
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CS

<400> SEQUENCE: 71

Gly Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Pro Lys
1 5 10 15

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
20 25 30

Met Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 72
<211> LENGTH: 38
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CT

<400> SEQUENCE: 72

Gly Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Gln Lys
1 5 10 15

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
20 25 30

Met Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 73
<211> LENGTH: 38
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CU

<400> SEQUENCE: 73

Gly Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Gln Gln
1 5 10 15

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
20 25 30

Met Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 74
<211> LENGTH: 38
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CW

<400> SEQUENCE: 74

Gly Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Pro
1 5 10 15

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
20 25 30

Met Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 75
<211> LENGTH: 38
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 75

Gly Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys
1 5 10 15

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
20 25 30

Met Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 76
<211> LENGTH: 37
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CQ

<400> SEQUENCE: 76

Gly His His Ser His Glu Gln His Pro His Gly Ala Asn Gln Gln Gly
1 5 10 15

Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met
20 25 30

Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 77
<211> LENGTH: 38
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CR

<400> SEQUENCE: 77

Gly Ala His His Pro His Glu His Asp Thr His Gly Ala Asn Gln Gln
1 5 10 15

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
20 25 30

Met Ser Gly Leu Gly Cys
35

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<210> SEQ ID NO 78
<211> LENGTH: 37
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequencce
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CX

<400> SEQUENCE: 78

Gly His His Ser His Glu Gln His Pro His Gly Ala Asn Pro Arg Gly
1 5 10 15

Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met
20 25 30

Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 79
<211> LENGTH: 36
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CF

<400> SEQUENCE: 79

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gly Leu
1 5 10 15

Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser
20 25 30

Gly Leu Gly Cys
35

<210> SEQ ID NO 80
<211> LENGTH: 39
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CY

<400> SEQUENCE: 80

Gly Gln His Lys Asp Asp Asn Pro Asn Leu Pro Arg Gly Ala Asn Pro
1 5 10 15

Arg Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly
20 25 30

Ser Met Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 81
<211> LENGTH: 37
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CE

<400> SEQUENCE: 81

Gly Glu Arg Ala Phe Lys Ala Trp Ala Val Ala Arg Leu Ser Gln Gly
1 5 10 15

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Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met
20 25 30

Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 82
<211> LENGTH: 35
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CZ

<400> SEQUENCE: 82

Phe Gly Ile Pro Met Asp Arg Ile Gly Arg Asn Pro Arg Gly Leu Ser
1 5 10 15

Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly
20 25 30

Leu Gly Cys
35

<210> SEQ ID NO 83
<211> LENGTH: 40
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog DA

<400> SEQUENCE: 83

Gly Lys Arg Thr Gly Gln Tyr Lys Leu Gly Ser Lys Thr Gly Pro Gly
1 5 10 15

Pro Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile
20 25 30

Gly Ser Met Ser Gly Leu Gly Cys
35 40

<210> SEQ ID NO 84
<211> LENGTH: 37
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CK

<400> SEQUENCE: 84

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Gly Ala Asn Gln Gln Gly
1 5 10 15

Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met
20 25 30

Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 85
<211> LENGTH: 36
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

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<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CL

<400> SEQUENCE: 85

Gly Val Pro Gln Val Ser Thr Ser Thr Gly Ala Asn Gln Gln Gly Leu
1 5 10 15

Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser
20 25 30

Gly Leu Gly Cys
35

<210> SEQ ID NO 86
<211> LENGTH: 37
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CM

<400> SEQUENCE: 86

Gly Gln Pro Ser Ser Ser Ser Gln Ser Thr Gly Ala Asn Gln Gln Gly
1 5 10 15

Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met
20 25 30

Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 87
<211> LENGTH: 37
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CN

<400> SEQUENCE: 87

Gly Gln Thr His Ser Ser Gly Thr Gln Ser Gly Ala Asn Gln Gln Gly
1 5 10 15

Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met
20 25 30

Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 88
<211> LENGTH: 37
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CO

<400> SEQUENCE: 88

Gly Ser Thr Gly Gln Trp His Ser Glu Ser Gly Ala Asn Gln Gln Gly
1 5 10 15

Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met
20 25 30

Ser Gly Leu Gly Cys
35

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<210> SEQ ID NO 89
<211> LENGTH: 37
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CP

<400> SEQUENCE: 89

Gly Ser Ser Ser Ser Ser Ser Ser Ser Ser Gly Ala Asn Gln Gln Gly
1 5 10 15

Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met
20 25 30

Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 90
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Gly is optionally modified with bone targeting compound, a bisphosphonates, polyAsp, polyGlu, osteopontin, osteocalcin, or sialoprotein peptides, negatively charged PEG molecules or natural polymers
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(6)
<223> OTHER INFORMATION: Xaa is Cys or another natural or unnatural amino acid
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Xaa is Phe or another natural or unnatural amino acid
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Xaa is Gly, a larger natural or unnatural amino acid or peptidomimetic
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (22)..(22)
<223> OTHER INFORMATION: Cys is optionally modified with bone targeting compound, a bisphosphonates, polyAsp, polyGlu, osteopontin, osteocalcin, or sialoprotein peptides, negatively charged PEG molecules or natural polymers

<400> SEQUENCE: 90

Gly Leu Ser Lys Gly Xaa Xaa Xaa Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 91
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog J
<220> FEATURE:
<221> NAME/KEY: MOD_RES

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<222> LOCATION: (6)..(6)
<223> OTHER INFORMATION: Xaa is CH2-NH
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: N-Me
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: N-Me

<400> SEQUENCE: 91

Gly Leu Ser Lys Gly Xaa Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 92
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog K
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: N-Me
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: N-Me

<400> SEQUENCE: 92

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 93
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog L
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: N-Me
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (11)..(11)
<223> OTHER INFORMATION: N-Me
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: N-Me

<400> SEQUENCE: 93

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 94
<211> LENGTH: 22

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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog M
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: N-Me
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (11)..(11)
<223> OTHER INFORMATION: N-Me

<400> SEQUENCE: 94

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 95
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog Z

<400> SEQUENCE: 95

Gly Leu Ser Arg Gly Cys Tyr Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 96
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog AA

<400> SEQUENCE: 96

Gly Leu Ser Arg Gly Cys Phe Val Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 97
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog AB

<400> SEQUENCE: 97

Gly Leu Ser Arg Gly Cys Phe Ser Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

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<210> SEQ ID NO 98
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog AC

<400> SEQUENCE: 98

Gly Leu Ser Arg Gly Cys Phe Thr Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 99
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog AD

<400> SEQUENCE: 99

Gly Leu Ser Arg Gly Cys Phe Gly Thr Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 100
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog AE

<400> SEQUENCE: 100

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Arg Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 101
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog AF
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: Xaa is Cit

<400> SEQUENCE: 101

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Xaa Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

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<210> SEQ ID NO 102
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog AG

<400> SEQUENCE: 102

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Val Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 103
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog AJ

<400> SEQUENCE: 103

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Val Gly Cys
20

<210> SEQ ID NO 104
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog AK
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Xaa is tBu-Ala

<400> SEQUENCE: 104

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Xaa Gly Cys
20

<210> SEQ ID NO 105
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog AT

<400> SEQUENCE: 105

Glu Leu Ser Glu Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

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<210> SEQ ID NO 106
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog AV
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: N-terminus modified with pentanoic acid

<400> SEQUENCE: 106

Glu Leu Ser Glu Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 107
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog AW
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Heptanoic acid modifying N-terminus

<400> SEQUENCE: 107

Glu Leu Ser Glu Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 108
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BB
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: N-terminus modified with heptanoic acid

<400> SEQUENCE: 108

Glu Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 109
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BC

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<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: N-terminus modified with pentanoic acid

<400> SEQUENCE: 109

Glu Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 110
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BF
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is Cit

<400> SEQUENCE: 110

Gly Leu Ser Arg Gly Cys Phe Gly Leu Xaa Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 111
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BG

<400> SEQUENCE: 111

Gly Leu Ser Arg Gly Cys Phe Gly Leu Gln Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 112
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BH

<400> SEQUENCE: 112

Gly Leu Ser Arg Gly Cys Phe Gly Leu Arg Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 113
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BJ

<400> SEQUENCE: 113

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Asn Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 114
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BK

<400> SEQUENCE: 114

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Ser Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 115
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog N
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(22)
<223> OTHER INFORMATION: All D-amino acids

<400> SEQUENCE: 115

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 116
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BD
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: N-Me

<400> SEQUENCE: 116

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 117

-continued

<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BN
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (11)..(11)
<223> OTHER INFORMATION: N-Me

<400> SEQUENCE: 117

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 118
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BE
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: N-Me

<400> SEQUENCE: 118

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 119
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog V
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: 3,4-dichloro

<400> SEQUENCE: 119

Gly Leu Ser Gly Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 120
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog X
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (7)..(7)

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<223> OTHER INFORMATION: 3-methyl

<400> SEQUENCE: 120

Gly Leu Ser Gly Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 121
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BP

<400> SEQUENCE: 121

Gly Leu Ser Arg Ser Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 122
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BO

<400> SEQUENCE: 122

Gly Leu Ser Arg Arg Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 123
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog P

<400> SEQUENCE: 123

Gly Leu Ser Gly Gly Cys Phe Gly Leu Arg Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 124
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BY

<400> SEQUENCE: 124

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Gly Leu Ser Arg Gly Cys Phe Gly Leu Ser Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 125
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BZ

<400> SEQUENCE: 125

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Gln
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 126
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BX

<400> SEQUENCE: 126

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Asn Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 127
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BQ

<400> SEQUENCE: 127

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Ser Leu Gly Cys
20

<210> SEQ ID NO 128
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BR

<400> SEQUENCE: 128

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Arg Leu Gly Cys

20

<210> SEQ ID NO 129
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BS

<400> SEQUENCE: 129

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Arg Gly Cys
20

<210> SEQ ID NO 130
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BT

<400> SEQUENCE: 130

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Ser Cys
20

<210> SEQ ID NO 131
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BU

<400> SEQUENCE: 131

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Thr Cys
20

<210> SEQ ID NO 132
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BW

<400> SEQUENCE: 132

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Arg Cys
20

<210> SEQ ID NO 133

<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CJ

<400> SEQUENCE: 133

Arg Ser Ser Cys Phe Gly Gly Arg Ile Asp Arg Ile Gly Ala Cys
1 5 10 15

<210> SEQ ID NO 134
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: N-terminal linked to CNP22 by disuccinimidyl glutarate

<400> SEQUENCE: 134

Gly Leu Ser Arg Gly Cys Phe Gly Leu Arg Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 135
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: N-terminal linked to CNP22 by Bis-PEO5

<400> SEQUENCE: 135

Gly Leu Ser Arg Gly Cys Phe Gly Leu Arg Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 136
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog E
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Xaa is D-Phe

<400> SEQUENCE: 136

Gly Leu Ser Lys Gly Cys Xaa Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 137

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<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Sythetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog G
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Xaa is 3-Cl-Phe

<400> SEQUENCE: 137

Gly Leu Ser Lys Gly Cys Xaa Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 138
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Xaa comprises one or more amino acids selected from Gly-Leu-Ser-Lys-Gly or substitutions at one or more of these amino acids and further comprises a hydrophilic or water soluable polymer

<400> SEQUENCE: 138

Xaa Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu
1 5 10 15

Gly Cys

<210> SEQ ID NO 139
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Cys is modified with a natural and/or synthetic polymer
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (17)..(17)
<223> OTHER INFORMATION: Cys is modified with a natural and/or synthetic polymer

<400> SEQUENCE: 139

Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly
1 5 10 15

Cys

<210> SEQ ID NO 140
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Gly is modified with a natural and/or synthetic polymer

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<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (22)..(22)
<223> OTHER INFORMATION: Cys is modified with a natural and/or synthetic polymer

<400> SEQUENCE: 140

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 141
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Cys is modified with a natural and/or synthetic polymeric group or combination thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (5)..(5)
<223> OTHER INFORMATION: Xaa is Lys or a conservative amino acid substitution thereof, any natural or unnatural amino acid, Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Glu or Ser
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (17)..(17)
<223> OTHER INFORMATION: Cys is optionally modified with a natural and/or synthetic polymeric group or combination thereof

<400> SEQUENCE: 141

Cys Phe Gly Leu Xaa Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly
1 5 10 15

Cys

<210> SEQ ID NO 142
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Xaa is optionally modified with bone- or cartilage-targeting moieties, moieties that reduce renal clearance, hydrophilic polymers, carbohydrates, hydrophobic acids and combinations thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is absent or may comprise 1 to 5 amino acids from Gly-Leu-Ser-Lys-Gly, optionally substituted with a natural or unnatural amino acid
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Xaa is Lys, any natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amine on a side chain
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (19)..(19)
<223> OTHER INFORMATION: Xaa is optionally modified with bone- or cartilage-targeting moieties, moieties that reduce renal clearance, hydrophilic polymers, carbohydrates, hydrophobic acids and combinations thereof

<400> SEQUENCE: 142

Xaa Xaa Cys Phe Gly Leu Xaa Leu Asp Arg Ile Gly Ser Met Ser Gly
1 5 10 15

Leu Gly Cys

<210> SEQ ID NO 143
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Gly is optionally modified with cartilage or bone targeting compounds, a bisphosphonate, polyAsp, polyGlu, osteopontin peptides, osteocalcin peptides, sialoprotein peptides, charged PEG molecules or hydrophilic polymers
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: Xaa is Lys or a conservative amino acid substitution thereof, any natural or unnatural amino acid, Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(6)
<223> OTHER INFORMATION: Xaa is Cys or peptide isosteres between positions 6 and 7
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Xaa is L-Phe, D-Phe, N-alkylated Phe, or Phe analog
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Xaa is Gly, tert-butyl-Gly, Thr, Ser, Val or Asn
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: Xaa is Leu, Ser, Thr or peptide bond isosteres thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is Lys or a conservative amino acid substitution thereof, any natural or unnatural amino acid, Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (11)..(11)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (14)..(14)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: Xaa is Gly, Arg, Ser or Asn
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (17)..(17)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Xaa is Leu, norleucine (Nle), homoleucine (Hleu), Val, tert-butyl-Ala (tBu-Ala), Arg, Thr, Ser or peptide bond isoteres thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (22)..(22)
<223> OTHER INFORMATION: Cys is optionally modified with cartilage or bone targeting compounds, a bisphosphonate, polyAsp, polyGlu, osteopontin peptides, osteocalcin peptides, sialoprotein peptides,

charged PEG molecules or hydrophilic polymers

<400> SEQUENCE: 143

Gly Leu Ser Xaa Gly Xaa Xaa Xaa Xaa Xaa Xaa Asp Arg Xaa Xaa Ser
1 5 10 15

Xaa Ser Gly Xaa Gly Cys
20

<210> SEQ ID NO 144
<211> LENGTH: 38
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 144

Gly His Lys Ser Glu Val Ala His Arg Phe Lys Gly Ala Asn Lys Lys
1 5 10 15

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
20 25 30

Met Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 145
<211> LENGTH: 39
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 145

Pro Gly Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys
1 5 10 15

Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly
20 25 30

Ser Met Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 146
<211> LENGTH: 52
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 146

Leu Arg Val Asp Thr Lys Ser Arg Ala Ala Trp Ala Arg Leu Leu Gln
1 5 10 15

Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu
20 25 30

Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser
35 40 45

Gly Leu Gly Cys
50

<210> SEQ ID NO 147
<211> LENGTH: 51
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 147

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Arg Val Asp Thr Lys Ser Arg Ala Ala Trp Ala Arg Leu Leu Gln Glu
1               5                   10                  15

His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser
            20                  25                  30

Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly
        35                  40                  45

Leu Gly Cys
    50


<210> SEQ ID NO 148
<211> LENGTH: 50
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 148

Val Asp Thr Lys Ser Arg Ala Ala Trp Ala Arg Leu Leu Gln Glu His
1               5                   10                  15

Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys
            20                  25                  30

Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu
        35                  40                  45

Gly Cys
    50


<210> SEQ ID NO 149
<211> LENGTH: 49
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 149

Asp Thr Lys Ser Arg Ala Ala Trp Ala Arg Leu Leu Gln Glu His Pro
1               5                   10                  15

Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly
            20                  25                  30

Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly
        35                  40                  45

Cys


<210> SEQ ID NO 150
<211> LENGTH: 48
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 150

Thr Lys Ser Arg Ala Ala Trp Ala Arg Leu Leu Gln Glu His Pro Asn
1               5                   10                  15

Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys
            20                  25                  30

Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
        35                  40                  45


<210> SEQ ID NO 151
<211> LENGTH: 47
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 151

Lys Ser Arg Ala Ala Trp Ala Arg Leu Leu Gln Glu His Pro Asn Ala
1 5 10 15

Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe
20 25 30

Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
35 40 45

<210> SEQ ID NO 152
<211> LENGTH: 46
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 152

Ser Arg Ala Ala Trp Ala Arg Leu Leu Gln Glu His Pro Asn Ala Arg
1 5 10 15

Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly
20 25 30

Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
35 40 45

<210> SEQ ID NO 153
<211> LENGTH: 45
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 153

Arg Ala Ala Trp Ala Arg Leu Leu Gln Glu His Pro Asn Ala Arg Lys
1 5 10 15

Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu
20 25 30

Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
35 40 45

<210> SEQ ID NO 154
<211> LENGTH: 44
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 154

Ala Ala Trp Ala Arg Leu Leu Gln Glu His Pro Asn Ala Arg Lys Tyr
1 5 10 15

Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys
20 25 30

Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
35 40

<210> SEQ ID NO 155
<211> LENGTH: 43
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 155

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Ala Trp Ala Arg Leu Leu Gln Glu His Pro Asn Ala Arg Lys Tyr Lys
1               5                   10                  15

Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu
            20                  25                  30

Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
        35                  40


<210> SEQ ID NO 156
<211> LENGTH: 42
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 156

Trp Ala Arg Leu Leu Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly
1               5                   10                  15

Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp
            20                  25                  30

Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
        35                  40


<210> SEQ ID NO 157
<211> LENGTH: 41
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 157

Ala Arg Leu Leu Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala
1               5                   10                  15

Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg
            20                  25                  30

Ile Gly Ser Met Ser Gly Leu Gly Cys
        35                  40


<210> SEQ ID NO 158
<211> LENGTH: 40
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 158

Arg Leu Leu Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn
1               5                   10                  15

Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile
            20                  25                  30

Gly Ser Met Ser Gly Leu Gly Cys
        35                  40


<210> SEQ ID NO 159
<211> LENGTH: 39
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 159

Leu Leu Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys
1               5                   10                  15

Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly
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            20                  25                  30

Ser Met Ser Gly Leu Gly Cys
        35


<210> SEQ ID NO 160
<211> LENGTH: 38
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 160

Leu Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys
1               5                   10                  15

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
            20                  25                  30

Met Ser Gly Leu Gly Cys
        35


<210> SEQ ID NO 161
<211> LENGTH: 36
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 161

Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu
1               5                   10                  15

Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser
            20                  25                  30

Gly Leu Gly Cys
        35


<210> SEQ ID NO 162
<211> LENGTH: 35
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 162

His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser
1               5                   10                  15

Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly
            20                  25                  30

Leu Gly Cys
        35


<210> SEQ ID NO 163
<211> LENGTH: 34
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 163

Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys
1               5                   10                  15

Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu
            20                  25                  30

Gly Cys
```

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<210> SEQ ID NO 164
<211> LENGTH: 33
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 164

Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly
1 5 10 15

Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly
20 25 30

Cys

<210> SEQ ID NO 165
<211> LENGTH: 32
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 165

Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys
1 5 10 15

Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25 30

<210> SEQ ID NO 166
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 166

Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe
1 5 10 15

Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25 30

<210> SEQ ID NO 167
<211> LENGTH: 30
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 167

Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly
1 5 10 15

Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25 30

<210> SEQ ID NO 168
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 168

Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu
1 5 10 15

Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys

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20 25

<210> SEQ ID NO 169
<211> LENGTH: 28
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 169

Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys
1 5 10 15

Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25

<210> SEQ ID NO 170
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 170

Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu
1 5 10 15

Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25

<210> SEQ ID NO 171
<211> LENGTH: 26
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 171

Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp
1 5 10 15

Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25

<210> SEQ ID NO 172
<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 172

Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg
1 5 10 15

Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25

<210> SEQ ID NO 173
<211> LENGTH: 24
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 173

Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile
1 5 10 15

Gly Ser Met Ser Gly Leu Gly Cys

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<210> SEQ ID NO 174
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 174

Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly
1 5 10 15

Ser Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 175
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 175

Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met
1 5 10 15

Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 176
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 176

Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser
1 5 10 15

Gly Leu Gly Cys
20

<210> SEQ ID NO 177
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 177

Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly
1 5 10 15

Leu Gly Cys

<210> SEQ ID NO 178
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 178

Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu
1 5 10 15

Gly Cys

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<210> SEQ ID NO 179
<211> LENGTH: 54
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 179

Gly Asp Leu Arg Val Asp Thr Lys Ser Arg Ala Ala Trp Ala Arg Leu
1 5 10 15

Leu Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys
20 25 30

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
35 40 45

Met Ser Gly Leu Gly Cys
50

<210> SEQ ID NO 180
<211> LENGTH: 53
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 180

Asp Leu Arg Val Asp Thr Lys Ser Arg Ala Ala Trp Ala Arg Leu Leu
1 5 10 15

Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly
20 25 30

Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Asn
35 40 45

Ser Gly Leu Gly Cys
50

<210> SEQ ID NO 181
<211> LENGTH: 38
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 181

Gly Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys
1 5 10 15

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
20 25 30

Asn Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 182
<211> LENGTH: 37
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 182

Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly
1 5 10 15

Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Asn
20 25 30

Ser Gly Leu Gly Cys

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35

<210> SEQ ID NO 183
<211> LENGTH: 38
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Sythetic Polypeptide

<400> SEQUENCE: 183

Gly His Lys Ser Glu Val Ala His Arg Phe Lys Gly Ala Asn Lys Lys
1 5 10 15

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
20 25 30

Asn Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 184
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 184

Gly Ala Asn Arg Arg Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu
1 5 10 15

Asp Arg Ile Gly Ser Asn Ser Gly Leu Gly Cys
20 25

<210> SEQ ID NO 185
<211> LENGTH: 54
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 185

Pro Asp Leu Arg Val Asp Thr Lys Ser Arg Ala Ala Trp Ala Arg Leu
1 5 10 15

Leu Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys
20 25 30

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
35 40 45

Met Ser Gly Leu Gly Cys
50

<210> SEQ ID NO 186
<211> LENGTH: 38
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 186

Pro Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys
1 5 10 15

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
20 25 30

Met Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 187

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<211> LENGTH: 34
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 187

Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys
1 5 10 15

Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu
20 25 30

Gly Cys

<210> SEQ ID NO 188
<211> LENGTH: 39
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 188

Pro Gly His Lys Ser Glu Val Ala His Arg Phe Lys Gly Ala Asn Lys
1 5 10 15

Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly
20 25 30

Ser Met Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 189
<211> LENGTH: 28
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 189

Pro Gly Ala Asn Arg Arg Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys
1 5 10 15

Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25

<210> SEQ ID NO 190
<211> LENGTH: 54
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 190

Met Asp Leu Arg Val Asp Thr Lys Ser Arg Ala Ala Trp Ala Arg Leu
1 5 10 15

Leu Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys
20 25 30

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
35 40 45

Met Ser Gly Leu Gly Cys
50

<210> SEQ ID NO 191
<211> LENGTH: 39
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

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<400> SEQUENCE: 191

Met Gly Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys
1 5 10 15

Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly
20 25 30

Ser Met Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 192
<211> LENGTH: 38
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 192

Met Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys
1 5 10 15

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
20 25 30

Met Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 193
<211> LENGTH: 39
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 193

Met Gly His Lys Ser Glu Val Ala His Arg Phe Lys Gly Ala Asn Lys
1 5 10 15

Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly
20 25 30

Ser Met Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 194
<211> LENGTH: 28
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 194

Met Gly Ala Asn Arg Arg Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys
1 5 10 15

Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25

<210> SEQ ID NO 195
<211> LENGTH: 30
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 195

Met Gly Ser Ser His His His His His His Ser Ser Gly Leu Val Pro
1 5 10 15

Arg Gly Ser His Thr Gly Asp Asp Asp Asp Lys His Met Asp

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           20                  25                  30

<210> SEQ ID NO 196
<211> LENGTH: 142
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 196

Met Val Leu Thr Lys Lys Lys Leu Gln Asp Leu Val Arg Glu Val Cys
1               5                   10                  15

Pro Asn Glu Gln Leu Asp Glu Asp Val Glu Glu Met Leu Leu Gln Ile
            20                  25                  30

Ala Asp Asp Phe Ile Glu Ser Val Val Thr Ala Ala Cys Gln Leu Ala
        35                  40                  45

Arg His Arg Lys Ser Ser Thr Leu Glu Val Lys Asp Val Gln Leu His
    50                  55                  60

Leu Glu Arg Gln Trp Asn Met Trp Ile Met Gly Ser Ser His His His
65                  70                  75                  80

His His His Ser Ser Gly Leu Val Pro Arg Gly Ser His Thr Gly Asp
                85                  90                  95

Asp Asp Asp Lys His Met Asp Pro Gly Gln Glu His Pro Asn Ala Arg
            100                 105                 110

Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly
        115                 120                 125

Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
    130                 135                 140


<210> SEQ ID NO 197
<211> LENGTH: 72
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 197

Val Leu Thr Lys Lys Lys Leu Gln Asp Leu Val Arg Glu Val Cys Pro
1               5                   10                  15

Asn Glu Gln Leu Asp Glu Asp Val Glu Glu Met Leu Leu Gln Ile Ala
            20                  25                  30

Asp Asp Phe Ile Glu Ser Val Val Thr Ala Ala Cys Gln Leu Ala Arg
        35                  40                  45

His Arg Lys Ser Ser Thr Leu Glu Val Lys Asp Val Gln Leu His Leu
    50                  55                  60

Glu Arg Gln Trp Asn Met Trp Ile
65                  70


<210> SEQ ID NO 198
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 198

Pro Gly Gln Glu His Pro Asn Ala Arg
1               5


<210> SEQ ID NO 199
<211> LENGTH: 30
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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 199

Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly
1 5 10 15

Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25 30

<210> SEQ ID NO 200
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 200

Gly Ala Asn Lys Pro
1 5

<210> SEQ ID NO 201
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 201

Gly Ala Asn Pro Lys
1 5

<210> SEQ ID NO 202
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 202

Gly Ala Asn Pro Gln
1 5

<210> SEQ ID NO 203
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 203

Gly Ala Asn Gln Lys
1 5

<210> SEQ ID NO 204
<211> LENGTH: 41
<212> TYPE: PRT
<213> ORGANISM: Triakis scyllium

<400> SEQUENCE: 204

Arg Leu Leu Lys Asp Leu Ser Asn Asn Pro Leu Arg Phe Arg Gly Arg
1 5 10 15

Ser Lys Lys Gly Pro Ser Arg Gly Cys Phe Gly Val Lys Leu Asp Arg
20 25 30

Ile Gly Ala Met Ser Gly Leu Gly Cys

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        35                  40


<210> SEQ ID NO 205
<211> LENGTH: 39
<212> TYPE: PRT
<213> ORGANISM: Triakis scyllium

<400> SEQUENCE: 205

Leu Lys Asp Leu Ser Asn Asn Pro Leu Arg Phe Arg Gly Arg Ser Lys
1               5                   10                  15

Lys Gly Pro Ser Arg Gly Cys Phe Gly Val Lys Leu Asp Arg Ile Gly
            20                  25                  30

Ala Met Ser Gly Leu Gly Cys
        35


<210> SEQ ID NO 206
<211> LENGTH: 38
<212> TYPE: PRT
<213> ORGANISM: Triakis scyllium

<400> SEQUENCE: 206

Lys Asp Leu Ser Asn Asn Pro Leu Arg Phe Arg Gly Arg Ser Lys Lys
1               5                   10                  15

Gly Pro Ser Arg Gly Cys Phe Gly Val Lys Leu Asp Arg Ile Gly Ala
            20                  25                  30

Met Ser Gly Leu Gly Cys
        35


<210> SEQ ID NO 207
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Triakis scyllium

<400> SEQUENCE: 207

Gly Pro Ser Arg Gly Cys Phe Gly Val Lys Leu Asp Arg Ile Gly Ala
1               5                   10                  15

Met Ser Gly Leu Gly Cys
            20


<210> SEQ ID NO 208
<211> LENGTH: 41
<212> TYPE: PRT
<213> ORGANISM: Lamna ditropis

<400> SEQUENCE: 208

Arg Leu Leu Lys Asp Leu Ser Asn Asn Pro Leu Arg Phe Lys Gly Arg
1               5                   10                  15

Ser Lys Lys Gly Pro Ser Arg Gly Cys Phe Gly Val Lys Leu Asp Arg
            20                  25                  30

Ile Gly Ala Met Ser Gly Leu Gly Cys
        35                  40


<210> SEQ ID NO 209
<211> LENGTH: 39
<212> TYPE: PRT
<213> ORGANISM: Lamna ditropis

<400> SEQUENCE: 209

Leu Lys Asp Leu Ser Asn Asn Pro Leu Arg Phe Lys Gly Arg Ser Lys
1               5                   10                  15

Lys Gly Pro Ser Arg Gly Cys Phe Gly Val Lys Leu Asp Arg Ile Gly
            20                  25                  30
```

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Ala Met Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 210
<211> LENGTH: 38
<212> TYPE: PRT
<213> ORGANISM: Lamna ditropis

<400> SEQUENCE: 210

Lys Asp Leu Ser Asn Asn Pro Leu Arg Phe Lys Gly Arg Ser Lys Lys
1 5 10 15

Gly Pro Ser Arg Gly Cys Phe Gly Val Lys Leu Asp Arg Ile Gly Ala
20 25 30

Met Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 211
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Lamna ditropis

<400> SEQUENCE: 211

Phe Lys Gly Arg Ser Lys Lys Gly Pro Ser Arg Gly Cys Phe Gly Val
1 5 10 15

Lys Leu Asp Arg Ile Gly Ala Met Ser Gly Leu Gly Cys
20 25

<210> SEQ ID NO 212
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Lamna ditropis

<400> SEQUENCE: 212

Gly Pro Ser Arg Gly Cys Phe Gly Val Lys Leu Asp Arg Ile Gly Ala
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 213
<211> LENGTH: 41
<212> TYPE: PRT
<213> ORGANISM: Squalus acanthias

<400> SEQUENCE: 213

Arg Leu Leu Gln Asp Leu Ser Asn Asn Pro Leu Arg Phe Lys Gly Arg
1 5 10 15

Ser Lys Lys Gly Pro Ser Arg Ser Cys Phe Gly Leu Lys Leu Asp Arg
20 25 30

Ile Gly Ala Met Ser Gly Leu Gly Cys
35 40

<210> SEQ ID NO 214
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Squalus acanthias

<400> SEQUENCE: 214

Gly Pro Ser Arg Ser Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ala
1 5 10 15

Met Ser Gly Leu Gly Cys
20

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<210> SEQ ID NO 215
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Oryzias latipes

<400> SEQUENCE: 215

Gly Trp Asn Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 216
<211> LENGTH: 24
<212> TYPE: PRT
<213> ORGANISM: Oryzias latipes

<400> SEQUENCE: 216

Pro Met Val Ala Gly Gly Gly Cys Phe Gly Met Lys Met Asp Arg Ile
1 5 10 15

Gly Ser Ile Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 217
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Arothron maculatus

<400> SEQUENCE: 217

Gly Arg Ser Ser Met Val Gly Gly Arg Gly Cys Phe Gly Met Lys Ile
1 5 10 15

Asp Arg Ile Gly Ser Ile Ser Gly Leu Gly Cys
20 25

<210> SEQ ID NO 218
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Oryzias latipes

<400> SEQUENCE: 218

Gly Gly Met Arg Ser Cys Phe Gly Val Arg Leu Glu Arg Ile Gly Ser
1 5 10 15

Phe Ser Gly Leu Gly Cys
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<210> SEQ ID NO 219
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Arothron maculatus

<400> SEQUENCE: 219

Gly Gly Leu Arg Ser Cys Phe Gly Val Arg Leu Ala Arg Ile Gly Ser
1 5 10 15

Phe Ser Gly Leu Gly Cys
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<210> SEQ ID NO 220
<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: Oryzias latipes

<400> SEQUENCE: 220

Gly Gly Ser Thr Ser Arg Ser Gly Cys Phe Gly His Lys Met Asp Arg
1 5 10 15

-continued

Ile Gly Thr Ile Ser Gly Met Gly Cys
20 25

<210> SEQ ID NO 221
<211> LENGTH: 24
<212> TYPE: PRT
<213> ORGANISM: Arothron maculatus

<400> SEQUENCE: 221

Gly Gly Ser Ser Arg Ser Gly Cys Phe Gly His Lys Met Asp Arg Ile
1 5 10 15

Gly Thr Ile Ser Gly Met Gly Cys
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<210> SEQ ID NO 222
<211> LENGTH: 39
<212> TYPE: PRT
<213> ORGANISM: Ornithorhynchus anatinus

<400> SEQUENCE: 222

Leu Leu His Asp His Pro Asn Pro Arg Lys Tyr Lys Pro Ala Asn Lys
1 5 10 15

Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly
20 25 30

Ser Thr Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 223
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Ornithorhynchus anatinus

<400> SEQUENCE: 223

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Thr Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 224
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 224

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ala
1 5 10 15

Met Ser Gly Leu Gly Cys
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<210> SEQ ID NO 225
<211> LENGTH: 22
<212> TYPE: PRT
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<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 225

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Gln Ser Gly Leu Gly Cys
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<210> SEQ ID NO 226
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 226

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Ala Ser Gly Leu Gly Cys
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<210> SEQ ID NO 227
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 227

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1 5 10 15

Ser Met Ser Gly Leu Gly Cys
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<210> SEQ ID NO 228
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 228

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys Asn Ser Phe Arg Tyr
20 25

<210> SEQ ID NO 229
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
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<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 229

Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Gln Ser Gly Leu Gly
1 5 10 15

Cys Asn Ser Phe Arg Tyr
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<210> SEQ ID NO 230
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<223> OTHER INFORMATION: Sythetnic Polypeptide
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (5)..(5)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid

<400> SEQUENCE: 230

Ile Glu Gly Arg Xaa

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1 5

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<220> FEATURE:
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<222> LOCATION: (6)..(6)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid

<400> SEQUENCE: 231

Asp Asp Asp Asp Lys Xaa
1 5

<210> SEQ ID NO 232
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
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<222> LOCATION: (2)..(3)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
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<222> LOCATION: (5)..(5)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid

<400> SEQUENCE: 232

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1 5

<210> SEQ ID NO 233
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 233

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<210> SEQ ID NO 234
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<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 234

Gly Ser Asp Arg
1

<210> SEQ ID NO 235
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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 235

Ile Thr Asp Arg
1

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<210> SEQ ID NO 236
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 236

Pro Gly Asp Arg
1

<210> SEQ ID NO 237
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 237

Ala Phe Leu Gly Pro Gly Asp Arg
1 5

What is claimed is:

1. A variant of C-type natriuretic peptide (CNP) selected from the group consisting of:

(SEQ ID NO: 179)
GDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMS GLGC (Gly-CNP53);

(SEQ ID NO: 185)
PDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMS GLGC (Pro-CNP53);

(SEQ ID NO: 190)
MDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMS GLGC (Met-CNP53)

(SEQ ID NO: 180)
DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNS GLGC [CNP-53(M48N)].

2. A pharmaceutical composition comprising a CNP variant of claim **1**, and a pharmaceutically acceptable excipient, carrier or diluent.

3. The composition of claim **2**, which is a lyophilized formulation prepared from a formulation that comprises a citric acid/citrate buffer or an acetic acid/acetate buffer having a pH from about 4 to about 6.

4. The composition of claim **3**, wherein the lyophilized formulation is prepared from a formulation that further comprises (a) an isotonicity-adjusting agent or a bulking agent or (b) an antioxidant.

5. A method of treating a bone-related disorder or skeletal dysplasia, comprising administering a CNP variant to a subject in need thereof, wherein the CNP variant is a CNP variant according to claim **1**, and wherein the bone-related disorder or skeletal dysplasia is selected from the group consisting of achondroplasia, hypochondroplasia, short stature, dwarfism and homozygous achondroplasia.

6. A method for recombinant production of a CNP variant, comprising culturing in a medium a host cell comprising a first polynucleotide encoding a CNP variant polypeptide linked to a second polynucleotide encoding a cleavable peptide or protein under conditions that result in expression of a fusion polypeptide encoded by the polynucleotides, wherein the fusion polypeptide comprises the CNP variant polypeptide directly linked to the cleavable peptide or protein or indirectly linked thereto via a linker, wherein the CNP variant is selected from the group consisting of:

(SEQ ID NO: 179)
GDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMS GLGC (Gly-CNP53);

(SEQ ID NO: 185)
PDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMS GLGC (Pro-CNP53);

(SEQ ID NO: 190)
MDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMS GLGC (Met-CNP53);

(SEQ ID NO: 180)
DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNS GLGC [CNP-53(M48N)].

7. The method of claim **6**, wherein the cleavable peptide or protein is selected from the group consisting of histidine tags, human transcription factor TAF12, TAF12 fragments, TAF12 histone fold domain, mutants of TAF12 and fragments thereof, TAF12(C/A), TAF12(D/E), TAF12(4D/4E), TAF12 (6D/6E), TAF12(10D/10E), TAF12(C/A & D/E), TAF12 (C/A & 4D/4E), TAF12(C/A & 6D/6E), TAF12(C/A & 10D/10E), ketosteroid isomerase, maltose-binding protein, β-galactosidase, glutathione-S-transferase, thioredoxin, chitin-binding domain, BMP-2, BMP-2 mutants, BMP-2(C/A), and mutants and fragments thereof.

8. The method of claim **6**, wherein the host cell is bacterial.

9. The method of claim **6**, wherein the fusion polypeptide is expressed as a soluble protein or as an inclusion body.

10. The method of claim **6**, further comprising isolating the expressed fusion polypeptide from the host cell or culture medium.

11. The method of claim **10**, further comprising contacting the isolated fusion polypeptide with a cleaving agent selected from the group consisting of formic acid, cyanogen bromide (CNBr), hydroxylamine, protein self cleavage, Factor Xa, enterokinase, ProTEV, and SUMO protease.

12. A CNP variant produced according to the method of claim **6**, wherein the CNP variant is selected from the group consisting of:

(SEQ ID NO: 179)
GDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMS
GLGC (Gly-CNP53));

(SEQ ID NO: 185)
PDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMS
GLGC (Pro-CNP53));

(SEQ ID NO: 190)
MDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMS
GLGC (Met-CNP53));

(SEQ ID NO: 180)
DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSG
LGC [CNP-53(M48N)]).

13. A method of increasing long bone growth, comprising administering a CNP variant according to claim **1** to a subject in need thereof, where the administration increases long bone growth.

14. A CNP variant according to claim **1** useful for increasing long bone growth or treating achondroplasia, hypochondroplasia, short stature, dwarfism, or homozygous achondroplasia in a subject in need thereof.

* * * * *

Docket No.: 30610/44556C1-R2
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Reissue Application of:
Daniel J. Wendt et al.

Application No.: Not Yet Assigned	Confirmation No.: Not Yet Assigned
Filed: Concurrently Herewith	Art Unit: Not Yet Assigned
For: Variants of C-Type Natriuretic Peptide	Examiner: Not Yet Assigned

PRELIMINARY AMENDMENT UNDER 37 C.F.R. § 1.115

MS Reissue
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

INTRODUCTORY COMMENTS

Prior to examination on the merits, please amend the above-identified U.S. patent application as follows:

Amendments to the Specification begin on page **2** of this paper.

Amendments to the Claims begin on page **3** of this paper.

Remarks/Arguments begin on page **28** of this paper.

AMENDMENT TO THE SPECIFICATION

Please replace the first paragraph of the specification with the following amended paragraph:

[[The present application]] This application is a continuation reissue of Reissue Application No. 14/604,262, which is an application for reissue of U.S. Patent No. 8,598,121, issued December 3, 2013, which is a continuation of U. S. Patent Application No. 12/784,117, now U. S. Patent 8,198,242, issued June 12, 2012, which claims the priority and benefit of US Provisional Application No. 61/254,563, filed on October 23, 2009, and US Provisional Application No. 61/180,112, filed on May 20, 2009, the disclosure of each of which is incorporated herein by reference in its entirety.

AMENDMENTS TO THE CLAIMS

Cancel claim 1.

Cancel claim 2.

Cancel claim 3.

Cancel claim 4.

Cancel claim 5.

Cancel claim 6.

Cancel claim 7.

Cancel claim 8.

Cancel claim 9.

Cancel claim 10.

Cancel claim 11.

Cancel claim 12.

Cancel claim 13.

Cancel claim 14.

15. (New) A macromolecule capable of releasing a CNP variant comprising a synthetic polymeric group coupled to the CNP variant through a hydrolysable linkage, wherein the CNP variant is selected from the group consisting of:
PGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-Gly-CNP37) (SEQ ID NO:145);
GDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP53) (SEQ ID NO: 179);
PDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP53) (SEQ ID NO: 185);
MDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP53) (SEQ ID NO: 190);
DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP-53(M48N)] (SEQ ID NO: 180);
LRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-52) (SEQ ID NO: 146);
RVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC

(CNP-51) (SEQ ID NO: 147);
VDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-50) (SEQ ID NO: 148);
DTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-49) (SEQ ID NO: 149);
TKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-48) (SEQ ID NO: 150);
KSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-47) (SEQ ID NO: 151);
SRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-46) (SEQ ID NO: 152);
RAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-45) (SEQ ID NO: 153);
AAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-44) (SEQ ID NO: 154);
AWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-43) (SEQ ID NO: 155);
WARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-42) (SEQ ID NO: 156);
ARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-41) (SEQ ID NO: 157);
RLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-40) (SEQ ID NO:158);
LLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-39)
(SEQ ID NO: 159);
LQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-38)
(SEQ ID NO: 160);
QEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-37)
(SEQ ID NO: 60);
EHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-36) (SEQ ID NO: 161);
HPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-35) (SEQ ID NO: 162);

PNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-34) (SEQ ID NO: 163);
NARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-33) (SEQ ID NO: 164);
ARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-32) (SEQ ID NO: 165);
RKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-31) (SEQ ID NO: 166);
KYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-30) (SEQ ID NO: 167);
YKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-29) (SEQ ID NO: 168);
KGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-28) (SEQ ID NO: 169);
GANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-27) (SEQ ID NO: 170);
ANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-26) (SEQ ID NO: 171);
NKKGLSKGCFGLKLDRIGSMSGLGC (CNP-25) (SEQ ID NO: 172);
KKGLSKGCFGLKLDRIGSMSGLGC (CNP-24) (SEQ ID NO: 173);
KGLSKGCFGLKLDRIGSMSGLGC (CNP-23) (SEQ ID NO: 174);
LSKGCFGLKLDRIGSMSGLGC (CNP-21) (SEQ ID NO: 175);
SKGCFGLKLDRIGSMSGLGC (CNP-20) (SEQ ID NO: 176);
KGCFGLKLDRIGSMSGLGC (CNP- 19) (SEQ ID NO: 177);
GCFGLKLDRIGSMSGLGC (CNP-18) (SEQ ID NO: 178);
QEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP37(M32N)] (SEQ ID NO: 182);
PQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP37) (SEQ ID NO: 186);
MQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP37) (SEQ ID NO: 192);
GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP37) (SEQ ID NO: 75);
GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [Gly-CNP37(M32N)] (SEQ ID NO: 181);
MGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-Gly-CNP37) (SEQ ID NO: 145);
GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (HSA-CNP27) (SEQ ID NO: 144);

GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSNSGLGC [HSA-CNP27(M22N)] (SEQ ID NO: 183);

PGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-HSA-CNP27) (SEQ ID NO: 188);

MGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-HSA-CNP27) (SEQ ID NO: 193);

GANRRGLSRGCFGLKLDRIGSMSGLGC [CNP27(K4,5,9R)] (SEQ ID NO: 36);

GANRRGLSRGCFGLKLDRIGSNSGLGC [CNP27(K4,5,9R, M22N)] (SEQ ID NO: 184);

PGANRRGLSRGCFGLKLDRIGSMSGLGC [Pro-CNP27(K4,5,9R)] (SEQ ID NO: 189);

MGANRRGLSRGCFGLKLDRIGSMSGLGC [Met-CNP27(K4,5,9R)] (SEQ ID NO: 194);

PEGIK-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-CNP27(K4,5,9R)] (SEQ ID NO: 36);

PEGIK-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEG1K-CNP27(K4,5,9R, M22N)] (SEQ ID NO: 184);

PEG1K-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-Pro-CNP27(K4,5,9R)] (SEQ ID NO: 189);

PEG1K-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-Met-CNP27(K4,5,9R)] (SEQ ID NO: 194);

PE012-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-CNP27(K4,5,9R)] (SEQ ID NO: 36);

PE012-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEO12-CNP27(K4,5,9R, M22N)] (SEQ ID NO: 184);

PEO12-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-Pro-CNP27(K4,5,9R)] (SEQ ID NO: 189);

PEO12-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-Met-CNP27(K4,5,9R)] (SEQ ID NO: 194);

PEO24-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-CNP27(K4,5,9R)] (SEQ ID NO: 36);

PEO24-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEO24-CNP27(K4,5,9R, M22N)] (SEQ ID NO: 184);
PEO24-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-Pro-CNP27(K4,5,9R)] (SEQ ID NO: 189); and
PEO24-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-Met-CNP27(K4,5,9R)] (SEQ ID NO: 194), and wherein hydrolysis of the hydrolysable linkage releases the CNP variant.

16. (New) The macromolecule of claim 15, wherein the synthetic polymeric group comprises a hydrophilic polymeric moiety.

17. (New) The macromolecule of claim 16, wherein the hydrophilic polymeric moiety comprises polyethylene glycol (PEG).

18. (New) A sustained release CNP variant formulation comprising a synthetic polymeric group coupled to the CNP variant through a hydrolysable linkage, wherein the CNP variant is selected from the group consisting of:
PGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-Gly-CNP37) (SEQ ID NO:145);
GDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP53) (SEQ ID NO: 179);
PDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP53) (SEQ ID NO: 185);
MDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP53) (SEQ ID NO: 190);
DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP-53(M48N)] (SEQ ID NO: 180);
LRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-52) (SEQ ID NO: 146);
RVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-51) (SEQ ID NO: 147);

VDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-50) (SEQ ID NO: 148);
DTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-49) (SEQ ID NO: 149);
TKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-48) (SEQ ID NO: 150);
KSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-47) (SEQ ID NO: 151);
SRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-46) (SEQ ID NO: 152);
RAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-45) (SEQ ID NO: 153);
AAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-44) (SEQ ID NO: 154);
AWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-43) (SEQ ID NO: 155);
WARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-42) (SEQ ID NO: 156);
ARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-41) (SEQ ID NO: 157);
RLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-40) (SEQ ID NO: 158);
LLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-39) (SEQ ID NO: 159);
LQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-38) (SEQ ID NO: 160);
QEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-37) (SEQ ID NO: 60);
EHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-36) (SEQ ID NO: 161);
HPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-35) (SEQ ID NO: 162);
PNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-34) (SEQ ID NO: 163);

NARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-33) (SEQ ID NO: 164);
ARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-32) (SEQ ID NO: 165);
RKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-31) (SEQ ID NO: 166);
KYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-30) (SEQ ID NO: 167);
YKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-29) (SEQ ID NO: 168);
KGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-28) (SEQ ID NO: 169);
GANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-27) (SEQ ID NO: 170);
ANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-26) (SEQ ID NO: 171);
NKKGLSKGCFGLKLDRIGSMSGLGC (CNP-25) (SEQ ID NO: 172);
KKGLSKGCFGLKLDRIGSMSGLGC (CNP-24) (SEQ ID NO: 173);
KGLSKGCFGLKLDRIGSMSGLGC (CNP-23) (SEQ ID NO: 174);
LSKGCFGLKLDRIGSMSGLGC (CNP-21) (SEQ ID NO: 175);
SKGCFGLKLDRIGSMSGLGC (CNP-20) (SEQ ID NO: 176);
KGCFGLKLDRIGSMSGLGC (CNP- 19) (SEQ ID NO: 177);
GCFGLKLDRIGSMSGLGC (CNP-18) (SEQ ID NO: 178);
QEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP37(M32N)] (SEQ ID NO: 182);
PQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP37) (SEQ ID NO: 186);
MQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP37) (SEQ ID NO: 192);
GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP37) (SEQ ID NO: 75);
GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [Gly-CNP37(M32N)] (SEQ ID NO: 181);
MGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-Gly-CNP37) (SEQ ID NO: 145);
GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (HSA-CNP27) (SEQ ID NO: 144);
GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSNSGLGC [HSA-CNP27(M22N)] (SEQ ID NO: 183);

PGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-HSA-CNP27) (SEQ ID NO: 188);

MGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-HSA-CNP27) (SEQ ID NO: 193);

GANRRGLSRGCFGLKLDRIGSMSGLGC [CNP27(K4,5,9R)] (SEQ ID NO: 36);

GANRRGLSRGCFGLKLDRIGSNSGLGC [CNP27(K4,5,9R, M22N)] (SEQ ID NO: 184);

PGANRRGLSRGCFGLKLDRIGSMSGLGC [Pro-CNP27(K4,5,9R)] (SEQ ID NO: 189);

MGANRRGLSRGCFGLKLDRIGSMSGLGC [Met-CNP27(K4,5,9R)] (SEQ ID NO: 194);

PEGIK-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-CNP27(K4,5,9R)] (SEQ ID NO: 36);

PEGIK-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEG1K-CNP27(K4,5,9R, M22N)] (SEQ ID NO: 184);

PEG1K-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-Pro-CNP27(K4,5,9R)] (SEQ ID NO: 189);

PEG1K-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-Met-CNP27(K4,5,9R)] (SEQ ID NO: 194);

PE012-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-CNP27(K4,5,9R)] (SEQ ID NO: 36);

PE012-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEO12-CNP27(K4,5,9R, M22N)] (SEQ ID NO: 184);

PEO12-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-Pro-CNP27(K4,5,9R)] (SEQ ID NO: 189);

PEO12-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-Met-CNP27(K4,5,9R)] (SEQ ID NO: 194);

PEO24-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-CNP27(K4,5,9R)] (SEQ ID NO: 36);

PEO24-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEO24-CNP27(K4,5,9R, M22N)] (SEQ ID NO: 184);

PEO24-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-Pro-CNP27(K4,5,9R)] (SEQ ID NO: 189); and
PEO24-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-Met-CNP27(K4,5,9R)] (SEQ ID NO: 194).

19. (New) The sustained release formulation of claim 18, wherein the synthetic polymeric group comprises a hydrophilic polymeric moiety.

20. (New) The sustained release formulation of claim 19, wherein the hydrophilic polymeric moiety comprises polyethylene glycol (PEG).

21. (New) A method of stimulating cGMP production in a cell expressing natriuretic peptide receptor B (NPR-B) comprising contacting the cell expressing NPR-B with a CNP variant selected from the group consisting of:
PGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-Gly-CNP37) (SEQ ID NO:145);
GDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP53) (SEQ ID NO: 179);
PDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP53) (SEQ ID NO: 185);
MDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP53) (SEQ ID NO: 190);
DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP-53(M48N)] (SEQ ID NO: 180);
LRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-52) (SEQ ID NO: 146);
RVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-51) (SEQ ID NO: 147);
VDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-50) (SEQ ID NO: 148);
DTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-

49) (SEQ ID NO: 149);
TKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-48)
(SEQ ID NO: 150);
KSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-47)
(SEQ ID NO: 151);
SRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-46)
(SEQ ID NO: 152);
RAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-45)
(SEQ ID NO: 153);
AAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-44) (SEQ
ID NO: 154);
AWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-43) (SEQ
ID NO: 155);
WARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-42) (SEQ ID
NO: 156);
ARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-41) (SEQ ID
NO: 157);
RLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-40) (SEQ ID
NO: 158);
LLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-39)
(SEQ ID NO: 159);
LQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-38)
(SEQ ID NO: 160);
QEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-37)
(SEQ ID NO: 60);
EHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-36) (SEQ ID NO: 161);
HPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-35) (SEQ ID NO: 162);
PNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-34) (SEQ ID NO: 163);
NARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-33) (SEQ ID NO: 164);
ARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-32) (SEQ ID NO: 165);
RKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-31) (SEQ ID NO: 166);

KYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-30) (SEQ ID NO: 167);
YKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-29) (SEQ ID NO: 168);
KGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-28) (SEQ ID NO: 169);
GANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-27) (SEQ ID NO: 170);
ANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-26) (SEQ ID NO: 171);
NKKGLSKGCFGLKLDRIGSMSGLGC (CNP-25) (SEQ ID NO: 172);
KKGLSKGCFGLKLDRIGSMSGLGC (CNP-24) (SEQ ID NO: 173);
KGLSKGCFGLKLDRIGSMSGLGC (CNP-23) (SEQ ID NO: 174);
LSKGCFGLKLDRIGSMSGLGC (CNP-21) (SEQ ID NO: 175);
SKGCFGLKLDRIGSMSGLGC (CNP-20) (SEQ ID NO: 176);
KGCFGLKLDRIGSMSGLGC (CNP- 19) (SEQ ID NO: 177);
GCFGLKLDRIGSMSGLGC (CNP-18) (SEQ ID NO: 178);
QEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP37(M32N)] (SEQ ID NO: 182);
PQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP37) (SEQ ID NO: 186);
MQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP37) (SEQ ID NO: 192);
GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP37) (SEQ ID NO: 75);
GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [Gly-CNP37(M32N)] (SEQ ID NO: 181);
MGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-Gly-CNP37) (SEQ ID NO: 145);
GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (HSA-CNP27) (SEQ ID NO: 144);
GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSNSGLGC [HSA-CNP27(M22N)] (SEQ ID NO: 183);
PGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-HSA-CNP27) (SEQ ID NO: 188);

MGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-HSA-CNP27) (SEQID NO: 193);

GANRRGLSRGCFGLKLDRIGSMSGLGC [CNP27(K4,5,9R)] (SEQ ID NO: 36);

GANRRGLSRGCFGLKLDRIGSNSGLGC [CNP27(K4,5,9R, M22N)] (SEQ ID NO: 184);

PGANRRGLSRGCFGLKLDRIGSMSGLGC [Pro-CNP27(K4,5,9R)] (SEQ ID NO: 189);

MGANRRGLSRGCFGLKLDRIGSMSGLGC [Met-CNP27(K4,5,9R)] (SEQ ID NO: 194);

PEGIK-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-CNP27(K4,5,9R)] (SEQ ID NO: 36);

PEGIK-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEG1K-CNP27(K4,5,9R, M22N)] (SEQ ID NO: 184);

PEG1K-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-Pro-CNP27(K4,5,9R)] (SEQ ID NO: 189);

PEG1K-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-Met-CNP27(K4,5,9R)] (SEQ ID NO: 194);

PE012-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-CNP27(K4,5,9R)] (SEQ ID NO: 36);

PE012-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEO12-CNP27(K4,5,9R, M22N)] (SEQ ID NO: 184);

PEO12-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-Pro-CNP27(K4,5,9R)] (SEQ ID NO: 189);

PEO12-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-Met-CNP27(K4,5,9R)] (SEQ ID NO: 194);

PEO24-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-CNP27(K4,5,9R)] (SEQ ID NO: 36);

PEO24-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEO24-CNP27(K4,5,9R, M22N)] (SEQ ID NO: 184);

PEO24-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-Pro-CNP27(K4,5,9R)] (SEQ ID NO: 189); and

PEO24-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-Met-CNP27(K4,5,9R)] (SEQ ID NO: 194).

22. (New) A method of overcoming cell growth arrest induced by a constitutively active mutant fibroblast growth factor receptor 3 (FGFR-3) comprising contacting a cell expressing the constitutively active FGFR-3 with a CNP variant selected from the group consisting of:
PGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-Gly-CNP37) (SEQ ID NO:145);
GDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP53) (SEQ ID NO: 179);
PDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP53) (SEQ ID NO: 185);
MDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP53) (SEQ ID NO: 190);
DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP-53(M48N)] (SEQ ID NO: 180);
LRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-52) (SEQ ID NO: 146);
RVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-51) (SEQ ID NO: 147);
VDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-50) (SEQ ID NO: 148);
DTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-49) (SEQ ID NO: 149);
TKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-48) (SEQ ID NO: 150);
KSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-47) (SEQ ID NO: 151);
SRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-46) (SEQ ID NO: 152);

RAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-45) (SEQ ID NO: 153);
AAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-44) (SEQ ID NO: 154);
AWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-43) (SEQ ID NO: 155);
WARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-42) (SEQ ID NO: 156);
ARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-41) (SEQ ID NO: 157);
RLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-40) (SEQ ID NO: 158);
LLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-39) (SEQ ID NO: 159);
LQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-38) (SEQ ID NO: 160);
QEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-37) (SEQ ID NO: 60);
EHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-36) (SEQ ID NO: 161);
HPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-35) (SEQ ID NO: 162);
PNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-34) (SEQ ID NO: 163);
NARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-33) (SEQ ID NO: 164);
ARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-32) (SEQ ID NO: 165);
RKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-31) (SEQ ID NO: 166);
KYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-30) (SEQ ID NO: 167);
YKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-29) (SEQ ID NO: 168);
KGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-28) (SEQ ID NO: 169);
GANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-27) (SEQ ID NO: 170);
ANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-26) (SEQ ID NO: 171);
NKKGLSKGCFGLKLDRIGSMSGLGC (CNP-25) (SEQ ID NO: 172);
KKGLSKGCFGLKLDRIGSMSGLGC (CNP-24) (SEQ ID NO: 173);

KGLSKGCFGLKLDRIGSMSGLGC (CNP-23) (SEQ ID NO: 174);
LSKGCFGLKLDRIGSMSGLGC (CNP-21) (SEQ ID NO: 175);
SKGCFGLKLDRIGSMSGLGC (CNP-20) (SEQ ID NO: 176);
KGCFGLKLDRIGSMSGLGC (CNP- 19) (SEQ ID NO: 177);
GCFGLKLDRIGSMSGLGC (CNP-18) (SEQ ID NO: 178);
QEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP37(M32N)] (SEQ ID NO: 182);
PQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP37) (SEQ ID NO: 186);
MQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP37) (SEQ ID NO: 192);
GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP37) (SEQ ID NO: 75);
GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [Gly-CNP37(M32N)] (SEQ ID NO: 181);
MGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-Gly-CNP37) (SEQ ID NO: 145);
GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (HSA-CNP27) (SEQ ID NO: 144);
GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSNSGLGC [HSA-CNP27(M22N)] (SEQ ID NO: 183);
PGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-HSA-CNP27) (SEQ ID NO: 188);
MGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-HSA-CNP27) (SEQ ID NO: 193);
GANRRGLSRGCFGLKLDRIGSMSGLGC [CNP27(K4,5,9R)] (SEQ ID NO: 36);
GANRRGLSRGCFGLKLDRIGSNSGLGC [CNP27(K4,5,9R, M22N)] (SEQ ID NO: 184);
PGANRRGLSRGCFGLKLDRIGSMSGLGC [Pro-CNP27(K4,5,9R)] (SEQ ID NO: 189);
MGANRRGLSRGCFGLKLDRIGSMSGLGC [Met-CNP27(K4,5,9R)]

(SEQ ID NO: 194);
PEGIK-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-CNP27(K4,5,9R)] (SEQ ID NO: 36);
PEGIK-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEG1K-CNP27(K4,5,9R, M22N)] (SEQ ID NO: 184);
PEG1K-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-Pro-CNP27(K4,5,9R)] (SEQ ID NO: 189);
PEG1K-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-Met-CNP27(K4,5,9R)] (SEQ ID NO: 194);
PE012-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-CNP27(K4,5,9R)] (SEQ ID NO: 36);
PE012-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEO12-CNP27(K4,5,9R, M22N)] (SEQ ID NO: 184);
PEO12-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-Pro-CNP27(K4,5,9R)] (SEQ ID NO: 189);
PEO12-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-Met-CNP27(K4,5,9R)] (SEQ ID NO: 194);
PEO24-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-CNP27(K4,5,9R)] (SEQ ID NO: 36);
PEO24-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEO24-CNP27(K4,5,9R, M22N)] (SEQ ID NO: 184);
PEO24-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-Pro-CNP27(K4,5,9R)] (SEQ ID NO: 189); and
PEO24-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-Met-CNP27(K4,5,9R)] (SEQ ID NO: 194).

23. (New) A method of treating a bone-related disorder or skeletal dysplasia, comprising administering a macromolecule capable of releasing a CNP variant comprising a synthetic polymeric group coupled to the CNP variant through a hydrolysable linkage, wherein the CNP variant is selected from the group consisting of:

PGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-Gly-CNP37) (SEQ ID NO:145);

GDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP53) (SEQ ID NO: 179);

PDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP53) (SEQ ID NO: 185);

MDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP53) (SEQ ID NO: 190);

DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP-53(M48N)] (SEQ ID NO: 180);

LRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-52) (SEQ ID NO: 146);

RVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-51) (SEQ ID NO: 147);

VDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-50) (SEQ ID NO: 148);

DTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-49) (SEQ ID NO: 149);

TKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-48) (SEQ ID NO: 150);

KSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-47) (SEQ ID NO: 151);

SRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-46) (SEQ ID NO: 152);

RAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-45) (SEQ ID NO: 153);

AAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-44) (SEQ ID NO: 154);

AWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-43) (SEQ ID NO: 155);

WARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-42) (SEQ ID NO: 156);
ARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-41) (SEQ ID NO: 157);
RLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-40) (SEQ ID NO: 158);
LLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-39) (SEQ ID NO: 159);
LQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-38) (SEQ ID NO: 160);
QEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-37) (SEQ ID NO: 60);
EHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-36) (SEQ ID NO: 161);
HPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-35) (SEQ ID NO: 162);
PNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-34) (SEQ ID NO: 163);
NARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-33) (SEQ ID NO: 164);
ARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-32) (SEQ ID NO: 165);
RKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-31) (SEQ ID NO: 166);
KYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-30) (SEQ ID NO: 167);
YKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-29) (SEQ ID NO: 168);
KGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-28) (SEQ ID NO: 169);
GANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-27) (SEQ ID NO: 170);
ANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-26) (SEQ ID NO: 171);
NKKGLSKGCFGLKLDRIGSMSGLGC (CNP-25) (SEQ ID NO: 172);
KKGLSKGCFGLKLDRIGSMSGLGC (CNP-24) (SEQ ID NO: 173);
KGLSKGCFGLKLDRIGSMSGLGC (CNP-23) (SEQ ID NO: 174);
LSKGCFGLKLDRIGSMSGLGC (CNP-21) (SEQ ID NO: 175);
SKGCFGLKLDRIGSMSGLGC (CNP-20) (SEQ ID NO: 176);
KGCFGLKLDRIGSMSGLGC (CNP- 19) (SEQ ID NO: 177);
GCFGLKLDRIGSMSGLGC (CNP-18) (SEQ ID NO: 178);

QEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP37(M32N)] (SEQ ID NO: 182);
PQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP37) (SEQ ID NO: 186);
MQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP37) (SEQ ID NO: 192);
GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP37) (SEQ ID NO: 75);
GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [Gly-CNP37(M32N)] (SEQ ID NO: 181);
MGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-Gly-CNP37) (SEQ ID NO: 145);
GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (HSA-CNP27) (SEQ ID NO: 144);
GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSNSGLGC [HSA-CNP27(M22N)] (SEQ ID NO: 183);
PGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-HSA-CNP27) (SEQ ID NO: 188);
MGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-HSA-CNP27) (SEQ ID NO: 193);
GANRRGLSRGCFGLKLDRIGSMSGLGC [CNP27(K4,5,9R)] (SEQ ID NO: 36);
GANRRGLSRGCFGLKLDRIGSNSGLGC [CNP27(K4,5,9R, M22N)] (SEQ ID NO: 184);
PGANRRGLSRGCFGLKLDRIGSMSGLGC [Pro-CNP27(K4,5,9R)] (SEQ ID NO: 189);
MGANRRGLSRGCFGLKLDRIGSMSGLGC [Met-CNP27(K4,5,9R)] (SEQ ID NO: 194);
PEGIK-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-CNP27(K4,5,9R)] (SEQ ID NO: 36);
PEGIK-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEG1K-CNP27(K4,5,9R, M22N)] (SEQ ID NO: 184);

PEG1K-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-Pro-CNP27(K4,5,9R)] (SEQ ID NO: 189);
PEG1K-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-Met-CNP27(K4,5,9R)] (SEQ ID NO: 194);
PE012-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-CNP27(K4,5,9R)] (SEQ ID NO: 36);
PE012-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEO12-CNP27(K4,5,9R, M22N)] (SEQ ID NO: 184);
PEO12-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-Pro-CNP27(K4,5,9R)] (SEQ ID NO: 189);
PEO12-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-Met-CNP27(K4,5,9R)] (SEQ ID NO: 194);
PEO24-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-CNP27(K4,5,9R)] (SEQ ID NO: 36);
PEO24-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEO24-CNP27(K4,5,9R, M22N)] (SEQ ID NO: 184);
PEO24-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-Pro-CNP27(K4,5,9R)] (SEQ ID NO: 189); and
PEO24-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-Met-CNP27(K4,5,9R)] (SEQ ID NO: 194), and wherein hydrolysis of the hydrolysable linkage releases the CNP variant.

24. (New) The method of claim 23, wherein the bone-related disorder or skeletal dysplasia is selected from the group consisting of achondroplasia, hypochondroplasia, short stature, dwarfism and homozygous achondroplasia.

25. (New) The method of claim 24, wherein the synthetic polymeric group comprises a hydrophilic polymeric moiety.

26. (New) The method of claim 25, wherein the hydrophilic polymeric moiety comprises polyethylene glycol (PEG).

27. (New) A method of treating a bone-related disorder or skeletal dysplasia, comprising administering a sustained CNP variant release formulation comprising a synthetic polymeric group coupled to the CNP variant through a hydrolysable linkage, wherein the CNP variant is selected from the group consisting of:

PGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-Gly-CNP37) (SEQ ID NO:145);

GDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP53) (SEQ ID NO: 179);

PDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP53) (SEQ ID NO: 185);

MDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP53) (SEQ ID NO: 190);

DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP-53(M48N)] (SEQ ID NO: 180);

LRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-52) (SEQ ID NO: 146);

RVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-51) (SEQ ID NO: 147);

VDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-50) (SEQ ID NO: 148);

DTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-49) (SEQ ID NO: 149);

TKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-48) (SEQ ID NO: 150);

KSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-47) (SEQ ID NO: 151);

SRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-46)

(SEQ ID NO: 152);
RAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-45)
(SEQ ID NO: 153);
AAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-44) (SEQ ID NO: 154);
AWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-43) (SEQ ID NO: 155);
WARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-42) (SEQ ID NO: 156);
ARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-41) (SEQ ID NO: 157);
RLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-40) (SEQ ID NO: 158);
LLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-39)
(SEQ ID NO: 159);
LQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-38)
(SEQ ID NO: 160);
QEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-37)
(SEQ ID NO: 60);
EHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-36) (SEQ ID NO: 161);
HPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-35) (SEQ ID NO: 162);
PNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-34) (SEQ ID NO: 163);
NARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-33) (SEQ ID NO: 164);
ARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-32) (SEQ ID NO: 165);
RKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-31) (SEQ ID NO: 166);
KYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-30) (SEQ ID NO: 167);
YKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-29) (SEQ ID NO: 168);
KGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-28) (SEQ ID NO: 169);
GANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-27) (SEQ ID NO: 170);
ANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-26) (SEQ ID NO: 171);
NKKGLSKGCFGLKLDRIGSMSGLGC (CNP-25) (SEQ ID NO: 172);

KKGLSKGCFGLKLDRIGSMSGLGC (CNP-24) (SEQ ID NO: 173);
KGLSKGCFGLKLDRIGSMSGLGC (CNP-23) (SEQ ID NO: 174);
LSKGCFGLKLDRIGSMSGLGC (CNP-21) (SEQ ID NO: 175);
SKGCFGLKLDRIGSMSGLGC (CNP-20) (SEQ ID NO: 176);
KGCFGLKLDRIGSMSGLGC (CNP- 19) (SEQ ID NO: 177);
GCFGLKLDRIGSMSGLGC (CNP-18) (SEQ ID NO: 178);
QEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP37(M32N)] (SEQ ID NO: 182);
PQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP37) (SEQ ID NO: 186);
MQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP37) (SEQ ID NO: 192);
GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP37) (SEQ ID NO: 75);
GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [Gly-CNP37(M32N)] (SEQ ID NO: 181);
MGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-Gly-CNP37) (SEQ ID NO: 145);
GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (HSA-CNP27) (SEQ ID NO: 144);
GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSNSGLGC [HSA-CNP27(M22N)] (SEQ ID NO: 183);
PGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-HSA-CNP27) (SEQ ID NO: 188);
MGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-HSA-CNP27) (SEQ ID NO: 193);
GANRRGLSRGCFGLKLDRIGSMSGLGC [CNP27(K4,5,9R)] (SEQ ID NO: 36);
GANRRGLSRGCFGLKLDRIGSNSGLGC [CNP27(K4,5,9R, M22N)] (SEQ ID NO: 184);
PGANRRGLSRGCFGLKLDRIGSMSGLGC [Pro-CNP27(K4,5,9R)] (SEQ ID NO: 189);

MGANRRGLSRGCFGLKLDRIGSMSGLGC [Met-CNP27(K4,5,9R)] (SEQ ID NO: 194);
PEGIK-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-CNP27(K4,5,9R)] (SEQ ID NO: 36);
PEGIK-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEG1K-CNP27(K4,5,9R, M22N)] (SEQ ID NO: 184);
PEG1K-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-Pro-CNP27(K4,5,9R)] (SEQ ID NO: 189);
PEG1K-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-Met-CNP27(K4,5,9R)] (SEQ ID NO: 194);
PE012-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-CNP27(K4,5,9R)] (SEQ ID NO: 36);
PE012-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEO12-CNP27(K4,5,9R, M22N)] (SEQ ID NO: 184);
PEO12-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-Pro-CNP27(K4,5,9R)] (SEQ ID NO: 189);
PEO12-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-Met-CNP27(K4,5,9R)] (SEQ ID NO: 194);
PEO24-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-CNP27(K4,5,9R)] (SEQ ID NO: 36);
PEO24-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEO24-CNP27(K4,5,9R, M22N)] (SEQ ID NO: 184);
PEO24-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-Pro-CNP27(K4,5,9R)] (SEQ ID NO: 189); and
PEO24-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-Met-CNP27(K4,5,9R)] (SEQ ID NO: 194).

28. (New) The method of claim 27, wherein the bone-related disorder or skeletal dysplasia is selected from the group consisting of achondroplasia, hypochondroplasia, short stature, dwarfism and homozygous achondroplasia.

29. (New) The method of claim 28, wherein the synthetic polymeric group comprises a hydrophilic polymeric moiety.

30. (New) The method of claim 29, wherein the hydrophilic polymeric moiety comprises polyethylene glycol (PEG).

REMARKS

Applicants request entry of this Preliminary Amendment prior to examination of the merits.

Amendments to the Specification

The first paragraph of the specification is amended to update the cross-reference to related applications and note that the present application is a continuation reissue application. The amendment includes no new matter.

Amendments to the Claims

By way of this amendment, claims 1-14 are canceled and claims 15-30 are new.

Support for the amendments to the claims is found throughout the specification as filed. For example, a macromolecule capable of releasing a CNP variant comprising a synthetic polymeric group coupled to the CNP variant through a hydrolysable linkage is described at col. 10, lines 9-16 of US Patent 8,598,121 ("the '121 patent"). CNP variants are described in cols. 49-53 of the '121 patent. Col. 86, lines 61-67 of the '121 patent describe the CNP variant can be in a sustained release formulation. At least Col. 51, lines 45-66, and Example 5 of the '121 patent describe a method of stimulating cGMP production in a cell expressing natriuretic peptide receptor B (NPR-B) comprising contacting the cell expressing NPR-B with a CNP variant. Example 7 describes a method of overcoming cell growth arrest induced by a constitutively active mutant fibroblast growth factor receptor 3 (FGFR-3) comprising contacting a cell expressing the constitutively active FGFR-3 with a CNP variant. Additionally, col. 24, lines 12-30, of the '121 patent discloses a method of treating a bone-related disorder or skeletal dysplasia, comprising administering a macromolecule capable of releasing a CNP variant or administering a sustained CNP variant release formulation comprising a synthetic polymeric group coupled to

the CNP variant through a hydrolysable linkage. The amendments include no new matter.

The Examiner is invited to contact the undersigned with any questions on this submission.

Dated: July 11, 2017

Respectfully submitted,
Electronic signature:
/Katherine L. Neville/
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Electronic Patent Application Fee Transmittal

Application Number:	
Filing Date:	
Title of Invention:	Variants of C-Type Natriuretic Peptide
First Named Inventor/Applicant Name:	Daniel J. Wendt
Filer:	Katherine Lynn Neville
Attorney Docket Number:	30610/44556C1-R2

Filed as Large Entity

Filing Fees for Reissue (Utility)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
UTILITY REISSUE BASIC	1014	1	280	280
DESIGN AND UTILITY REISSUE BASIC	1114	1	600	600
DESIGN AND UTILITY REISSUE BASIC	1314	1	2160	2160
Pages:				
Claims:				
INDEPENDENT CLAIMS REISSUE	1204	3	420	1260
Miscellaneous-Filing:				
Petition:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
		Total in USD ($)		**4300**

Electronic Acknowledgement Receipt

EFS ID:	29748553
Application Number:	15646822
International Application Number:	
Confirmation Number:	7727
Title of Invention:	Variants of C-Type Natriuretic Peptide
First Named Inventor/Applicant Name:	Daniel J. Wendt
Customer Number:	90849
Filer:	Katherine Lynn Neville
Filer Authorized By:	
Attorney Docket Number:	30610/44556C1-R2
Receipt Date:	11-JUL-2017
Filing Date:	
Time Stamp:	16:40:51
Application Type:	Reissue (Utility)

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$4300
RAM confirmation Number	071217INTEFSW16420700
Deposit Account	132855
Authorized User	Katherine Neville
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: 37 CFR 1.16 (National application filing, search, and examination fees) 37 CFR 1.17 (Patent application and reexamination processing fees)	

37 CFR 1.20 (Post Issuance fees)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Sequence Listing (Text File)	Sequence_Listing_-_From_Parent_44556C1.TXT	108298	no	-
Warnings:					
Information:					
2	Oath or Declaration filed	Declaration_Pursuant_To_37_CFR_1821f.PDF	15944 80c118f0669d2f79660d88ec5d837a1739cb5ef9	no	1
Warnings:					
Information:					
3	Application Data Sheet	Application_Data_Sheet.PDF	30483 a341e2d28dfdf0dc6653e4525d50105ebe7a9f96	no	6
Warnings:					
Information:					
This is not an USPTO supplied ADS fillable form					
4	Transmittal Reissue Application	Reissue_Patent_Application_Transmittal.PDF	34000 e8dd8447853b0ec6d21b1bf9bfd176390bab5b2f	no	1
Warnings:					
Information:					
5	Assignee showing of ownership per 37 CFR 3.73	Statement_By_Assignee_to_Establish_Ownership_37_CFR_373c.PDF	24640 658ccef78b226bf96fbeb823a9a607afcdc63267	no	2
Warnings:					
Information:					
6	Consent of Assignee accompanying the declaration	0111_01_USCN1R_Executed_Consent_of_Assignee.PDF	87819 a8429ff5c5a27d5541a8f937bfd3305bc3259fc5	no	1
Warnings:					

Information:					
7	Power of Attorney	Executed_General_Power_of_Attorney.PDF	49523 75202356b357ffc972ac9d5bb1efe61858370bbc	no	1
Warnings:					
Information:					
8	Specification	Pat_No_8598121_B2_-_2nd_Reissue_Application_as_filed.PDF	14571503 ef64f51ac720dbec3266e5cc466ad52bf59f1beb	no	197
Warnings:					
Information:					
9		44556C1-R2_preliminary_amendment.PDF	82680 4dad8d49a5e19f62d7f2520f90d6ba1bb90f0fe6	yes	29

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Preliminary Amendment	1	1
	Specification	2	2
	Claims	3	27
	Applicant Arguments/Remarks Made in an Amendment	28	29

Warnings:					
Information:					
10	Fee Worksheet (SB06)	fee-info.pdf	36772 c2c366662faa38a4435094bff65f0e3ac6e4d195	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	15041662		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Docket No.: 30610/44556C1-R2
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Reissue Application of:
Daniel J. Wendt et al.

Application No.: Not Yet Assigned	Confirmation No.: Not Yet Assigned
Filed: Concurrently Herewith	Art Unit: Not Yet Assigned
For: Variants of C-Type Natriuretic Peptide	Examiner: Not Yet Assigned

DECLARATION PURSUANT TO 37 C.F.R. § 1.821(f)

MS Sequence
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Submitted herewith for filing in connection with the above-referenced continuation reissue application is a labeled, computer readable copy of the Sequence Listing included in the application.

I hereby state that I have reviewed the paper copy of the Sequence Listing contained in columns 139 to 263 of said Sequence Listing, as required by 37 C.F.R. § 1.821(c), and have reviewed the computer readable form of the Sequence Listing, as required by 37 C.F.R. § 1.821(e), and that the content of the paper and computer readable copies for the above-referenced reissue application are the same as required by 37 C.F.R. § 1.821(f).

Early favorable consideration of the patent application is respectfully solicited.

Dated: July 11, 2017

Respectfully submitted,

Electronic signature:
/Katherine L. Neville/
Katherine L. Neville
Registration No.: 53,379
MARSHALL, GERSTEIN & BORUN LLP
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Attorney for Applicant

Application Data Sheet

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Country of mailing address::	US
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Correspondence Information

Correspondence Customer Number::	90849

Application Information

Application Type::	Reissue
Subject Matter::	Utility
CD-ROM or CD-R?::	None
Sequence submission?::	Yes
Computer Readable Form (CRF)?::	Yes
Title::	Variants of C-Type Natriuretic Peptide
Attorney Docket Number::	30610/44556C1-R2
Request for Early Publication?::	No
Request for Non-Publication?::	No
Total Drawing Sheets::	57
Small Entity?::	No
Petition included?::	No
Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2::	No

This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013:: No

Representative Information

Representative Customer Number:: 90849

Domestic Priority Information

Application::	Continuity Type::	Parent Application::	Parent Filing Date::	Status::
This Application	Continuation Reissue	14/604,262	1/23/2015	Pending
14/604,262	Reissue of	13/466,672	5/08/2012	Patented
13/466,672	Continuation of	12/784,117	5/20/2010	Patented
12/784,117	An application claiming the benefit under 35 USC 119(e)	61/254,563	10/23/2009	Expired
12/784,117	An application claiming the benefit under 35 USC 119(e)	61/180,112	5/20/2009	Expired

Applicant Information

Applicant Number:: 1

Applicant Type:: Assignee

Organization Name:: BioMarin Pharmaceutical Inc.

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City of mailing address:: Novato

State or Province of mailing address:: California

Country of mailing address:: US

Postal or Zip Code of mailing address:: 94949

Authorization or Opt-Out of Authorization to Permit Access

When this Application Data Sheet is properly signed and filed with the application, applicant has provided written authority to permit a participating foreign intellectual property (IP) office access to the instant application-as-filed (see paragraph A in subsection 1 below) and the European Patent Office (EPO) access to any search results from the instant application (see paragraph B in subsection 1 below).

Should applicant choose not to provide an authorization identified in subsection 1 below, applicant **must opt-out** of the authorization by checking the corresponding box A or B or both in subsection 2 below.

NOTE: This section of the Application Data Sheet is **ONLY** reviewed and processed with the **INITIAL** filing of an application. After the initial filing of an application, an Application Data Sheet cannot be used to provide or rescind authorization for access by a foreign IP office(s). Instead, Form PTO/SB/39 or PTO/SB/69 must be used as appropriate.

1. Authorization to Permit Access by a Foreign Intellectual Property Office(s)

A. Priority Document Exchange (PDX) - Unless box A in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the State Intellectual Property Office of the People's Republic of China (SIPO), the World Intellectual Property Organization (WIPO), and any other foreign intellectual property office participating with the USPTO in a bilateral or multilateral priority document exchange agreement in which a foreign application claiming priority to the instant patent application is filed, access to: (1) the instant patent application-as-filed and its related bibliographic data, (2) any foreign or domestic application to which priority or benefit is claimed by the instant application and its related bibliographic data, and (3) the date of filing of this Authorization. See 37 CFR 1.14(h)(1).

B. Search Results from U.S. Application to EPO - Unless box B in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the EPO access to the bibliographic data and search results from the instant patent application when a European patent application claiming priority to the instant patent application is filed. See 37 CFR 1.14(h)(2).

The applicant is reminded that the EPO's Rule 141(1) EPC (European Patent Convention) requires applicants to submit a copy of search results from the instant application without delay in a European patent application that claims priority to the instant application.

2. Opt-Out of Authorizations to Permit Access by a Foreign Intellectual Property Office(s)

☐ A. Applicant **DOES NOT** authorize the USPTO to permit a participating foreign IP office access to the instant application-as-filed. If this box is checked, the USPTO will not be providing a participating foreign IP office with any documents and information identified in subsection 1A above.

☐ B. Applicant **DOES NOT** authorize the USPTO to transmit to the EPO any search results from the instant patent application. If this box is checked, the USPTO will not be providing the EPO with search results from the instant application.

NOTE: Once the application has published or is otherwise publicly available, the USPTO may provide access to the application in accordance with 37 CFR 1.14.

Signature:

NOTE: This Application Data Sheet must be signed in accordance with 37 CFR 1.33(b). **However, if this Application Data Sheet is submitted with the INITIAL filing of the application and either box A or B is not checked in subsection 2 of the "Authorization or Opt-Out of Authorization to Permit Access" section, then this form must also be signed in accordance with 37 CFR 1.14(c).**

This Application Data Sheet **must** be signed by a patent practitioner if one or more of the applicants is a **juristic entity** (e.g., corporation or association). If the applicant is two or more joint inventors, this form must be signed by a patent practitioner, **all** joint inventors who are the applicant, or one or more joint inventor-applicants who have been given power of attorney (e.g., see USPTO Form PTO/AIA/81) on behalf of **all** joint inventor-applicants.

See 37 CFR 1.4(d) for the manner of making signatures and certifications.

Signature	/Katherine L. Neville/	Date (YYYY-MM-DD)	2017-07-11
Name	Katherine L. Neville	Registration Number	53,379

DocCode – SEQ.TXT

SCORE Placeholder Sheet for IFW Content

Application Number: 15646822 Document Date: 07/11/2017

The presence of this form in the IFW record indicates that the following document type was received in electronic format on the date identified above. This content is stored in the SCORE database.

Since this was an electronic submission, there is no physical artifact folder, no artifact folder is recorded in PALM, and no paper documents or physical media exist. The TIFF images in the IFW record were created from the original documents that are stored in SCORE.

- Sequence Listing

At the time of document entry (noted above):

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Form Revision Date: August 26, 2013

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
15/646,822	07/11/2017	Daniel J. Wendt	30610/44556C1-R2

CONFIRMATION NO. 7727

90849
Marshall, Gerstein & Borun LLP (Biomarin)
233 South Wacker Drive
6300 Willis Tower
Chicago, IL 60606

FORMALITIES LETTER

OC000000092740958

Date Mailed: 07/13/2017

NOTICE TO FILE MISSING PARTS OF REISSUE APPLICATION

Filing Date Granted

An application number and filing date have been accorded to this reissue application. The item(s) indicated below, however, are missing. Applicant is given **TWO MONTHS** from the date of this Notice within which to file all required items and pay any fees required below to avoid abandonment. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

- The inventor's oath or declaration has not been submitted.

  An inventor's oath or declaration in compliance with 37 CFR 1.63 or 1.64 (and 37 CFR 1.175) executed by or with respect to each inventor is required. For a reissue application, the inventor's oath or declaration must be submitted prior to examination and cannot be postponed. See 37 CFR 1.175(e). An assignee of the entire interest can execute the oath or declaration if: (1) the application does not seek to enlarge the scope of the claims of the original patent; or (2) the application for the original patent was filed under 37 CFR 1.46 by the assignee of the entire interest. A substitute statement under 37 CFR 1.64 in lieu of an oath or declaration may be submitted in the circumstances provided for in 35 U.S.C. 115(d) and 37 CFR 1.64.

- Surcharge as set forth in 37 CFR 1.16(f) must be submitted.
  The surcharge is due for any one of:
  - late submission of the basic filing fee, search fee, or examination fee,
  - late submission of inventor's oath or declaration,
  - filing an application that does not contain at least one claim on filing, or
  - submission of an application filed by reference to a previously filed application.

SUMMARY OF FEES DUE:

The fee(s) required within **TWO MONTHS** from the date of this Notice to avoid abandonment is/are itemized below. No entity status discount is in effect. If applicant is qualified for small entity status, a written assertion of small entity status must be submitted to establish small entity status. (See 37 CFR 1.27). If applicant is qualified for micro entity status, an acceptable Certification of Micro Entity Status must be submitted to establish micro entity status. (See 37 CFR 1.29 and forms PTO/SB/15A and 15B.)

- $ **1200** for **124** pages in excess of **100** application size fee.
- $ **140** surcharge.
- $(**0**) previous unapplied payment amount.
- $ **1340** TOTAL FEE BALANCE DUE.

Replies must be received in the USPTO within the set time period or must include a proper Certificate of Mailing or Transmission under 37 CFR 1.8 with a mailing or transmission date within the set time period. For more information and a suggested format, see Form PTO/SB/92 and MPEP 512.

Replies should be mailed to:

Mail Stop Missing Parts
Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

Registered users of EFS-Web may alternatively submit their reply to this notice via EFS-Web, including a copy of this Notice and selecting the document description "Applicant response to Pre-Exam Formalities Notice". https://sportal.uspto.gov/authenticate/AuthenticateUserLocalEPF.html

For more information about EFS-Web please call the USPTO Electronic Business Center at 1-866-217-9197 or visit our website at http://www.uspto.gov/ebc.

If you are not using EFS-Web to submit your reply, you must include a copy of this notice.

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at **(571) 272-4000** or **(571) 272-4200** or **1-888-786-0101**.

/resydnor-mcpherson/

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
15/646,822	07/11/2017	1629	4300	30610/44556C1-R2	16	6

CONFIRMATION NO. 7727

FILING RECEIPT

90849
Marshall, Gerstein & Borun LLP (Biomarin)
233 South Wacker Drive
6300 Willis Tower
Chicago, IL 60606

OC000000092740957

Date Mailed: 07/13/2017

Receipt is acknowledged of this reissue patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

Inventor(s)

Daniel J. Wendt, Novato, CA;
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Sianna Castillo, Novato, CA;
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Mika Aoyagi-Scharber, Novato, CA;
Michel Claude Vellard, Novato, CA;
Augustus O. Okamafe, Concord, CA;

Applicant(s)

BioMarin Pharmaceutical Inc., Novato, CA, Assignee (with 37 CFR 1.172 Interest);

Assignment For Published Patent Application

BioMarin Pharmaceutical Inc., Novato, CA

Power of Attorney: The patent practitioners associated with Customer Number 90849

Domestic Priority data as claimed by applicant

This application is a CON of 14/604,262 01/23/2015
which is a REI of 13/466,672 05/08/2012 PAT 8598121
which is a CON of 12/784,117 05/20/2010 PAT 8198242
which claims benefit of 61/180,112 05/20/2009
and claims benefit of 61/254,563 10/23/2009

Foreign Applications for which priority is claimed (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.) - None.
Foreign application information must be provided in an Application Data Sheet in order to constitute a claim to foreign priority. See 37 CFR 1.55 and 1.76.

Permission to Access Application via Priority Document Exchange: Yes

Permission to Access Search Results: Yes

Applicant may provide or rescind an authorization for access using Form PTO/SB/39 or Form PTO/SB/69 as appropriate.

If Required, Foreign Filing License Granted: 07/12/2017

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 15/646,822**

Projected Publication Date: None, application is not eligible for pre-grant publication

Non-Publication Request: No

Early Publication Request: No

Title

Variants of C-Type Natriuretic Peptide

Preliminary Class

514

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4258).

LICENSE FOR FOREIGN FILING UNDER

Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where the conditions for issuance of a license have been met, regardless of whether or not a license may be required as set forth in 37 CFR 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign AssetsControl, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

SelectUSA

The United States represents the largest, most dynamic marketplace in the world and is an unparalleled location for business investment, innovation, and commercialization of new technologies. The U.S. offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to promote and facilitate business investment. SelectUSA provides information assistance to the international investor

community; serves as an ombudsman for existing and potential investors; advocates on behalf of U.S. cities, states, and regions competing for global investment; and counsels U.S. economic development organizations on investment attraction best practices. To learn more about why the United States is the best country in the world to develop technology, manufacture products, deliver services, and grow your business, visit http://www.SelectUSA.gov or call +1-202-482-6800.

PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 15/646,822

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	280
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	600
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	2160
TOTAL CLAIMS (37 CFR 1.16(i))	16 minus 20=	*			OR	x 80 =	0.00
INDEPENDENT CLAIMS (37 CFR 1.16(h))	6 minus 3 =	* 3				x 420 =	1260
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						1200
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							0.00
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	5500

APPLICATION AS AMENDED - PART II

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT A	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT B	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.

** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".

*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".

The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.

==========

Sequence Listing could not be accepted.
If you need help call the Patent Electronic Business Center at (866) 217-9197 (toll free).
Reviewer: Kathleen Kalafus
Timestamp: [year=2017; month=7; day=13; hr=10; min=27; sec=45; ms=665;]

==========

****

Reviewer Comments:
NOTE: This error report refers to the sequence listing CRF filed on 7/11/2017.

1.

W447 n or Xaa used, for: SEQID(48) on line number 1269
W447 n or Xaa used, for: SEQID(49) on line number 1353

An “Xaa” can only be used to represent a single amino acid or modified amino acid. An “Xaa” cannot be used to represent a chemical molecule attached to an amino acid.

In SEQ ID NOs: 48 and 49, an “Xaa” was inappropriately used to represent a non-amino acid molecule.

In SEQ ID NO:48, the Xaa at position 1 was defined as “synthetic or natural polymeric group or combination thereof.” This definition includes non-amino acid molecules such as polyethylene glycol (PEG).

In SEQ ID NO:49, the Xaa at position 1 was defined as (among other things) a “water soluble polymer.” A water soluble polymer includes molecules that are not amino acids.

Amino acids can be annotated to indicate that they are attached to non-amino acid molecules using a feature section. A feature consists of numeric identifiers <220>, <221>, <222>, and <223>. Numeric identifier <220> should remain blank, numeric Identifier <221> should be selected from “WIPO Standard ST.25 (1998), Appendix 2, Tables 5 and 6,” numeric identifier <222> should specify the location of the feature within sequence, and numeric identifier <223> should describe the feature.

For example, for SEQ ID NO:48, the Xaa at position 1 could be removed, making the Xaa currently at position 2 position 1. A feature could be added describing the Xaa at position 1 as being attached to "a synthetic or natural polymeric group or combination thereof." A similar feature section was used to describe the modifications to the Cys residue in position 19.

Please make all necessary changes in the sequences identified above, and review the entirety of the sequence listing for additional instances of similar errors.

2.

The messages shown below do not indicate non-compliance with the sequence rules, and do not require correction.

W213 Artificial or Unknown found in <213> in SEQ ID (1)
W213 Artificial or Unknown found in <213> in SEQ ID (2)
W213 Artificial or Unknown found in <213> in SEQ ID (3)
W213 Artificial or Unknown found in <213> in SEQ ID (4)
W213 Artificial or Unknown found in <213> in SEQ ID (5)
W213 Artificial or Unknown found in <213> in SEQ ID (6)
W447 n or Xaa used, for: SEQID(6) on line number 199
W213 Artificial or Unknown found in <213> in SEQ ID (7)
W213 Artificial or Unknown found in <213> in SEQ ID (8)
W213 Artificial or Unknown found in <213> in SEQ ID (9)
W213 Artificial or Unknown found in <213> in SEQ ID (10)
W213 Artificial or Unknown found in <213> in SEQ ID (11)
W213 Artificial or Unknown found in <213> in SEQ ID (12)
W213 Artificial or Unknown found in <213> in SEQ ID (13)
W213 Artificial or Unknown found in <213> in SEQ ID (14)
W213 Artificial or Unknown found in <213> in SEQ ID (15)
W213 Artificial or Unknown found in <213> in SEQ ID (16)
W213 Artificial or Unknown found in <213> in SEQ ID (17)
W213 Artificial or Unknown found in <213> in SEQ ID (18)
W213 Artificial or Unknown found in <213> in SEQ ID (19)
W213 Artificial or Unknown found in <213> in SEQ ID (20) This error has occured more than 20 times, will not be displayed
W447 n or Xaa used, for: SEQID(34) on line number 637
W447 n or Xaa used, for: SEQID(44) on line number 931

W447 n or Xaa used, for: SEQID(45) on line number 960
W447 n or Xaa used, for: SEQID(46) on line number 1054
W447 n or Xaa used, for: SEQID(46) on line number 1058
W447 n or Xaa used, for: SEQID(47) on line number 1224
W447 n or Xaa used, for: SEQID(47) on line number 1228
W447 n or Xaa used, for: SEQID(50) on line number 1450
W447 n or Xaa used, for: SEQID(50) on line number 1454
W447 n or Xaa used, for: SEQID(51) on line number 1474
W447 n or Xaa used, for: SEQID(52) on line number 1498
W447 n or Xaa used, for: SEQID(53) on line number 1522
W447 n or Xaa used, for: SEQID(54) on line number 1550
W447 n or Xaa used, for: SEQID(55) on line number 1574
W447 n or Xaa used, for: SEQID(57) on line number 1626
W447 n or Xaa used, for: SEQID(59) on line number 1682
W402 Undefined organism found in <213> in SEQ ID (68)
W402 Undefined organism found in <213> in SEQ ID (78)
W447 n or Xaa used, for: SEQID(90) on line number 2520 This error has occurred more than 20 times, will not be displayed

****

For any sequence listing-related questions or concerns the Applicant is invited to contact the USPTO Sequence Help Desk at 571-272-2510.

The errors listed above are exemplary and may not be a complete list of all errors in the sequence listing file.

To correct the sequence listing errors noted in this report - The recommended method for correction of errors is to access the sequence listing working file using the software program in which the listing was originally prepared, e.g., the project file in PatentIn, make any necessary corrections within that program, then generate a new sequence listing file. Use of a word processing program to correct errors directly in the original sequence listing file is strongly discouraged, since such programs often introduce unintended changes to the sequence listing, rendering the listing unacceptable. When the working file or original program is not available for correction, then use of a common or plain text-only editor, such as NotePad, to edit the original sequence listing file may suffice.

A sequence listing text file submitted via EFS-Web that complies with the requirements of 37 CFR 1.824(a) (2)-(6) and (b) (i.e., is a compliant sequence listing ASCII text file), serves as both the paper

copy required by 37 CFR 1.821(c) and the CRF required by 37 CFR 1.821(e).

****

Validated By CRFValidator v 1.0.5

Application No: 15646822 Version No: 1.0

Input Set:

Output Set:

Started: 2017-07-11 16:42:15.240
Finished: 2017-07-11 16:42:19.622
Elapsed: 0 hr(s) 0 min(s) 4 sec(s) 382 ms
Total Warnings: 251
Total Errors: 0
No. of SeqIDs Defined: 237
Actual SeqID Count: 237

Error code		Error Description
W	213	Artificial or Unknown found in <213> in SEQ ID (1)
W	213	Artificial or Unknown found in <213> in SEQ ID (2)
W	213	Artificial or Unknown found in <213> in SEQ ID (3)
W	213	Artificial or Unknown found in <213> in SEQ ID (4)
W	213	Artificial or Unknown found in <213> in SEQ ID (5)
W	213	Artificial or Unknown found in <213> in SEQ ID (6)
W	447	n or Xaa used, for: SEQID(6) on line number 199
W	213	Artificial or Unknown found in <213> in SEQ ID (7)
W	213	Artificial or Unknown found in <213> in SEQ ID (8)
W	213	Artificial or Unknown found in <213> in SEQ ID (9)
W	213	Artificial or Unknown found in <213> in SEQ ID (10)
W	213	Artificial or Unknown found in <213> in SEQ ID (11)
W	213	Artificial or Unknown found in <213> in SEQ ID (12)
W	213	Artificial or Unknown found in <213> in SEQ ID (13)
W	213	Artificial or Unknown found in <213> in SEQ ID (14)
W	213	Artificial or Unknown found in <213> in SEQ ID (15)
W	213	Artificial or Unknown found in <213> in SEQ ID (16)
W	213	Artificial or Unknown found in <213> in SEQ ID (17)
W	213	Artificial or Unknown found in <213> in SEQ ID (18)
W	213	Artificial or Unknown found in <213> in SEQ ID (19)

Input Set:

Output Set:

Started: 2017-07-11 16:42:15.240
Finished: 2017-07-11 16:42:19.622
Elapsed: 0 hr(s) 0 min(s) 4 sec(s) 382 ms
Total Warnings: 251
Total Errors: 0
No. of SeqIDs Defined: 237
Actual SeqID Count: 237

Error code		Error Description
W	213	Artificial or Unknown found in <213> in SEQ ID (20) This error has occured more than 20 times, will not be displayed
W	447	n or Xaa used, for: SEQID(34) on line number 637
W	447	n or Xaa used, for: SEQID(44) on line number 931
W	447	n or Xaa used, for: SEQID(45) on line number 960
W	447	n or Xaa used, for: SEQID(46) on line number 1054
W	447	n or Xaa used, for: SEQID(46) on line number 1058
W	447	n or Xaa used, for: SEQID(47) on line number 1224
W	447	n or Xaa used, for: SEQID(47) on line number 1228
W	447	n or Xaa used, for: SEQID(48) on line number 1269
W	447	n or Xaa used, for: SEQID(49) on line number 1353
W	447	n or Xaa used, for: SEQID(50) on line number 1450
W	447	n or Xaa used, for: SEQID(50) on line number 1454
W	447	n or Xaa used, for: SEQID(51) on line number 1474
W	447	n or Xaa used, for: SEQID(52) on line number 1498
W	447	n or Xaa used, for: SEQID(53) on line number 1522
W	447	n or Xaa used, for: SEQID(54) on line number 1550
W	447	n or Xaa used, for: SEQID(55) on line number 1574
W	447	n or Xaa used, for: SEQID(57) on line number 1626
W	447	n or Xaa used, for: SEQID(59) on line number 1682
W	402	Undefined organism found in <213> in SEQ ID (68)
W	402	Undefined organism found in <213> in SEQ ID (78)
W	447	n or Xaa used, for: SEQID(90) on line number 2520

Input Set:

Output Set:

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Finished: 2017-07-11 16:42:19.622
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Total Warnings: 251
Total Errors: 0
No. of SeqIDs Defined: 237
Actual SeqID Count: 237

Error code	Error Description

SEQUENCE LISTING

<110> BioMarin Pharmaceutical Inc.
Wendt, et al.

<120> Variants of C-Type Natriuretic Peptide

<130> 30610/44556C1

<140> US 15/646,822
<141> 2017-07-11

<150> 12/784,117
<151> 2010-05-20

<150> 61/254,563
<151> 2009-10-23

<150> 61/180,112
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<160> 237

<170> PatentIn version 3.5

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<223> Cysteines in positions 6 and 22 form cyclic domain via disulfide bond

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Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
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<223> CNP-17

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Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly
1 5 10 15

Cys

<210> 3
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<400> 3

Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu
1 5 10 15

Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25

<210> 4
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<400> 4

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Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly
            20                  25                  30

Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met
        35                  40                  45

Ser Gly Leu Gly Cys
    50
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<210> 5
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<220>
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<223> Gly is optionally modified with an amino acid sequence derived from a natriuretic polypeptide, a non-natriuretic polypeptide

<220>
<221> MOD_RES
<222> (22)..(22)
<223> Cys is optionally modified with an amino acid sequence derived from a natriuretic polypeptide, a non-natriuretic polypeptide

<400> 5

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Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1               5                   10                  15

Met Ser Gly Leu Gly Cys
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<210> 6
<211> 22
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<222> (1)..(1)
<223> Gly is modified with a synthetic or natural polymeric group or combination thereof

<220>
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<222> (4)..(4)
<223> Xaa is Lys or a conservative amino acid substitution thereof, any natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amine on a side chain, Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Glu or Ser

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<220>
<221> MOD_RES
<222> (22)..(22)
<223> Cys is optionally modified with a synthetic or natural polymeric group or combination thereof

<400> 6

Gly Leu Ser Xaa Gly Cys Phe Gly Leu Xaa Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
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<400> 7

Gly Ala Asn Lys Lys
1 5

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Gly Ala Asn Arg Arg
1   5

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Gly Ala Asn Pro Arg
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Gly Ala Asn Gln Gln
1   5

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<400> 11

Gly Ala Asn Ser Ser
1   5

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<400> 12

Gly Ala Asn Arg Gln
1 5

<210> 13
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<400> 13

Gly Ala Asn Arg Met
1 5

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<400> 14

Gly Ala Asn Arg Thr
1 5

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Gly Ala Asn Arg Ser
1 5

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1 5 10

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Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys
1 5 10 15

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1 5 10 15

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1 5 10 15

Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys
20 25 30

<210> 22
<211> 31
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<400> 22

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1 5 10 15

Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Arg Arg
20 25 30

<210> 23
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<400> 23

Ser Leu Arg Arg Ser Ser
1 5

<210> 24

<211> 5
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<400> 24

Asn Ser Phe Arg Tyr
1 5

<210> 25
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<400> 25

Ser Pro Lys Met Val Gln Gly Ser Gly
1 5

<210> 26
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Met Val Gln Gly Ser Gly
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Lys Val Leu Arg Arg Tyr
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<210> 28
<211> 6

<212> PRT
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<400> 28

Lys Val Leu Arg Arg His
1 5

<210> 29
<211> 12
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<400> 29

Gly Gln His Lys Asp Asp Asn Pro Asn Leu Pro Arg
1 5 10

<210> 30
<211> 9
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<220>
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<400> 30

Gly Val Pro Gln Val Ser Thr Ser Thr
1 5

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<211> 15
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Gly Glu Arg Ala Phe Lys Ala Trp Ala Val Ala Arg Leu Ser Gln
1 5 10 15

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Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
1 5 10

<210> 33
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<223> Amino acid extention

<400> 33

Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys
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<210> 34
<211> 37
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<220>
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<223> CNP-22 with amino acid extention

<220>
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<220>
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<222> (25)..(25)
<223> Xaa is Lys or a conservative amino acid substitution thereof, any natural or unnatural amino acid, Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Glu or Ser

<220>
<221> MISC_FEATURE
<222> (37)..(37)
<223> Cys is optionally modified with a synthetic, natural or
combination polymeric group

<400> 34

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1 5 10 15

Leu Ser Xaa Gly Cys Phe Gly Leu Xaa Leu Asp Arg Ile Gly Ser Met
20 25 30

Ser Gly Leu Gly Cys
35

<210> 35
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<220>
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<223> CNP22 (K4R)

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Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
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<210> 36
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<223> GANRR-CNP22(K4R) - CNP27(Arg4)

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Gly Ala Asn Arg Arg Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu
1   5   10   15

Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
            20   25

<210> 37
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<223> GANPR-CNP22(K4R) - CNP27(Pro4) - Analog CI

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Gly Ala Asn Pro Arg Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu
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Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
            20   25

<210> 38
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<223> ER-CNP22

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1   5   10   15

Gly Ser Met Ser Gly Leu Gly Cys
            20

<210> 39
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<220>
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<223> Analog BA - ER-CNP22(K4R)

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Gly Ser Met Ser Gly Leu Gly Cys
20

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<223> R-CNP22

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Arg Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly
1 5 10 15

Ser Met Ser Gly Leu Gly Cys
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<210> 41
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<223> Analog AZ - R-CNP22(K4R)

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Arg Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly
1   5   10   15

Ser Met Ser Gly Leu Gly Cys
            20

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<220>
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<223> CNP22 (K4R)(K10R)

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Gly Leu Ser Arg Gly Cys Phe Gly Leu Arg Leu Asp Arg Ile Gly Ser
1   5   10   15

Met Ser Gly Leu Gly Cys
            20

<210> 43
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<223> Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -CH2-NH-

<220>
<221> MOD_RES
<222> (6)..(7)
<223> Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -C(=O)-N(R)-
where R is methyl, ethyl, n-propyl, isopropyl, cyclopropyl,
n-butyl, isobutyl, sec-butyl or tert-butyl

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<222> (6)..(7)
<223> Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -C(=O)-NH-CH2-

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<223> Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -CH2-S-

<220>
<221> MOD_RES
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<223> Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -CH2-S(O)n
where n is 1 or 2

<220>
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<223> Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -CH2-CH2-

<220>
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<223> Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -CH=CH-

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<223> Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -CH(CN)-NH-

<220>
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<223> Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -CH(OH)-CH2-

<220>
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<223> Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -O-C(=O)-NH-

<220>
<221> MOD_RES
<222> (6)..(7)
<223> Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with NHC(=O)NH-

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Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
            20

<210> 44
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<222> (7)..(7)
<223> Xaa is 3-amino-2-phenylpropionic acid or D-Phe

<400> 44

Gly Leu Ser Lys Gly Cys Xaa Gly Leu Lys Leu Asp Arg Ile Gly Ser
1   5   10   15

Met Ser Gly Leu Gly Cys
            20

<210> 45
<211> 22
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<220>
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<223> CNP22 - modified Cys6

<220>
<221> MOD_RES
<222> (6)..(6)
<223> Xaa is Cys, homocysteine, penicillamine, 2-mercaptopropionic
       acid, or 3-mercaptopropionic acid.

<400> 45

Gly Leu Ser Lys Gly Xaa Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1   5   10   15

Met Ser Gly Leu Gly Cys
            20

<210> 46
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<223> Gly is optionally modified with a bone or cartilage targeting compound, a biphosphonate, polyAsp, polyGlu, osteopontin, osteocalcin, or sialoprotein peptides, polymers, PEG, carbohydrates, hydrophobic acids, or amino acids

<220>
<221> MOD_RES
<222> (4)..(4)
<223> Xaa is Lys or a conservative amino acid substitution thereof, any natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amine on a side chain, Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser, or Glu

<220>
<221> MOD_RES
<222> (6)..(7)
<223> Xaa is Cys with peptide bond isosteres between positions 6 and 7

<220>
<221> MOD_RES
<222> (7)..(7)
<223> Xaa is L-Phe, D-Phe, 3-amino-2-phenylpropi

DocCode – SEQ.TXT

SCORE Placeholder Sheet for IFW Content

Application Number: 15646822 Document Date: 08/02/2017

The presence of this form in the IFW record indicates that the following document type was received in electronic format on the date identified above. This content is stored in the SCORE database.

Since this was an electronic submission, there is no physical artifact folder, no artifact folder is recorded in PALM, and no paper documents or physical media exist. The TIFF images in the IFW record were created from the original documents that are stored in SCORE.

- Sequence Listing

At the time of document entry (noted above):

- USPTO employees may access SCORE content via eDAN using the Supplemental Content tab, or via the SCORE web page.
- External customers may access SCORE content via PAIR using the Supplemental Content tab.

Form Revision Date: August 26, 2013

Docket No.: 30610/44556C1-R2
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Reissue Application of:
Daniel J. Wendt et al.

Application No.: 15/646,822 Confirmation No.: 7727

Filed: July 11, 2017 Art Unit: 1629

For: Variants of C-Type Natriuretic Peptide Examiner: Not Yet Assigned

PRELIMINARY AMENDMENT UNDER 37 C.F.R. § 1.115

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

This paper is filed in response to the Computer Readable Form Defective Notice, dated June 15, 2017. Prior to examination on the merits, please amend the above-identified U.S. patent application as follows:

Amendments to the Specification begin on page 2 of this paper.

Remarks/Arguments begin on page 3 of this paper.

AMENDMENTS TO THE SPECIFICATION

IN THE SEQUENCE LISTING

Please replace the Sequence Listing currently of record with the computer-readable form of the Substitute Sequence Listing submitted herewith.
(filename: 44556C1_R2_SubSeqListing.txt; 108,579 bytes; created July 28, 2017)

REMARKS

In response to the Computer Readable Form Defective Notice, dated June 15, 2017, the Specification has been amended to replace the Sequence Listing currently of record with the Substitute Sequence Listing submitted herewith in computer-readable form and paper copy. The Substitute Sequence Listing has been amended to correct the alleged errors found in SEQ ID NOs: 48 and 49. Specifically, the modification "synthetic or natural polymeric group or combination thereof" previously described as Xaa1 of SEQ ID NOs: 48 and 49 have been amended to more appropriately modify the N-terminus. Further, Xaa1-Xaa5 have been added to SEQ ID NO: 48 to account for the optional "Gly-Leu-Ser-Lys-Gly" peptide previously noted as a feature at Xaa2. No new matter has been added by way of this amendment.

No fees are believed to be due in connection with this response. The Director is hereby authorized to charge any deficiency in fees filed, asserted to be filed or which should have been filed herewith (or hereafter in this application by this firm) to our Deposit Account No. 13-2855, under Order No. 30610/44556C1-R2.

Dated: August 2, 2017

Respectfully submitted,

By /Katherine L. Neville/
Katherine L. Neville
Registration No.: 53,379
MARSHALL, GERSTEIN & BORUN LLP
233 S. Wacker Drive
6300 Willis Tower
Chicago, Illinois 60606-6357
(312) 474-6300
Attorney for Applicant

Electronic Acknowledgement Receipt

EFS ID:	29964434
Application Number:	15646822
International Application Number:	
Confirmation Number:	7727
Title of Invention:	Variants of C-Type Natriuretic Peptide
First Named Inventor/Applicant Name:	Daniel J. Wendt
Customer Number:	90849
Filer:	Katherine Lynn Neville/Kristen Dola
Filer Authorized By:	Katherine Lynn Neville
Attorney Docket Number:	30610/44556C1-R2
Receipt Date:	02-AUG-2017
Filing Date:	
Time Stamp:	16:14:26
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Sequence Listing (Text File)	44556C1_R2_SubSeqListing.TXT	108579	no	-

Warnings:

Information:					
2	Sequence Listing	44556C1-R2_SubSeqListing.PDF	146725 e194ac27498a0eeea39761c103ee113527e3cf74	no	103
Warnings:					
Information:					
3	CRF Statement Paper and CRF are the same	Seq_Stmt.PDF	13725 057213ca13429476a19e6d32c96b6bdb689170c0	no	1
Warnings:					
Information:					
4		Resp_Notice_to_Comply_NTC.PDF	21213 14438b7e7f01dc1ee655fefcaea61329c0f0aebf	yes	3

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Response to Pre-Exam Sequence Notice	1	1
	Specification	2	2
	Applicant Arguments/Remarks Made in an Amendment	3	3

Warnings:	
Information:	
Total Files Size (in bytes):	290242

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

SEQUENCE LISTING

<110> BioMarin Pharmaceutical Inc.
Wendt, et al.

<120> Variants of C-Type Natriuretic Peptide

<130> 30610/44556C1-R2

<140> 15/646,822
<141> 2017-07-11

<150> 14/604,232
<151> 2015-01-23

<150> 13/466,672
<151> 2012-05-08

<150> 12/784,117
<151> 2010-05-20

<150> 61/254,563
<151> 2009-10-23

<150> 61/180,112
<151> 2009-05-20

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<170> PatentIn version 3.5

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<223> Cysteines in positions 6 and 22 form cyclic domain via disulfide bond

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1 5 10 15

Met Ser Gly Leu Gly Cys

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1 5 10 15

Cys

<210> 3
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Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu
1 5 10 15

Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25

<210> 4
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<400> 4

Asp Leu Arg Val Asp Thr Lys Ser Arg Ala Ala Trp Ala Arg Leu Leu
1 5 10 15

Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly
20 25 30

Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met
35 40 45

Ser Gly Leu Gly Cys
50

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<223> Cys is optionally modified with an amino acid sequence derived from a natriuretic polypeptide, a non-natriuretic polypeptide

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Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
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<210> 6
<211> 22
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<220>
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<223> Gly is modified with a synthetic or natural polymeric group or combination thereof

<220>
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<223> Xaa is Lys or a conservative amino acid substitution thereof, any natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amine on a side chain, Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Glu or Ser

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<223> Cys is optionally modified with a synthetic or natural polymeric group or combination thereof

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1 5 10 15

Met Ser Gly Leu Gly Cys
20

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1 5

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Gly Ala Asn Arg Arg
1 5

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Gly Ala Asn Pro Arg
1 5

<210> 10
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Gly Ala Asn Gln Gln
1 5

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1 5

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Gly Ala Asn Arg Gln
1 5

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Gly Ala Asn Arg Met
1 5

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Gly Ala Asn Arg Thr
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Gly Ala Asn Arg Ser
1 5

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Ala Ala Trp Ala Arg Leu Leu Gln Glu His Pro Asn Ala
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Ala Ala Trp Ala Arg Leu Leu Gln Glu His Pro Asn Ala Arg
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<210> 18
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Asp Leu Arg Val Asp Thr Lys Ser Arg Ala Ala Trp Ala Arg
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Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys
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<210> 20
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Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Arg Arg
1 5 10 15

<210> 21
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Asp Leu Arg Val Asp Thr Lys Ser Arg Ala Ala Trp Ala Arg Leu Leu
1 5 10 15

Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys
20 25 30

<210> 22
<211> 31
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1 5 10 15

Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Arg Arg
20 25 30

<210> 23
<211> 6
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<400> 23

Ser Leu Arg Arg Ser Ser
1   5

<210> 24
<211> 5
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<220>
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<400> 24

Asn Ser Phe Arg Tyr
1   5

<210> 25
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Ser Pro Lys Met Val Gln Gly Ser Gly
1   5

<210> 26
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Met Val Gln Gly Ser Gly
1   5

<210> 27
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<400> 27

Lys Val Leu Arg Arg Tyr
1   5

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<400> 28

Lys Val Leu Arg Arg His
1   5

<210> 29
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Gly Gln His Lys Asp Asp Asn Pro Asn Leu Pro Arg
1   5   10

<210> 30
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Gly Val Pro Gln Val Ser Thr Ser Thr
1   5

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Gly Glu Arg Ala Phe Lys Ala Trp Ala Val Ala Arg Leu Ser Gln

1 5 10 15

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<211> 14
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<210> 34
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<220>
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<220>
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<222> (37)..(37)
<223> Cys is optionally modified with a synthetic, natural or combination polymeric group

<400> 34

Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly
1 5 10 15

Leu Ser Xaa Gly Cys Phe Gly Leu Xaa Leu Asp Arg Ile Gly Ser Met
20 25 30

Ser Gly Leu Gly Cys
35

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Met Ser Gly Leu Gly Cys
20

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Gly Ala Asn Arg Arg Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu
1 5 10 15

Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25

<210> 37
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<223> GANPR-CNP22(K4R) - CNP27(Pro4) - Analog CI

<400> 37

Gly Ala Asn Pro Arg Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu
1 5 10 15

Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25

<210> 38
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<400> 38

Glu Arg Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile
1 5 10 15

Gly Ser Met Ser Gly Leu Gly Cys
            20

<210> 39
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<223> Analog BA - ER-CNP22(K4R)

<400> 39

Glu Arg Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile
1   5   10   15

Gly Ser Met Ser Gly Leu Gly Cys
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<210> 40
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<220>
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1   5   10   15

Ser Met Ser Gly Leu Gly Cys
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<210> 41
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<223> Analog AZ - R-CNP22(K4R)

<400> 41

Arg Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly
1 5 10 15

Ser Met Ser Gly Leu Gly Cys
20

<210> 42
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<220>
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<223> CNP22 (K4R)(K10R)

<400> 42

Gly Leu Ser Arg Gly Cys Phe Gly Leu Arg Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> 43
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<222> (6)..(7)
<223> Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -CH2-NH-

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where R is methyl, ethyl, n-propyl, isopropyl, cyclopropyl,
n-butyl, isobutyl, sec-butyl or tert-butyl

<220>
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<223> Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -C(=O)-NH-CH2-

<220>
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<223> Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -CH2-S-

<220>
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<222> (6)..(7)
<223> Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -CH2-S(O)n
where n is 1 or 2

<220>
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<223> Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -CH2-CH2-

<220>
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<223> Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -CH=CH-

<220>
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<223> Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -C(=O)-CH2-

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<223> Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -CH(CN)-NH-

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<222> (6)..(7)
<223> Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with NHC(=O)NH-

<400> 43

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
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1 5 10 15

Met Ser Gly Leu Gly Cys
20

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<223> Xaa is Cys, homocysteine, penicillamine, 2-mercaptopropionic acid, or 3-mercaptopropionic acid.

<400> 45

Gly Leu Ser Lys Gly Xaa Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser

1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> 46
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<223> Gly is optionally modified with a bone or cartilage targeting compound, a biphosphonate, polyAsp, polyGlu, osteopontin, osteocalcin, or sialoprotein peptides, polymers, PEG, carbohydrates, hydrophobic acids, or amino acids

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<223> Xaa is Lys or a conservative amino acid substitution thereof, any natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amine on a side chain, Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser, or Glu

<220>
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<222> (6)..(7)
<223> Xaa is Cys with peptide bond isosteres between positions 6 and 7

<220>
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<223> Xaa is L-Phe, D-Phe, 3-amino-2-phenylpropionic acid, or N-alkylated derivatives of Phe

<220>
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<220>
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<223> Xaa is Leu, Ser, Thr or peptide bond isosteres thereof

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<223> Xaa is Lys or a conservative amino acid substitution thereof, any

natural or unnatural amino acid, Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu

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<222> (11)..(11)
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<220>
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<222> (20)..(20)
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<220>
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<223> Cys is optionally modified with a bone or cartilage targeting compound, a bisphosphonate, polyAsp, polyGlu, osteopontin, osteocalcin or sialoprotein peptides, or amino acid sequences derived from non-CNP polypeptides

<400> 46

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1 5 10 15

Xaa Ser Gly Xaa Gly Cys
20

<210> 47
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<223> Synthetic Polypeptide

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<221> MOD_RES
<222> (1)..(1)
<223> Gly is optionally modified with a peptide sequence containing one

to five amino acids from a natriuretic or non-natriuretic polypeptide

<220>
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<222> (1)..(1)
<223> Gly is optionally modified with a peptide sequence containing one to five amino acids from a natriuretic or non-natriuretic polypeptide

<220>
<221> MOD_RES
<222> (4)..(4)
<223> Xaa is Lys or a conservative amino acid substitution thereof, any natural or unnatural amino acid or peptidomimetic, Arg, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser, or Glu

<220>
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<222> (6)..(6)
<223> Xaa is Cys or decarboxy Cys

<220>
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<223> Peptide bond between positions 6 and 7 may be replaced with -CH2-NH-

<220>
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<223> Peptide bond between positions 6 and 7 may be replaced with -C(=O)-N(R)- where R is methyl, ethyl, n-propyl, isopropyl, cyclopropyl, n-butyl, isobutyl, sec-butyl or tert-butyl

<220>
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<220>
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<222> (6)..(7)
<223> Peptide bond between positions 6 and 7 may be replaced with -CH2-S-

<220>
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<220>
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-CH2-CH2-

<220>
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<223> Peptide bond between positions 6 and 7 may be replaced with -CH=CH-

<220>
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<223> Peptide bond between positions 6 and 7 may be replaced with -C(=O)-CH2-

<220>
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<223> Xaa is Gly, tert-butyl-Gly (tBu-Gly), Val, Ser, Thr or Asn

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<223> Xaa is Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu

<220>
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<223> Xaa is Met, Val, Asn, beta-Cl-Ala, 2-aminobutyric acid (Abu) or 2-amino-isobutyric acid (Aib)

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<221> MOD_RES
<222> (22)..(22)
<223> Cys is optionally modified with a synthetic bone targeting compound, a bisphosphonate, polyAsp, polyGlu, osteopontin, osteocalcin or sialoprotein peptides, or charged PEG molecules

<400> 47

Gly Leu Ser Xaa Gly Xaa Xaa Xaa Xaa Xaa Xaa Asp Arg Xaa Gly Ser
1 5 10 15

Xaa Ser Gly Xaa Gly Cys
20

<210> 48
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MOD_RES
<222> (1)..(1)
<223> N-terminus is optionally modified with a synthetic or natural polymeric group of combination thereof

<220>
<221> MOD_RES
<222> (1)..(1)
<223> Xaa is Gly or is absent

<220>
<221> MOD_RES
<222> (2)..(2)
<223> Xaa is Leu or is absent

<220>
<221> MOD_RES
<222> (3)..(3)
<223> Xaa is Ser or is absent

<220>
<221> MOD_RES
<222> (4)..(4)
<223> Xaa is Lys or is absent

<220>
<221> MOD_RES
<222> (5)..(5)
<223> Xaa is Gly or is absent

<220>
<221> MOD_RES
<222> (10)..(10)
<223> Xaa is Lys, a conservative amino acid substitution, any natural or unnatural amino acid or peptidomimetic that does have a reactive primary amine on a side chain

<220>
<221> MOD_RES
<222> (22)..(22)
<223> Cys is optionally modified with a synthetic or natural polymeric group or a combination thereof

<400> 48

Xaa Xaa Xaa Xaa Xaa Cys Phe Gly Leu Xaa Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> 49
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MOD_RES
<222> (1)..(1)
<223> N-terminus is optionally modified with a peptide fragment or water soluble polymer

<220>
<221> MOD_RES
<222> (1)..(1)
<223> Xaa is Cys or peptide bond isosteres thereof

<220>
<221> MOD_RES
<222> (2)..(2)
<223> Xaa is L-Phe, D-Phe, 3-amino-2-phenylpropionic acid or N-alkylated derivatives of Phe

<220>
<221> MOD_RES
<222> (3)..(3)
<223> Xaa is Gly, tert-butyl-Gly (tBu-Gly), Val, Ser, Thr or Asn

<220>
<221> MOD_RES
<222> (4)..(4)
<223> Xaa is Leu, Ser, Thr or N-Me-Leu

<220>
<221> MOD_RES
<222> (5)..(5)
<223> Xaa is Lys or a conservative amino acid substitution thereof, any natural or unnatural amino acid, Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu

<220>
<221> MOD_RES
<222> (6)..(6)
<223> Xaa is Leu and peptide bond isosteres thereof

<220>
<221> MOD_RES
<222> (9)..(9)
<223> Xaa is Ile, tBu-Gly or peptide bond isosteres thereof

<220>
<221> MOD_RES
<222> (12)..(12)
<223> Xaa is Met, Val, Asn, beta-Cl-Ala, 2-aminobutyric acid (Abu) or 2-amino-isobutyric acid (Aib)

<220>
<221> MOD_RES
<222> (15)..(15)
<223> Xaa is Leu, norleucine (Nle), homoleucine (Hleu), Val, tert-butyl-Ala (tBu-Ala), Ser, Thr, Arg or peptide bond isosteres thereof

<220>
<221> MOD_RES
<222> (17)..(17)
<223> Cys is optionally modified with amino acid sequences useful in bone/cartilage targeting, polyAsp or polyGlu, osteopontin, osteocalcin, sialoprotein or sequences derived from NPPC or non-CNP polypeptides

<400> 49

Xaa Xaa Xaa Xaa Xaa Xaa Asp Arg Xaa Gly Ser Xaa Ser Gly Xaa Gly
1 5 10 15

Cys

<210> 50
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MOD_RES
<222> (1)..(1)
<223> Gly is optionally modified with bone targeting compound, moieties to reduce renal clearance, charged PEG molecules or hydrophilic polymers

<220>
<221> MOD_RES
<222> (4)..(4)
<223> Xaa is Lys or a conservative amino acid substitution thereof, any natural or unnatural amino acid, Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu

<220>
<221> MOD_RES
<222> (6)..(6)
<223> Xaa is Cys or peptide isosteres between positions 6 and 7

<220>
<221> MOD_RES
<222> (7)..(7)
<223> Xaa is L-Phe, D-Phe, 3-amino-2-phenylpropionic acid or N-alkylated derivatives of Phe

<220>
<221> MOD_RES
<222> (8)..(8)
<223> Xaa is Gly, tert-butyl-Gly, Thr, Ser, Val or Asn

<220>
<221> MOD_RES
<222> (9)..(9)
<223> Xaa is Leu, Ser, Thr or peptide bond isosteres thereof

<220>
<221> MOD_RES
<222> (10)..(10)
<223> Xaa is Lys or a conservative amino acid substitution thereof, any natural or unnatural amino acid, Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu

<220>
<221> MOD_RES
<222> (11)..(11)
<223> Xaa is Leu, Asn or peptide bond isosteres thereof

<220>
<221> MOD_RES
<222> (14)..(14)
<223> Xaa is Ile, tert-butyl-Gly (tBu-Gly), Asn or peptide bond isosteres thereof

<220>
<221> MOD_RES
<222> (15)..(15)
<223> Xaa is Gly, Arg, Ser or Asn

<220>
<221> MOD_RES
<222> (17)..(17)
<223> Xaa is Met, Val, Asn, beta-Cl-Ala, 2-aminobutyric acid (Abu) or 2-amino-isobutyric acid (Aib)

<220>
<221> MOD_RES
<222> (20)..(20)
<223> Xaa is Leu, norleucine (Nle), homoleucine (Hleu), Val, tert-butyl-Ala (tBu-Ala), Arg, Thr, Ser or peptide bond isoteres thereof

<220>
<221> MOD_RES
<222> (22)..(22)
<223> Cys is optionally modified with bone targeting compound, moieties to reduce renal clearance, charged PEG molecules or hydrophilic polymers

<400> 50

Gly Leu Ser Xaa Gly Xaa Xaa Xaa Xaa Xaa Xaa Asp Arg Xaa Xaa Ser
1 5 10 15

Xaa Ser Gly Xaa Gly Cys
20

<210> 51
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MOD_RES
<222> (1)..(1)
<223> Xaa is Arg or Glu

<400> 51

Xaa Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> 52
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MOD_RES
<222> (5)..(5)
<223> Xaa is Arg, Gln or Ser

<400> 52

Gly Leu Ser Lys Xaa Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> 53
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MOD_RES
<222> (15)..(15)
<223> Xaa is Ser, Asn, Arg, or Cit

<400> 53

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Xaa Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> 54
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MOD_RES
<222> (19)..(19)
<223> Xaa is Ser, Arg, or Asn

<400> 54

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Xaa Leu Gly Cys
20

<210> 55
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MOD_RES
<222> (21)..(21)
<223> Xaa is Ser, Thr, or Arg

<400> 55

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1   5   10   15

Met Ser Gly Leu Xaa Cys
            20

<210> 56
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog A

<220>
<221> BINDING
<222> (6)..(6)
<223> CH2NH

<400> 56

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1   5   10   15

Met Ser Gly Leu Gly Cys
            20

<210> 57
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analogs B

<220>
<221> BINDING
<222> (7)..(7)
<223> Xaa replaced with N-Me-Phe

<400> 57

Gly Leu Ser Lys Gly Cys Xaa Gly Leu Lys Leu Asp Arg Ile Gly Ser

1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> 58
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog F

<220>
<221> BINDING
<222> (8)..(8)
<223> tBu

<400> 58

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> 59
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog H

<220>
<221> BINDING
<222> (7)..(7)
<223> Xaa is replaced with NHCH2-CH(Phe)CO

<400> 59

Gly Leu Ser Lys Gly Cys Xaa Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
            20

<210> 60
<211> 37
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog BL - CNP37

<400> 60

Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly
1   5   10   15

Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met
            20   25   30

Ser Gly Leu Gly Cys
        35

<210> 61
<211> 35
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog CA

<400> 61

Ala Ala Trp Ala Arg Leu Leu Gln Glu His Pro Asn Ala Gly Leu Ser
1   5   10   15

Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly
            20   25   30

Leu Gly Cys

35

<210> 62
<211> 36
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog CB

<400> 62

Ala Ala Trp Ala Arg Leu Leu Gln Glu His Pro Asn Ala Arg Gly Leu
1 5 10 15

Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser
20 25 30

Gly Leu Gly Cys
35

<210> 63
<211> 36
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog CC

<400> 63

Asp Leu Arg Val Asp Thr Lys Ser Arg Ala Ala Trp Ala Arg Gly Leu
1 5 10 15

Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser
20 25 30

Gly Leu Gly Cys
35

<210> 64
<211> 27
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> GANQQ-CNP22

<400> 64

Gly Ala Asn Gln Gln Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu
1 5 10 15

Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25

<210> 65
<211> 27
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> GANRR-CNP22

<400> 65

Gly Ala Asn Arg Arg Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu
1 5 10 15

Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25

<210> 66
<211> 27
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE

<223> GANPR-CNP22

<400> 66

Gly Ala Asn Pro Arg Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu
1 5 10 15

Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25

<210> 67
<211> 27
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> GANSS-CNP22

<400> 67

Gly Ala Asn Ser Ser Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu
1 5 10 15

Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25

<210> 68
<211> 32
<212> PRT
<213> Artificial Sequene

<220>
<223> Sythetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog CD

<220>
<221> MOD_RES
<222> (1)..(1)
<223> N-terminus BNP tail

<220>
<221> MOD_RES
<222> (32)..(32)
<223> N-terminus BNP tail

<400> 68

Ser Pro Lys Met Val Gln Gly Ser Gly Cys Phe Gly Leu Lys Leu Asp
1 5 10 15

Arg Ile Gly Ser Met Ser Gly Leu Gly Cys Lys Val Leu Arg Arg His
20 25 30

<210> 69
<211> 27
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> GANQQ-CNP22(K4R) - Analog CH

<400> 69

Gly Ala Asn Gln Gln Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu
1 5 10 15

Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25

<210> 70
<211> 27
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> GANSS-CNP22(K4R) - Analog CG

<400> 70

Gly Ala Asn Ser Ser Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu
1 5 10 15

Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25

<210> 71
<211> 38
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog CS

<400> 71

Gly Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Pro Lys
1 5 10 15

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
20 25 30

Met Ser Gly Leu Gly Cys
35

<210> 72
<211> 38
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog CT

<400> 72

Gly Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Gln Lys
1 5 10 15

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
20 25 30

Met Ser Gly Leu Gly Cys
35

<210> 73
<211> 38
<212> PRT

<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog CU

<400> 73

Gly Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Gln Gln
1 5 10 15

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
20 25 30

Met Ser Gly Leu Gly Cys
35

<210> 74
<211> 38
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog CW

<400> 74

Gly Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Pro
1 5 10 15

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
20 25 30

Met Ser Gly Leu Gly Cys
35

<210> 75
<211> 38
<212> PRT
<213> Artificial Sequence

<220>

<223> Synthetic Polypeptide

<400> 75

Gly Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys
1 5 10 15

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
20 25 30

Met Ser Gly Leu Gly Cys
35

<210> 76
<211> 37
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog CQ

<400> 76

Gly His His Ser His Glu Gln His Pro His Gly Ala Asn Gln Gln Gly
1 5 10 15

Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met
20 25 30

Ser Gly Leu Gly Cys
35

<210> 77
<211> 38
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog CR

<400> 77

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Gly Ala His His Pro His Glu His Asp Thr His Gly Ala Asn Gln Gln
1               5                   10                  15

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
            20                  25                  30

Met Ser Gly Leu Gly Cys
        35
```

<210> 78
<211> 37
<212> PRT
<213> Artificial Sequencce

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog CX

<400> 78

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Gly His His Ser His Glu Gln His Pro His Gly Ala Asn Pro Arg Gly
1               5                   10                  15

Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met
            20                  25                  30

Ser Gly Leu Gly Cys
        35
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<210> 79
<211> 36
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog CF

<400> 79

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Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gly Leu
1               5                   10                  15
```

Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser
            20   25   30

Gly Leu Gly Cys
        35

<210> 80
<211> 39
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog CY

<400> 80

Gly Gln His Lys Asp Asp Asn Pro Asn Leu Pro Arg Gly Ala Asn Pro
1   5   10   15

Arg Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly
            20   25   30

Ser Met Ser Gly Leu Gly Cys
        35

<210> 81
<211> 37
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog CE

<400> 81

Gly Glu Arg Ala Phe Lys Ala Trp Ala Val Ala Arg Leu Ser Gln Gly
1   5   10   15

Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met

```
            20                  25                  30

Ser Gly Leu Gly Cys
        35


<210>  82
<211>  35
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic Polypeptide


<220>
<221>  MISC_FEATURE
<223>  Analog CZ

<400>  82

Phe Gly Ile Pro Met Asp Arg Ile Gly Arg Asn Pro Arg Gly Leu Ser
1               5                   10                  15


Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly
            20                  25                  30


Leu Gly Cys
        35


<210>  83
<211>  40
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic Polypeptide


<220>
<221>  MISC_FEATURE
<223>  Analog DA

<400>  83

Gly Lys Arg Thr Gly Gln Tyr Lys Leu Gly Ser Lys Thr Gly Pro Gly
1               5                   10                  15


Pro Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile
            20                  25                  30
```

Gly Ser Met Ser Gly Leu Gly Cys
35 40

<210> 84
<211> 37
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog CK

<400> 84

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Gly Ala Asn Gln Gln Gly
1 5 10 15

Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met
20 25 30

Ser Gly Leu Gly Cys
35

<210> 85
<211> 36
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog CL

<400> 85

Gly Val Pro Gln Val Ser Thr Ser Thr Gly Ala Asn Gln Gln Gly Leu
1 5 10 15

Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser
20 25 30

Gly Leu Gly Cys
35

<210> 86
<211> 37
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog CM

<400> 86

Gly Gln Pro Ser Ser Ser Ser Gln Ser Thr Gly Ala Asn Gln Gln Gly
1 5 10 15

Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met
20 25 30

Ser Gly Leu Gly Cys
35

<210> 87
<211> 37
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog CN

<400> 87

Gly Gln Thr His Ser Ser Gly Thr Gln Ser Gly Ala Asn Gln Gln Gly
1 5 10 15

Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met
20 25 30

Ser Gly Leu Gly Cys
35

<210> 88
<211> 37

<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog CO

<400> 88

Gly Ser Thr Gly Gln Trp His Ser Glu Ser Gly Ala Asn Gln Gln Gly
1 5 10 15

Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met
20 25 30

Ser Gly Leu Gly Cys
35

<210> 89
<211> 37
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog CP

<400> 89

Gly Ser Ser Ser Ser Ser Ser Ser Ser Ser Gly Ala Asn Gln Gln Gly
1 5 10 15

Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met
20 25 30

Ser Gly Leu Gly Cys
35

<210> 90
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MOD_RES
<222> (1)..(1)
<223> Gly is optionally modified with bone targeting compound, a bisphosphonates, polyAsp, polyGlu, osteopontin, osteocalcin, or sialoprotein peptides, negatively charged PEG molecules or natural polymers

<220>
<221> MOD_RES
<222> (6)..(6)
<223> Xaa is Cys or another natural or unnatural amino acid

<220>
<221> MOD_RES
<222> (7)..(7)
<223> Xaa is Phe or another natural or unnatural amino acid

<220>
<221> MOD_RES
<222> (8)..(8)
<223> Xaa is Gly, a larger natural or unnatural amino acid or peptidomimetic

<220>
<221> MOD_RES
<222> (22)..(22)
<223> Cys is optionally modified with bone targeting compound, a bisphosphonates, polyAsp, polyGlu, osteopontin, osteocalcin, or sialoprotein peptides, negatively charged PEG molecules or natural polymers

<400> 90

Gly Leu Ser Lys Gly Xaa Xaa Xaa Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> 91
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE

<223> Analog J

<220>
<221> MOD_RES
<222> (6)..(6)
<223> Xaa is CH2-NH

<220>
<221> BINDING
<222> (9)..(9)
<223> N-Me

<220>
<221> BINDING
<222> (20)..(20)
<223> N-Me

<400> 91

Gly Leu Ser Lys Gly Xaa Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> 92
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog K

<220>
<221> BINDING
<222> (9)..(9)
<223> N-Me

<220>
<221> BINDING
<222> (20)..(20)
<223> N-Me

<400> 92

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys

20

<210> 93
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog L

<220>
<221> BINDING
<222> (9)..(9)
<223> N-Me

<220>
<221> BINDING
<222> (11)..(11)
<223> N-Me

<220>
<221> BINDING
<222> (20)..(20)
<223> N-Me

<400> 93

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> 94
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog M

<220>
<221> BINDING
<222> (9)..(9)

<223> N-Me

<220>
<221> BINDING
<222> (11)..(11)
<223> N-Me

<400> 94

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1   5   10   15

Met Ser Gly Leu Gly Cys
            20

<210> 95
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog Z

<400> 95

Gly Leu Ser Arg Gly Cys Tyr Gly Leu Lys Leu Asp Arg Ile Gly Ser
1   5   10   15

Met Ser Gly Leu Gly Cys
            20

<210> 96
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog AA

<400> 96

Gly Leu Ser Arg Gly Cys Phe Val Leu Lys Leu Asp Arg Ile Gly Ser
1   5   10   15

Met Ser Gly Leu Gly Cys
            20

<210> 97
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog AB

<400> 97

Gly Leu Ser Arg Gly Cys Phe Ser Leu Lys Leu Asp Arg Ile Gly Ser
1   5   10   15

Met Ser Gly Leu Gly Cys
            20

<210> 98
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog AC

<400> 98

Gly Leu Ser Arg Gly Cys Phe Thr Leu Lys Leu Asp Arg Ile Gly Ser
1   5   10   15

Met Ser Gly Leu Gly Cys
            20

<210> 99
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog AD

<400> 99

Gly Leu Ser Arg Gly Cys Phe Gly Thr Lys Leu Asp Arg Ile Gly Ser
1   5   10   15

Met Ser Gly Leu Gly Cys
            20

<210> 100
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog AE

<400> 100

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Arg Ser
1   5   10   15

Met Ser Gly Leu Gly Cys
            20

<210> 101
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog AF

<220>
<221> MOD_RES
<222> (15)..(15)

<223> Xaa is Cit

<400> 101

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Xaa Ser
1   5   10   15

Met Ser Gly Leu Gly Cys
            20

<210> 102
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog AG

<400> 102

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1   5   10   15

Val Ser Gly Leu Gly Cys
            20

<210> 103
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog AJ

<400> 103

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1   5   10   15

Met Ser Gly Val Gly Cys
            20

<210> 104
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog AK

<220>
<221> MOD_RES
<222> (20)..(20)
<223> Xaa is tBu-Ala

<400> 104

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Xaa Gly Cys
20

<210> 105
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog AT

<400> 105

Glu Leu Ser Glu Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> 106
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog AV

<220>
<221> MOD_RES
<222> (1)..(1)
<223> N-terminus modified with pentanoic acid

<400> 106

Glu Leu Ser Glu Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1   5   10   15

Met Ser Gly Leu Gly Cys
            20

<210> 107
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog AW

<220>
<221> MOD_RES
<222> (1)..(1)
<223> Heptanoic acid modifying N-terminus

<400> 107

Glu Leu Ser Glu Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1   5   10   15

Met Ser Gly Leu Gly Cys
            20

<210> 108
<211> 22
<212> PRT
<213> Artificial Sequence

<220>

<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog BB

<220>
<221> MOD_RES
<222> (1)..(1)
<223> N-terminus modified with heptanoic acid

<400> 108

Glu Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> 109
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog BC

<220>
<221> MOD_RES
<222> (1)..(1)
<223> N-terminus modified with pentanoic acid

<400> 109

Glu Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> 110
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog BF

<220>
<221> MOD_RES
<222> (10)..(10)
<223> Xaa is Cit

<400> 110

Gly Leu Ser Arg Gly Cys Phe Gly Leu Xaa Leu Asp Arg Ile Gly Ser
1   5   10   15

Met Ser Gly Leu Gly Cys
            20

<210> 111
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog BG

<400> 111

Gly Leu Ser Arg Gly Cys Phe Gly Leu Gln Leu Asp Arg Ile Gly Ser
1   5   10   15

Met Ser Gly Leu Gly Cys
            20

<210> 112
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog BH

<400> 112

Gly Leu Ser Arg Gly Cys Phe Gly Leu Arg Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> 113
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog BJ

<400> 113

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Asn Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> 114
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog BK

<400> 114

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Ser Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> 115

<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog N

<220>
<221> MISC_FEATURE
<222> (1)..(22)
<223> All D-amino acids

<400> 115

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> 116
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog BD

<220>
<221> BINDING
<222> (9)..(9)
<223> N-Me

<400> 116

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> 117
<211> 22

<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog BN

<220>
<221> BINDING
<222> (11)..(11)
<223> N-Me

<400> 117

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> 118
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog BE

<220>
<221> BINDING
<222> (20)..(20)
<223> N-Me

<400> 118

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> 119
<211> 22
<212> PRT

<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog V

<220>
<221> BINDING
<222> (7)..(7)
<223> 3,4-dichloro

<400> 119

Gly Leu Ser Gly Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> 120
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog X

<220>
<221> BINDING
<222> (7)..(7)
<223> 3-methyl

<400> 120

Gly Leu Ser Gly Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> 121
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog BP

<400> 121

Gly Leu Ser Arg Ser Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1   5   10   15

Met Ser Gly Leu Gly Cys
            20

<210> 122
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog BO

<400> 122

Gly Leu Ser Arg Arg Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1   5   10   15

Met Ser Gly Leu Gly Cys
            20

<210> 123
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog P

<400> 123

Gly Leu Ser Gly Gly Cys Phe Gly Leu Arg Leu Asp Arg Ile Gly Ser
1   5   10   15

Met Ser Gly Leu Gly Cys
            20

<210> 124
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog BY

<400> 124

Gly Leu Ser Arg Gly Cys Phe Gly Leu Ser Leu Asp Arg Ile Gly Ser
1   5   10   15

Met Ser Gly Leu Gly Cys
            20

<210> 125
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog BZ

<400> 125

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Gln
1   5   10   15

Met Ser Gly Leu Gly Cys
            20

<210> 126
<211> 22
<212> PRT

<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog BX

<400> 126

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Asn Ser Gly Leu Gly Cys
20

<210> 127
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog BQ

<400> 127

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Ser Leu Gly Cys
20

<210> 128
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog BR

<400> 128

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1   5   10   15

Met Ser Arg Leu Gly Cys
            20

<210> 129
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog BS

<400> 129

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1   5   10   15

Met Ser Gly Arg Gly Cys
            20

<210> 130
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog BT

<400> 130

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1   5   10   15

Met Ser Gly Leu Ser Cys
            20

<210> 131
<211> 22

<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog BU

<400> 131

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1   5   10   15

Met Ser Gly Leu Thr Cys
            20

<210> 132
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog BW

<400> 132

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1   5   10   15

Met Ser Gly Leu Arg Cys
            20

<210> 133
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog CJ

<400> 133

Arg Ser Ser Cys Phe Gly Gly Arg Ile Asp Arg Ile Gly Ala Cys
1 5 10 15

<210> 134
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MOD_RES
<222> (1)..(1)
<223> N-terminal linked to CNP22 by disuccinimidyl glutarate

<400> 134

Gly Leu Ser Arg Gly Cys Phe Gly Leu Arg Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> 135
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MOD_RES
<222> (1)..(1)
<223> N-terminal linked to CNP22 by Bis-PEO5

<400> 135

Gly Leu Ser Arg Gly Cys Phe Gly Leu Arg Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> 136
<211> 22
<212> PRT

<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog E

<220>
<221> MOD_RES
<222> (7)..(7)
<223> Xaa is D-Phe

<400> 136

Gly Leu Ser Lys Gly Cys Xaa Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> 137
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Sythetic Polypeptide

<220>
<221> MISC_FEATURE
<223> Analog G

<220>
<221> MOD_RES
<222> (7)..(7)
<223> Xaa is 3-Cl-Phe

<400> 137

Gly Leu Ser Lys Gly Cys Xaa Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> 138
<211> 18
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MOD_RES
<222> (1)..(1)
<223> Xaa comprises one or more amino acids selected from
       Gly-Leu-Ser-Lys-Gly or substitutions at one or more of these
       amino acids and further comprises a hydrophilic or water soluable
       polymer

<400> 138

Xaa Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu
1   5   10   15

Gly Cys

<210> 139
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MOD_RES
<222> (1)..(1)
<223> Cys is modified with a natural and/or synthetic polymer

<220>
<221> MOD_RES
<222> (17)..(17)
<223> Cys is modified with a natural and/or synthetic polymer

<400> 139

Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly
1   5   10   15

Cys

<210> 140
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MOD_RES
<222> (1)..(1)
<223> Gly is modified with a natural and/or synthetic polymer

<220>
<221> MOD_RES
<222> (22)..(22)
<223> Cys is modified with a natural and/or synthetic polymer

<400> 140

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1   5   10   15

Met Ser Gly Leu Gly Cys
            20

<210> 141
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MOD_RES
<222> (1)..(1)
<223> Cys is modified with a natural and/or synthetic polymeric group
       or combination thereof

<220>
<221> MOD_RES
<222> (5)..(5)
<223> Xaa is Lys or a conservative amino acid substitution thereof, any
       natural or unnatural amino acid, Arg, Gly, 6-hydroxy-norleucine,
       citrulline (Cit), Gln, Glu or Ser

<220>
<221> MOD_RES
<222> (17)..(17)
<223> Cys is optionally modified with a natural and/or synthetic
       polymeric group or combination thereof

<400> 141

Cys Phe Gly Leu Xaa Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly
1   5   10   15

Cys

<210> 142
<211> 19
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MOD_RES
<222> (1)..(1)
<223> Xaa is optionally modified with bone- or cartilage-targeting moieties, moieties that reduce renal clearance, hydrophilic polymers, carbohydrates, hydrophobic acids and combinations thereof

<220>
<221> MOD_RES
<222> (2)..(2)
<223> Xaa is absent or may comprise 1 to 5 amino acids from Gly-Leu-Ser-Lys-Gly, optionally substituted with a natural or unnatural amino acid

<220>
<221> MOD_RES
<222> (7)..(7)
<223> Xaa is Lys, any natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amine on a side chain

<220>
<221> MOD_RES
<222> (19)..(19)
<223> Xaa is optionally modified with bone- or cartilage-targeting moieties, moieties that reduce renal clearance, hydrophilic polymers, carbohydrates, hydrophobic acids and combinations thereof

<400> 142

Xaa Xaa Cys Phe Gly Leu Xaa Leu Asp Arg Ile Gly Ser Met Ser Gly
1 5 10 15

Leu Gly Cys

<210> 143
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MOD_RES
<222> (1)..(1)
<223> Gly is optionally modified with cartilage or bone targeting compounds, a bisphosphonate, polyAsp, polyGlu, osteopontin peptides, osteocalcin peptides, sialoprotein peptides, charged PEG molecules or hydrophilic polymers

<220>
<221> MOD_RES
<222> (4)..(4)
<223> Xaa is Lys or a conservative amino acid substitution thereof, any natural or unnatural amino acid, Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu

<220>
<221> MOD_RES
<222> (6)..(6)
<223> Xaa is Cys or peptide isosteres between positions 6 and 7

<220>
<221> MOD_RES
<222> (7)..(7)
<223> Xaa is L-Phe, D-Phe, N-alkylated Phe, or Phe analog

<220>
<221> MOD_RES
<222> (8)..(8)
<223> Xaa is Gly, tert-butyl-Gly, Thr, Ser, Val or Asn

<220>
<221> MOD_RES
<222> (9)..(9)
<223> Xaa is Leu, Ser, Thr or peptide bond isosteres thereof

<220>
<221> MOD_RES
<222> (10)..(10)
<223> Xaa is Lys or a conservative amino acid substitution thereof, any natural or unnatural amino acid, Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu

<220>
<221> misc_feature
<222> (11)..(11)
<223> Xaa can be any naturally occurring amino acid

<220>
<221> misc_feature
<222> (14)..(14)
<223> Xaa can be any naturally occurring amino acid

<220>
<221> MOD_RES
<222> (15)..(15)
<223> Xaa is Gly, Arg, Ser or Asn

<220>
<221> misc_feature
<222> (17)..(17)
<223> Xaa can be any naturally occurring amino acid

<220>
<221> MOD_RES
<222> (20)..(20)
<223> Xaa is Leu, norleucine (Nle), homoleucine (Hleu), Val, tert-butyl-Ala (tBu-Ala), Arg, Thr, Ser or peptide bond isoteres thereof

<220>
<221> MOD_RES
<222> (22)..(22)
<223> Cys is optionally modified with cartilage or bone targeting compounds, a bisphosphonate, polyAsp, polyGlu, osteopontin peptides, osteocalcin peptides, sialoprotein peptides, charged PEG molecules or hydrophilic polymers

<400> 143

Gly Leu Ser Xaa Gly Xaa Xaa Xaa Xaa Xaa Xaa Asp Arg Xaa Xaa Ser
1 5 10 15

Xaa Ser Gly Xaa Gly Cys
20

<210> 144
<211> 38
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 144

Gly His Lys Ser Glu Val Ala His Arg Phe Lys Gly Ala Asn Lys Lys
1 5 10 15

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
20 25 30

Met Ser Gly Leu Gly Cys
35

<210> 145
<211> 39
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 145

Pro Gly Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys
1 5 10 15

Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly
20 25 30

Ser Met Ser Gly Leu Gly Cys
35

<210> 146
<211> 52
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 146

Leu Arg Val Asp Thr Lys Ser Arg Ala Ala Trp Ala Arg Leu Leu Gln
1 5 10 15

Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu
20 25 30

Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser
35 40 45

Gly Leu Gly Cys
50

<210> 147
<211> 51
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 147

```
Arg Val Asp Thr Lys Ser Arg Ala Ala Trp Ala Arg Leu Leu Gln Glu
1               5                   10                  15

His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser
            20                  25                  30

Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly
        35                  40                  45

Leu Gly Cys
    50
```

<210> 148
<211> 50
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 148

```
Val Asp Thr Lys Ser Arg Ala Ala Trp Ala Arg Leu Leu Gln Glu His
1               5                   10                  15

Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys
            20                  25                  30

Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu
        35                  40                  45

Gly Cys
    50
```

<210> 149
<211> 49
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 149

```
Asp Thr Lys Ser Arg Ala Ala Trp Ala Arg Leu Leu Gln Glu His Pro
1               5                   10                  15
```

```
Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly
            20                  25                  30

Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly
        35                  40                  45

Cys
```

<210> 150
<211> 48
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 150

```
Thr Lys Ser Arg Ala Ala Trp Ala Arg Leu Leu Gln Glu His Pro Asn
1               5                   10                  15

Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys
            20                  25                  30

Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
        35                  40                  45
```

<210> 151
<211> 47
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 151

```
Lys Ser Arg Ala Ala Trp Ala Arg Leu Leu Gln Glu His Pro Asn Ala
1               5                   10                  15

Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe
            20                  25                  30

Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
        35                  40                  45
```

<210> 152

<211> 46
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 152

Ser Arg Ala Ala Trp Ala Arg Leu Leu Gln Glu His Pro Asn Ala Arg
1 5 10 15

Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly
20 25 30

Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
35 40 45

<210> 153
<211> 45
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 153

Arg Ala Ala Trp Ala Arg Leu Leu Gln Glu His Pro Asn Ala Arg Lys
1 5 10 15

Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu
20 25 30

Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
35 40 45

<210> 154
<211> 44
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 154

Ala Ala Trp Ala Arg Leu Leu Gln Glu His Pro Asn Ala Arg Lys Tyr
1 5 10 15

```
Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys
            20                  25                  30

Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
        35                  40
```

<210> 155
<211> 43
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 155

```
Ala Trp Ala Arg Leu Leu Gln Glu His Pro Asn Ala Arg Lys Tyr Lys
1               5                   10                  15

Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu
            20                  25                  30

Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
        35                  40
```

<210> 156
<211> 42
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 156

```
Trp Ala Arg Leu Leu Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly
1               5                   10                  15

Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp
            20                  25                  30

Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
        35                  40
```

<210> 157
<211> 41
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 157

Ala Arg Leu Leu Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala
1   5   10   15

Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg
            20   25   30

Ile Gly Ser Met Ser Gly Leu Gly Cys
        35   40

<210> 158
<211> 40
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 158

Arg Leu Leu Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn
1   5   10   15

Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile
            20   25   30

Gly Ser Met Ser Gly Leu Gly Cys
        35   40

<210> 159
<211> 39
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 159

Leu Leu Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys
1   5   10   15

Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly
            20   25   30

Ser Met Ser Gly Leu Gly Cys
        35

<210> 160
<211> 38
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 160

Leu Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys
1   5   10   15

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
            20   25   30

Met Ser Gly Leu Gly Cys
        35

<210> 161
<211> 36
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 161

Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu
1   5   10   15

Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser
            20   25   30

Gly Leu Gly Cys
        35

<210> 162
<211> 35
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 162

```
His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser
1               5                   10                  15

Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly
            20                  25                  30

Leu Gly Cys
        35
```

<210> 163
<211> 34
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 163

```
Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys
1               5                   10                  15

Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu
            20                  25                  30

Gly Cys
```

<210> 164
<211> 33
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 164

```
Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly
1               5                   10                  15

Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly
            20                  25                  30

Cys
```

<210> 165
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 165

Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys
1 5 10 15

Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25 30

<210> 166
<211> 31
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 166

Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe
1 5 10 15

Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25 30

<210> 167
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 167

Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly
1 5 10 15

Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25 30

<210> 168
<211> 29
<212> PRT

<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 168

Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu
1 5 10 15

Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25

<210> 169
<211> 28
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 169

Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys
1 5 10 15

Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25

<210> 170
<211> 27
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 170

Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu
1 5 10 15

Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25

<210> 171
<211> 26
<212> PRT
<213> Artificial Sequence

<220>

<223> Synthetic Polypeptide

<400> 171

Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp
1 5 10 15

Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25

<210> 172
<211> 25
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 172

Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg
1 5 10 15

Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25

<210> 173
<211> 24
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 173

Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile
1 5 10 15

Gly Ser Met Ser Gly Leu Gly Cys
20

<210> 174
<211> 23
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 174

Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly
1   5   10   15

Ser Met Ser Gly Leu Gly Cys
            20

<210> 175
<211> 21
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 175

Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met
1   5   10   15

Ser Gly Leu Gly Cys
            20

<210> 176
<211> 20
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 176

Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser
1   5   10   15

Gly Leu Gly Cys
            20

<210> 177
<211> 19
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 177

Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly
1   5   10   15

Leu Gly Cys

<210> 178
<211> 18
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 178

Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu
1 5 10 15

Gly Cys

<210> 179
<211> 54
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 179

Gly Asp Leu Arg Val Asp Thr Lys Ser Arg Ala Ala Trp Ala Arg Leu
1 5 10 15

Leu Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys
20 25 30

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
35 40 45

Met Ser Gly Leu Gly Cys
50

<210> 180
<211> 53
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 180

Asp Leu Arg Val Asp Thr Lys Ser Arg Ala Ala Trp Ala Arg Leu Leu
1 5 10 15

Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly
20 25 30

Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Asn
35 40 45

Ser Gly Leu Gly Cys
50

<210> 181
<211> 38
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 181

Gly Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys
1 5 10 15

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
20 25 30

Asn Ser Gly Leu Gly Cys
35

<210> 182
<211> 37
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 182

Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly
1 5 10 15

Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Asn
20 25 30

Ser Gly Leu Gly Cys
        35

<210> 183
<211> 38
<212> PRT
<213> Artificial Sequence

<220>
<223> Sythetic Polypeptide

<400> 183

Gly His Lys Ser Glu Val Ala His Arg Phe Lys Gly Ala Asn Lys Lys
1   5   10   15

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
            20   25   30

Asn Ser Gly Leu Gly Cys
        35

<210> 184
<211> 27
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 184

Gly Ala Asn Arg Arg Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu
1   5   10   15

Asp Arg Ile Gly Ser Asn Ser Gly Leu Gly Cys
            20   25

<210> 185
<211> 54
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 185

Pro Asp Leu Arg Val Asp Thr Lys Ser Arg Ala Ala Trp Ala Arg Leu

1 5 10 15

Leu Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys
20 25 30

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
35 40 45

Met Ser Gly Leu Gly Cys
50

<210> 186
<211> 38
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 186

Pro Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys
1 5 10 15

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
20 25 30

Met Ser Gly Leu Gly Cys
35

<210> 187
<211> 34
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 187

Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys
1 5 10 15

Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu
20 25 30

Gly Cys

<210> 188
<211> 39
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 188

Pro Gly His Lys Ser Glu Val Ala His Arg Phe Lys Gly Ala Asn Lys
1 5 10 15

Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly
20 25 30

Ser Met Ser Gly Leu Gly Cys
35

<210> 189
<211> 28
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 189

Pro Gly Ala Asn Arg Arg Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys
1 5 10 15

Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25

<210> 190
<211> 54
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 190

Met Asp Leu Arg Val Asp Thr Lys Ser Arg Ala Ala Trp Ala Arg Leu
1 5 10 15

Leu Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys

```
            20                  25                  30

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
        35                  40                  45

Met Ser Gly Leu Gly Cys
    50
```

<210> 191
<211> 39
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 191

```
Met Gly Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys
1               5                   10                  15

Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly
            20                  25                  30

Ser Met Ser Gly Leu Gly Cys
        35
```

<210> 192
<211> 38
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 192

```
Met Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys
1               5                   10                  15

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
            20                  25                  30

Met Ser Gly Leu Gly Cys
        35
```

<210> 193
<211> 39

<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 193

Met Gly His Lys Ser Glu Val Ala His Arg Phe Lys Gly Ala Asn Lys
1 5 10 15

Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly
20 25 30

Ser Met Ser Gly Leu Gly Cys
35

<210> 194
<211> 28
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 194

Met Gly Ala Asn Arg Arg Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys
1 5 10 15

Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25

<210> 195
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 195

Met Gly Ser Ser His His His His His His Ser Ser Gly Leu Val Pro
1 5 10 15

Arg Gly Ser His Thr Gly Asp Asp Asp Asp Lys His Met Asp
20 25 30

<210> 196

<211> 142
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 196

Met Val Leu Thr Lys Lys Lys Leu Gln Asp Leu Val Arg Glu Val Cys
1 5 10 15

Pro Asn Glu Gln Leu Asp Glu Asp Val Glu Glu Met Leu Leu Gln Ile
20 25 30

Ala Asp Asp Phe Ile Glu Ser Val Val Thr Ala Ala Cys Gln Leu Ala
35 40 45

Arg His Arg Lys Ser Ser Thr Leu Glu Val Lys Asp Val Gln Leu His
50 55 60

Leu Glu Arg Gln Trp Asn Met Trp Ile Met Gly Ser Ser His His His
65 70 75 80

His His His Ser Ser Gly Leu Val Pro Arg Gly Ser His Thr Gly Asp
85 90 95

Asp Asp Asp Lys His Met Asp Pro Gly Gln Glu His Pro Asn Ala Arg
100 105 110

Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly
115 120 125

Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
130 135 140

<210> 197
<211> 72
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 197

Val Leu Thr Lys Lys Lys Leu Gln Asp Leu Val Arg Glu Val Cys Pro
1 5 10 15

```
Asn Glu Gln Leu Asp Glu Asp Val Glu Glu Met Leu Leu Gln Ile Ala
            20                  25                  30

Asp Asp Phe Ile Glu Ser Val Val Thr Ala Ala Cys Gln Leu Ala Arg
        35                  40                  45

His Arg Lys Ser Ser Thr Leu Glu Val Lys Asp Val Gln Leu His Leu
    50                  55                  60

Glu Arg Gln Trp Asn Met Trp Ile
65                  70
```

<210> 198
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 198

```
Pro Gly Gln Glu His Pro Asn Ala Arg
1               5
```

<210> 199
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 199

```
Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly
1               5                   10                  15

Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
            20                  25                  30
```

<210> 200
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 200

Gly Ala Asn Lys Pro
1 5

<210> 201
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 201

Gly Ala Asn Pro Lys
1 5

<210> 202
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 202

Gly Ala Asn Pro Gln
1 5

<210> 203
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 203

Gly Ala Asn Gln Lys
1 5

<210> 204
<211> 41
<212> PRT
<213> Triakis scyllium

<400> 204

Arg Leu Leu Lys Asp Leu Ser Asn Asn Pro Leu Arg Phe Arg Gly Arg

```
1               5                   10                  15

Ser Lys Lys Gly Pro Ser Arg Gly Cys Phe Gly Val Lys Leu Asp Arg
            20                  25                  30

Ile Gly Ala Met Ser Gly Leu Gly Cys
        35                  40
```

<210> 205
<211> 39
<212> PRT
<213> Triakis scyllium

<400> 205

```
Leu Lys Asp Leu Ser Asn Asn Pro Leu Arg Phe Arg Gly Arg Ser Lys
1               5                   10                  15

Lys Gly Pro Ser Arg Gly Cys Phe Gly Val Lys Leu Asp Arg Ile Gly
            20                  25                  30

Ala Met Ser Gly Leu Gly Cys
        35
```

<210> 206
<211> 38
<212> PRT
<213> Triakis scyllium

<400> 206

```
Lys Asp Leu Ser Asn Asn Pro Leu Arg Phe Arg Gly Arg Ser Lys Lys
1               5                   10                  15

Gly Pro Ser Arg Gly Cys Phe Gly Val Lys Leu Asp Arg Ile Gly Ala
            20                  25                  30

Met Ser Gly Leu Gly Cys
        35
```

<210> 207
<211> 22
<212> PRT
<213> Triakis scyllium

<400> 207

```
Gly Pro Ser Arg Gly Cys Phe Gly Val Lys Leu Asp Arg Ile Gly Ala
```

```
1               5                   10                  15

Met Ser Gly Leu Gly Cys
            20
```

<210> 208
<211> 41
<212> PRT
<213> Lamna ditropis

<400> 208

```
Arg Leu Leu Lys Asp Leu Ser Asn Asn Pro Leu Arg Phe Lys Gly Arg
1               5                   10                  15

Ser Lys Lys Gly Pro Ser Arg Gly Cys Phe Gly Val Lys Leu Asp Arg
            20                  25                  30

Ile Gly Ala Met Ser Gly Leu Gly Cys
        35                  40
```

<210> 209
<211> 39
<212> PRT
<213> Lamna ditropis

<400> 209

```
Leu Lys Asp Leu Ser Asn Asn Pro Leu Arg Phe Lys Gly Arg Ser Lys
1               5                   10                  15

Lys Gly Pro Ser Arg Gly Cys Phe Gly Val Lys Leu Asp Arg Ile Gly
            20                  25                  30

Ala Met Ser Gly Leu Gly Cys
        35
```

<210> 210
<211> 38
<212> PRT
<213> Lamna ditropis

<400> 210

```
Lys Asp Leu Ser Asn Asn Pro Leu Arg Phe Lys Gly Arg Ser Lys Lys
1               5                   10                  15

Gly Pro Ser Arg Gly Cys Phe Gly Val Lys Leu Asp Arg Ile Gly Ala
```

```
            20                  25                  30

Met Ser Gly Leu Gly Cys
        35
```

<210> 211
<211> 29
<212> PRT
<213> Lamna ditropis

<400> 211

```
Phe Lys Gly Arg Ser Lys Lys Gly Pro Ser Arg Gly Cys Phe Gly Val
1               5                   10                  15

Lys Leu Asp Arg Ile Gly Ala Met Ser Gly Leu Gly Cys
            20                  25
```

<210> 212
<211> 22
<212> PRT
<213> Lamna ditropis

<400> 212

```
Gly Pro Ser Arg Gly Cys Phe Gly Val Lys Leu Asp Arg Ile Gly Ala
1               5                   10                  15

Met Ser Gly Leu Gly Cys
            20
```

<210> 213
<211> 41
<212> PRT
<213> Squalus acanthias

<400> 213

```
Arg Leu Leu Gln Asp Leu Ser Asn Asn Pro Leu Arg Phe Lys Gly Arg
1               5                   10                  15

Ser Lys Lys Gly Pro Ser Arg Ser Cys Phe Gly Leu Lys Leu Asp Arg
            20                  25                  30

Ile Gly Ala Met Ser Gly Leu Gly Cys
        35                  40
```

<210> 214

<211> 22
<212> PRT
<213> Squalus acanthias

<400> 214

Gly Pro Ser Arg Ser Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ala
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> 215
<211> 22
<212> PRT
<213> Oryzias latipes

<400> 215

Gly Trp Asn Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> 216
<211> 24
<212> PRT
<213> Oryzias latipes

<400> 216

Pro Met Val Ala Gly Gly Gly Cys Phe Gly Met Lys Met Asp Arg Ile
1 5 10 15

Gly Ser Ile Ser Gly Leu Gly Cys
20

<210> 217
<211> 27
<212> PRT
<213> Arothron maculatus

<400> 217

Gly Arg Ser Ser Met Val Gly Gly Arg Gly Cys Phe Gly Met Lys Ile
1 5 10 15

Asp Arg Ile Gly Ser Ile Ser Gly Leu Gly Cys
20 25

<210> 218
<211> 22
<212> PRT
<213> Oryzias latipes

<400> 218

Gly Gly Met Arg Ser Cys Phe Gly Val Arg Leu Glu Arg Ile Gly Ser
1 5 10 15

Phe Ser Gly Leu Gly Cys
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<210> 220
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<213> Oryzias latipes

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<400> 221

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<210> 222
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<400> 224

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Met Ser Gly Leu Gly Cys
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<210> 225
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Gln Ser Gly Leu Gly Cys
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<210> 226
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<210> 227
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<400> 228

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1 5 10 15

Met Ser Gly Leu Gly Cys Asn Ser Phe Arg Tyr
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<210> 229
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<400> 229

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1 5 10 15

Cys Asn Ser Phe Arg Tyr
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<210> 230
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<400> 237

Ala Phe Leu Gly Pro Gly Asp Arg
1   5

Docket No.: 30610/44556C1-R2
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Reissue Application of:
Daniel J. Wendt et al.

Application No.: 15/646,822 | Confirmation No.: 7727

Filed: July 11, 2017 | Art Unit: 1629

For: Variants of C-Type Natriuretic Peptide | Examiner: Not Yet Assigned

SEQUENCE STATEMENT

MS Sequence
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I hereby state that the content of the paper and computer-readable copies of the Substitute Sequence Listing filed concurrently herewith are the same and do not include new matter.

Dated: August 2, 2017

Respectfully submitted,

By /Katherine L. Neville/
Katherine L. Neville
Registration No.: 53,379
MARSHALL, GERSTEIN & BORUN LLP
233 S. Wacker Drive
6300 Willis Tower
Chicago, Illinois 60606-6357
(312) 474-6300
Attorney for Applicant

```
============================================================
Sequence Listing was accepted.
If you need help call the Patent Electronic Business Center at (866)
217-9197 (toll free).
Reviewer: Anjum, Durreshwar
Timestamp: [year=2017; month=8; day=7; hr=13; min=47; sec=38; ms=919; ]
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Validated By CRFValidator v 1.0.5

Application No: 15646822 Version No: 2.0

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Output Set:

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Finished: 2017-08-02 16:15:08.736
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Total Errors: 0
No. of SeqIDs Defined: 237
Actual SeqID Count: 237

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Input Set:

Output Set:

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Finished: 2017-08-02 16:15:08.736
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Total Warnings: 251
Total Errors: 0
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Actual SeqID Count: 237

Error code		Error Description
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W	447	n or Xaa used, for: SEQID(44) on line number 940
W	447	n or Xaa used, for: SEQID(45) on line number 969
W	447	n or Xaa used, for: SEQID(46) on line number 1063
W	447	n or Xaa used, for: SEQID(46) on line number 1067
W	447	n or Xaa used, for: SEQID(47) on line number 1233
W	447	n or Xaa used, for: SEQID(47) on line number 1237
W	447	n or Xaa used, for: SEQID(48) on line number 1296
W	447	n or Xaa used, for: SEQID(49) on line number 1380
W	447	n or Xaa used, for: SEQID(50) on line number 1477
W	447	n or Xaa used, for: SEQID(50) on line number 1481
W	447	n or Xaa used, for: SEQID(51) on line number 1501
W	447	n or Xaa used, for: SEQID(52) on line number 1525
W	447	n or Xaa used, for: SEQID(53) on line number 1549
W	447	n or Xaa used, for: SEQID(54) on line number 1577
W	447	n or Xaa used, for: SEQID(55) on line number 1601
W	447	n or Xaa used, for: SEQID(57) on line number 1653
W	447	n or Xaa used, for: SEQID(59) on line number 1709
W	402	Undefined organism found in <213> in SEQ ID (68)
W	402	Undefined organism found in <213> in SEQ ID (78)
W	447	n or Xaa used, for: SEQID(90) on line number 2547

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Output Set:	
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Finished:	2017-08-02 16:15:08.736
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Actual SeqID Count:	237

Error code	Error Description

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Wendt, et al.

<120> Variants of C-Type Natriuretic Peptide

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<140> US 15/646,822
<141> 2017-08-02

<150> 14/604,232
<151> 2015-01-23

<150> 13/466,672
<151> 2012-05-08

<150> 12/784,117
<151> 2010-05-20

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<151> 2009-10-23

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1 5 10 15

Met Ser Gly Leu Gly Cys

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Cys

<210> 3
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20 25

<210> 4
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1   5   10   15

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            20   25   30

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Ser Gly Leu Gly Cys
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<223> Cys is optionally modified with an amino acid sequence derived
       from a natriuretic polypeptide, a non-natriuretic polypeptide

<400> 5

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1   5   10   15

Met Ser Gly Leu Gly Cys
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<223> Xaa is Lys or a conservative amino acid substitution thereof, any natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amine on a side chain, Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Glu or Ser

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<220>
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<222> (22)..(22)
<223> Cys is optionally modified with a synthetic or natural polymeric group or combination thereof

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Gly Ala Asn Ser Ser
1 5

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1 5

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<400> 13

Gly Ala Asn Arg Met
1 5

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1 5 10 15

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1 5 10 15

<210> 21
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<220>
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Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys
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<220>
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<210> 23
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<220>
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<400> 23

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Ser Pro Lys Met Val Gln Gly Ser Gly
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Lys Val Leu Arg Arg Tyr
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1 5

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1 5 10 15

<210> 32
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<400> 32

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
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<220>
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<220>
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<223> Xaa is Lys or a conservative amino acid substitution thereof, any natural or unnatural amino acid, Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Glu or Ser

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<222> (37)..(37)
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20 25 30

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            20   25

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1   5   10   15

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Arg Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly
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1   5   10   15

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<210> 43
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<222> (6)..(7)
<223> Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -C(=O)-N(R)-
where R is methyl, ethyl, n-propyl, isopropyl, cyclopropyl,
n-butyl, isobutyl, sec-butyl or tert-butyl

<220>
<221> MOD_RES
<222> (6)..(7)
<223> Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -C(=O)-NH-CH2-

<220>
<221> MOD_RES
<222> (6)..(7)
<223> Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -CH2-S-

<220>
<221> MOD_RES
<222> (6)..(7)
<223> Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -CH2-S(O)n
where n is 1 or 2

<220>
<221> MOD_RES
<222> (6)..(7)
<223> Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -CH2-CH2-

<220>
<221> MOD_RES
<222> (6)..(7)
<223> Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -CH=CH-

<220>
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<222> (6)..(7)
<223> Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -C(=O)-CH2-

<220>
<221> MOD_RES
<222> (6)..(7)
<223> Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -CH(CN)-NH-

<220>
<221> MOD_RES
<222> (6)..(7)
<223> Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -CH(OH)-CH2-

<220>
<221> MOD_RES
<222> (6)..(7)
<223> Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -O-C(=O)-NH-

<220>
<221> MOD_RES
<222> (6)..(7)
<223> Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with NHC(=O)NH-

<400> 43

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> 44
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MOD_RES
<222> (7)..(7)
<223> Xaa is 3-amino-2-phenylpropionic acid or D-Phe

<400> 44

Gly Leu Ser Lys Gly Cys Xaa Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> 45
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MISC_FEATURE
<223> CNP22 - modified Cys6

<220>
<221> MOD_RES
<222> (6)..(6)
<223> Xaa is Cys, homocysteine, penicillamine, 2-mercaptopropionic
acid, or 3-mercaptopropionic acid.

<400> 45

Gly Leu Ser Lys Gly Xaa Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser

1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> 46
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<220>
<221> MOD_RES
<222> (1)..(1)
<223> Gly is optionally modified with a bone or cartilage targeting compound, a biphosphonate, polyAsp, polyGlu, osteopontin, osteocalcin, or sialoprotein peptides, polymers, PEG, carbohydrates, hydrophobic acids, or amino acids

<220>
<221> MOD_RES
<222> (4)..(4)
<223> Xaa is Lys or a conservative amino acid substitution thereof, any natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amine on a side chain, Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser, or Glu

<220>
<221> MOD_RES
<222> (6)..(7)
<223> Xaa is Cys with peptide bond isosteres between positions 6 and 7

<220>
<221> MOD_RES
<222> (7)..(7)
<223> Xaa is L-Phe, D-Phe, 3-amino-2-phenylpropionic acid, or N-alkylated derivatives of Phe

<220>
<221> MOD_RES

Electronic Acknowledgement Receipt	
EFS ID:	29748553
Application Number:	15646822
International Application Number:	
Confirmation Number:	7727
Title of Invention:	Variants of C-Type Natriuretic Peptide
First Named Inventor/Applicant Name:	Daniel J Wendt
Customer Number:	90849
Filer:	Katherine Lynn Neville
Filer Authorized By:	
Attorney Docket Number:	30610/44556C1-R2
Receipt Date:	11-JUL-2017
Filing Date:	
Time Stamp:	16:40:51
Application Type:	Reissue (Utility)

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$4300
RAM confirmation Number	071217INTEFSW16420700
Deposit Account	132855
Authorized User	Katherine Neville
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: 37 CFR 1.16 (National application filing, search, and examination fees) 37 CFR 1.17 (Patent application and reexamination processing fees)	

08/08/2017 SDIRETA1 00000004 132855 15646822
01 FC:1051 140.00 DA
02 FC:1004 1200.00 DA

PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 15/646,822

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	280
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	600
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	2160
TOTAL CLAIMS (37 CFR 1.16(i))	16 minus 20=	*			OR	x 80 =	0.00
INDEPENDENT CLAIMS (37 CFR 1.16(h))	6 minus 3 =	* 3				x 420 =	1260
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						1200
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							0.00
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	5500

APPLICATION AS AMENDED - PART II

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT A	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT B	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.

** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".

*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".

The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
15/646,822	07/11/2017	1629	5640	30610/44556C1-R2	16	6

CONFIRMATION NO. 7727

FILING RECEIPT

90849
Marshall, Gerstein & Borun LLP (Biomarin)
233 South Wacker Drive
6300 Willis Tower
Chicago, IL 60606

OC000000093352579

Date Mailed: 08/10/2017

Receipt is acknowledged of this reissue patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

Inventor(s)

Daniel J. Wendt, Novato, CA;
Shinong Long, Novato, CA;
Sianna Castillo, Novato, CA;
Christopher P. Price, Aachen, GERMANY;
Mika Aoyagi-Scharber, Novato, CA;
Michel Claude Vellard, Novato, CA;
Augustus O. Okamafe, Concord, CA;

Applicant(s)

BioMarin Pharmaceutical Inc., Novato, CA, Assignee (with 37 CFR 1.172 Interest);

Assignment For Published Patent Application

BioMarin Pharmaceutical Inc., Novato, CA

Power of Attorney: The patent practitioners associated with Customer Number 90849

Domestic Priority data as claimed by applicant

This application is a CON of 14/604,262 01/23/2015
which is a REI of 13/466,672 05/08/2012 PAT 8598121
which is a CON of 12/784,117 05/20/2010 PAT 8198242
which claims benefit of 61/180,112 05/20/2009
and claims benefit of 61/254,563 10/23/2009

Foreign Applications for which priority is claimed (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.) - None.
Foreign application information must be provided in an Application Data Sheet in order to constitute a claim to foreign priority. See 37 CFR 1.55 and 1.76.

Permission to Access Application via Priority Document Exchange: Yes

Permission to Access Search Results: Yes

Applicant may provide or rescind an authorization for access using Form PTO/SB/39 or Form PTO/SB/69 as appropriate.

If Required, Foreign Filing License Granted: 07/12/2017

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 15/646,822**

Projected Publication Date: None, application is not eligible for pre-grant publication

Non-Publication Request: No

Early Publication Request: No

Title

Variants of C-Type Natriuretic Peptide

Preliminary Class

514

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4258).

LICENSE FOR FOREIGN FILING UNDER

Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where the conditions for issuance of a license have been met, regardless of whether or not a license may be required as set forth in 37 CFR 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign AssetsControl, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

SelectUSA

The United States represents the largest, most dynamic marketplace in the world and is an unparalleled location for business investment, innovation, and commercialization of new technologies. The U.S. offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to promote and facilitate business investment. SelectUSA provides information assistance to the international investor

community; serves as an ombudsman for existing and potential investors; advocates on behalf of U.S. cities, states, and regions competing for global investment; and counsels U.S. economic development organizations on investment attraction best practices. To learn more about why the United States is the best country in the world to develop technology, manufacture products, deliver services, and grow your business, visit http://www.SelectUSA.gov or call +1-202-482-6800.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
15/646,822	07/11/2017	Daniel J. Wendt	30610/44556C1-R2

CONFIRMATION NO. 7727

90849
Marshall, Gerstein & Borun LLP (Biomarin)
233 South Wacker Drive
6300 Willis Tower
Chicago, IL 60606

FORMALITIES LETTER

OC000000093352580

Date Mailed: 08/10/2017

NOTICE TO FILE MISSING PARTS OF REISSUE APPLICATION

Filing Date Granted

An application number and filing date have been accorded to this reissue application. The item(s) indicated below, however, are missing. Applicant is given **TWO MONTHS** from the date of this Notice within which to file all required items and pay any fees required below to avoid abandonment. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

- The inventor's oath or declaration has not been submitted.

  An inventor's oath or declaration in compliance with 37 CFR 1.63 or 1.64 (and 37 CFR 1.175) executed by or with respect to each inventor is required. For a reissue application, the inventor's oath or declaration must be submitted prior to examination and cannot be postponed. See 37 CFR 1.175(e). An assignee of the entire interest can execute the oath or declaration if: (1) the application does not seek to enlarge the scope of the claims of the original patent; or (2) the application for the original patent was filed under 37 CFR 1.46 by the assignee of the entire interest. A substitute statement under 37 CFR 1.64 in lieu of an oath or declaration may be submitted in the circumstances provided for in 35 U.S.C. 115(d) and 37 CFR 1.64.

SUMMARY OF FEES DUE:

The fee(s) required within **TWO MONTHS** from the date of this Notice to avoid abandonment is/are itemized below. No entity status discount is in effect. If applicant is qualified for small entity status, a written assertion of small entity status must be submitted to establish small entity status. (See 37 CFR 1.27). If applicant is qualified for micro entity status, an acceptable Certification of Micro Entity Status must be submitted to establish micro entity status. (See 37 CFR 1.29 and forms PTO/SB/15A and 15B.)

- $ **1200** for **124** pages in excess of **100** application size fee.
- $(**0**) previous unapplied payment amount.
- $ **1200** TOTAL FEE BALANCE DUE.

Replies must be received in the USPTO within the set time period or must include a proper Certificate of Mailing or Transmission under 37 CFR 1.8 with a mailing or transmission date within the set time period. For more information and a suggested format, see Form PTO/SB/92 and MPEP 512.

Replies should be mailed to:

Mail Stop Missing Parts
Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

Registered users of EFS-Web may alternatively submit their reply to this notice via EFS-Web, including a copy of this Notice and selecting the document description "Applicant response to Pre-Exam Formalities Notice". https://sportal.uspto.gov/authenticate/AuthenticateUserLocalEPF.html

For more information about EFS-Web please call the USPTO Electronic Business Center at 1-866-217-9197 or visit our website at http://www.uspto.gov/ebc.

If you are not using EFS-Web to submit your reply, you must include a copy of this notice.

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at **(571) 272-4000** or **(571) 272-4200** or **1-888-786-0101**.

/kesmith/

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
15/646,822	07/11/2017	Daniel J. Wendt	30610/44556C1-R2

CONFIRMATION NO. 7727

90849
Marshall, Gerstein & Borun LLP (Biomarin)
233 South Wacker Drive
6300 Willis Tower
Chicago, IL 60606

WITHDRAWAL NOTICE

OC000000092777923

Date Mailed: 08/10/2017

Letter Regarding a New Notice and/or the Status of the Application

If a new notice or Filing Receipt is enclosed, applicant may disregard the previous notice mailed on 07/13/2017. The time period for reply runs from the mail date of the new notice. Within the time period for reply, applicant is required to file a reply in compliance with the requirements set forth in the new notice to avoid abandonment of the application.

Registered users of EFS-Web may alternatively submit their reply to this notice via EFS-Web. https://sportal.uspto.gov/authenticate/AuthenticateUserLocalEPF.html

For more information about EFS-Web please call the USPTO Electronic Business Center at **1-866-217-9197** or visit our website at http://www.uspto.gov/ebc.

If the reply is not filed electronically via EFS-Web, the reply must be accompanied by a copy of the new notice.

If the Office previously granted a petition to withdraw the holding of abandonment or a petition to revive under 37 CFR 1.137, the status of the application has been returned to pending status.

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at **(571) 272-4000** or **(571) 272-4200** or **1-888-786-0101**.

/kesmith/

PTO/AIA/80 (07-17)
Approved for use through 01/31/2018. OMB 0651-0035
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no person is required to respond to a collection of information unless it displays a valid OMB control number

POWER OF ATTORNEY TO PROSECUTE APPLICATIONS BEFORE THE USPTO

I hereby revoke all previous powers of attorney given in the application identified in the attached statement under 37 CFR 3.73(c).

I hereby appoint:

[X] Practitioners associated with Customer Number: 35893

OR

[ ] Practitioner(s) named below (if more than ten patent practitioners are to be named, then a customer number must be used):

Name	Registration Number

Name	Registration Number

As attorney(s) or agent(s) to represent the undersigned before the United States Patent and Trademark Office (USPTO) in connection with any and all patent applications assigned only to the undersigned according to the USPTO assignment records or assignment documents attached to this form in accordance with 37 CFR 3.73(c).

Please change the correspondence address for the application identified in the attached statement under 37 CFR 3.73(c) to:

[X] The address associated with Customer Number: 35893

OR

[ ]

Firm or individual name		
Address		
City	State	Zip
Country		
Telephone	Email	

Assignee name and address:

BIOMARIN PHARMACEUTICAL INC.
105 DIGITAL DRIVE
Novato, California 94949

A copy of this form, together with a statement under 37 CFR 3.73(c) (Form PTO/AIA/96 or equivalent) is required to be filed in each application in which this form is used. The statement under 37 CFR 3.73(c) may be completed by one of the practitioners appointed in this form, and must identify the application in which this Power of Attorney is to be filed.

SIGNATURE of Assignee of Record

The individual whose signature and title is supplied below is authorized to act on behalf of the assignee.

Signature	Date 09-15-2017
Name Luisa Bigbusia	Telephone 415-506-6709
Title VP, Intellectual Property	

This collection of information is required by 37 CFR 1.31, 1.32, and 1.33. The information is required to obtain or retain a benefit by the public, which is to update (and by the USPTO to process) the file of a patent or reexamination proceeding. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 18 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
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Electronic Acknowledgement Receipt

EFS ID:	30492372
Application Number:	15646822
International Application Number:	
Confirmation Number:	7727
Title of Invention:	Variants of C-Type Natriuretic Peptide
First Named Inventor/Applicant Name:	Daniel J. Wendt
Customer Number:	90849
Filer:	Melissa Hunter-Ensor/Nancy Bechet
Filer Authorized By:	Melissa Hunter-Ensor
Attorney Docket Number:	30610/44556C1-R2
Receipt Date:	27-SEP-2017
Filing Date:	11-JUL-2017
Time Stamp:	15:30:38
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Power of Attorney	BIOMARINGENERAL.pdf	667907 312b42388ba924521085f0d48769302fc5b8f0b3	no	2
Warnings:					

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New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

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Bib Data Sheet

CONFIRMATION NO. 7727

SERIAL NUMBER	FILING OR 371(c) DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
15/646,822	07/11/2017 RULE	514	3991	30610/44556C1-R2

AIA (First Inventor to File): YES

INVENTORS

Daniel J. Wendt, Novato, CA;
Shinong Long, Novato, CA;
Sianna Castillo, Novato, CA;
Christopher P. Price, Aachen, GERMANY;
Mika Aoyagi-Scharber, Novato, CA;
Michel Claude Vellard, Novato, CA;
Augustus O. Okamafe, Concord, CA;

APPLICANTS

BioMarin Pharmaceutical Inc., Novato, CA, Assignee (with 37 CFR 1.172 Interest);

**** CONTINUING DATA ***************************

This application is a CON of 14/604,262 01/23/2015 PAT RE46707
which is a REI of 13/466,672 05/08/2012 PAT 8598121
which is a CON of 12/784,117 05/20/2010 PAT 8198242
which claims benefit of 61/180,112 05/20/2009
and claims benefit of 61/254,563 10/23/2009

**** FOREIGN APPLICATIONS *********************

IF REQUIRED, FOREIGN FILING LICENSE GRANTED ** 07/12/2017

Foreign Priority claimed	STATE OR COUNTRY	SHEETS DRAWING	TOTAL CLAIMS	INDEPENDENT CLAIMS
☐ yes ☐ no	CA	61	16	6
35 USC 119 (a-d) conditions met ☐ yes ☐ no ☐ Met after Allowance				
Verified and Acknowledged Examiner's Signature Initials				

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TITLE

Variants of C-Type Natriuretic Peptide

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Docket No.: 135576.010627/RE/CON
(Prior Docket No.: 30610/44556C1-R2)
(Patent)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Daniel J. Wendt et al.

Application No.: 15/646,822 | Confirmation No.: 7727

Filed: July 11, 2017 | Art Unit: To be assigned

For: *VARIANTS OF C-TYPE NATRIURETIC PEPTIDE* | Examiner: To be assigned

SUBMITTED VIA EFS-Web
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Alexandria, VA 22313-1450

RESPONSE TO NOTICE TO FILE MISSING PARTS OF REISSUE APPLICATION
(Filing Date Granted)

Dear Commissioner:

This response is submitted in reply to the Notice to File Missing Parts of Reissue Application, dated August 10, 2017, ("Notice"), as received in the above-identified continuation reissue patent application to which an application number and a filing date have been accorded. The Notice indicates that a fee of $1200 is due for 124 pages in the application that are in excess of 100 pages of application size.

Applicants submit herewith the $1200 fee for the excess pages in the application. The Commissioner is hereby authorized to charge the $1200 fee, and any additional fees that may be due or required in this application, to Deposit Account No. 50-2678, Ref. No. 135576.010627/REI/CON.

Dated: October 4, 2017

Respectfully submitted,
Greenberg Traurig, LLP
By: /Leslie Serunian/
Leslie Serunian
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Greenberg Traurig, LLP
One International Place
Boston, MA 02110
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BOS 48406020v1

Electronic Patent Application Fee Transmittal

Application Number:	15646822
Filing Date:	11-Jul-2017
Title of Invention:	Variants of C-Type Natriuretic Peptide
First Named Inventor/Applicant Name:	Daniel J. Wendt
Filer:	Leslie A. Serunian
Attorney Docket Number:	30610/44556C1-R2

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
UTILITY APPL SIZE FEE PER 50 SHEETS >100	1081	3	400	1200
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension-of-Time:				
Miscellaneous:				
			Total in USD ($)	**1200**

Electronic Acknowledgement Receipt

EFS ID:	30559843
Application Number:	15646822
International Application Number:	
Confirmation Number:	7727
Title of Invention:	Variants of C-Type Natriuretic Peptide
First Named Inventor/Applicant Name:	Daniel J. Wendt
Customer Number:	90849
Filer:	Leslie A. Serunian
Filer Authorized By:	
Attorney Docket Number:	30610/44556C1-R2
Receipt Date:	04-OCT-2017
Filing Date:	11-JUL-2017
Time Stamp:	13:52:32
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	$1200
RAM confirmation Number	100517INTEFSW00000167502678
Deposit Account	502678
Authorized User	Leslie Serunian
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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Applicant Response to Pre-Exam Formalities Notice	135576_010627RECON_RespMissParts.pdf	139707 aea8be343d34043ce1a0a0367171489ba69ff328	no	1
Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	30242 8126a5558ee7ec8bb91c872037f1fcb426c53658	no	2
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New Applications Under 35 U.S.C. 111
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National Stage of an International Application under 35 U.S.C. 371
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New International Application Filed with the USPTO as a Receiving Office
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PTO/SB/08a (03-15)
Approved for use through 07/31/2016. OMB 0651-0031
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT
(Not for submission under 37 CFR 1.99)

Application Number	15646822
Filing Date	2017-07-11
First Named Inventor	Daniel J. Wendt
Art Unit	1629
Examiner Name	N/A
Attorney Docket Number	135576-010627/REI/CON

U.S.PATENTS Remove

Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	5252714		1993-10-12	Harris et al.	
	2	5352770		1994-10-04	Matsuo	
	3	5434133		1995-07-18	Tanaka et al.	
	4	5824784		1998-10-20	Kinstler et al.	
	5	5846932		1998-12-08	Lowe et al.	
	6	6020168		2000-02-01	Matsuo et al.	
	7	6034231		2000-03-07	Tanaka et al.	
	8	6136040		2000-10-24	Ornitz et al.	

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	Examiner Name	N/A
	Attorney Docket Number	135576-010627/REI/CON

	9	6265632		2001-07-24	Yayon et al.	
	10	6329375		2001-12-11	Tang et al.	
	11	6344459		2002-02-05	Bridges et al.	
	12	6743425		2004-06-01	Nakao	
	13	6849714		2005-02-01	Bridon et al.	
	14	6858580		2005-02-22	Ekwuribe et al.	
	15	6861236		2005-03-01	Moll et al.	
	16	7276481		2007-10-02	Golembo et al.	
	17	8198242		2012-06-12	Wendt et al.	
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	Application Number	15646822
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	First Named Inventor	Daniel J. Wendt
	Art Unit	1629
	Examiner Name	N/A
	Attorney Docket Number	135576-010627/REI/CON

Examiner Initial*	Cite No	Publication Number	Kind Code[1]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	20040138134		2004-07-15	Golembo et al.	
	2	20070197434		2007-08-23	Nakao et al.	
	3	20070292966		2007-12-20	Prickett et al.	
	4	20080194682		2008-08-14	Golembo et al.	

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	1	0466174	EP	A1	1992-01-15	Matsuo Hisayuki		
	2	0497368	EP	A1	1992-08-05	Suntory Ltd et al.		
	3	1743653	EP	A1	2007-01-17	Nakao Kazuwa		
	4	1994/20534	WO	A1	1994-09-15	Mayo Foundation		

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	15646822
	Filing Date	2017-07-11
	First Named Inventor	Daniel J. Wendt
	Art Unit	1629
	Examiner Name	N/A
	Attorney Docket Number	135576-010627/REI/CON

	5	2000/61631	WO		2000-10-19	AstraZeneca Ab et al.		
	6	2002/074234	WO	A2	2002-09-26	Prochon Biotech Ltd et al.		
	7	2004/047871	WO	A2	2004-06-10	Nobex Corp		
	8	2009/67639	WO		2009-05-28	Biomarin Pharmaceutical Inc.		

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Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]
	1	AGOSTON et al., C-type natriuretic peptide regulates endochondral bone growth through p38 MAP kinase-dependent and -independent pathways, BMC Dev. Biol., 7:18 (2007).	
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	Art Unit	1629
	Examiner Name	N/A
	Attorney Docket Number	135576-010627/REI/CON

	6	BARTON et al., Endothelium-independent relaxation and hyperpolarization to C-type natriuretic peptide in porcine coronary arteries, J. Cardiovasc. Pharmacol., 31 :377-83 (1998).	
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
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	26	HE et al., Allosteric activation of a spring-loaded natriuretic peptide receptor dimer by hormone, Science, 293:1657-62 (2001).	
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
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	First Named Inventor	Daniel J. Wendt
	Art Unit	1629
	Examiner Name	N/A
	Attorney Docket Number	135576-010627/REI/CON

	28	HOFMANN et al., Recent advances in the application of expressed protein ligation to protein engineering, Curr. Opin. Biotechnol., 13:297-303 (2002).	
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	33	IGAKI et al., Effects of intravenously administered C-type natriuretic peptide in humans: comparison with atrial natriuretic peptide, Hvpertens. Res., 21 :7-13 (1998).	
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	35	International Preliminary Report on Patentability for corresponding International Application No. PCT/US08/84270, dated May 25, 2010.	
	36	International Preliminary Report on Patentability for corresponding International Application No. PCT/US2010/035586, mailinq date November 22, 2011.	
	37	International Search Report and Written Opinion for corresponding International Application No. PCT/US2008/084270, dated May 28, 2009.	
	38	International Search Report and Written Opinion for corresponding International Application No. PCT/US2010/035586, mailing date November 24, 2010.	

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	First Named Inventor	Daniel J. Wendt
	Art Unit	1629
	Examiner Name	N/A
	Attorney Docket Number	135576-010627/REI/CON

	39	ITOH et al., C-type natriuretic peptide ameliorates monocrotaline-induced pulmonary hypertension in rats, Am. J. Respir. Grit. Care Med., 170:1204-11 (2004).	
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
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	First Named Inventor	Daniel J. Wendt
	Art Unit	1629
	Examiner Name	N/A
	Attorney Docket Number	135576-010627/REI/CON

	50	MELO et al., Chronic regulation of arterial blood pressure by ANP: role of endogenous vasoactive endothelial factors, Am. J. Physiol., 275:H1826-33 (1998).	

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	Filing Date	2017-07-11
	First Named Inventor	Daniel J. Wendt
	Art Unit	1629
	Examiner Name	N/A
	Attorney Docket Number	135576-010627/REI/CON

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

See attached certification statement.

The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Leslie Serunian/	Date (YYYY-MM-DD)	2017-10-11
Name/Print	Leslie Serunian	Registration Number	35,353

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these record s.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Doc code: IDS
Doc description: Information Disclosure Statement (IDS) Filed

PTO/SB/08a (03-15)
Approved for use through 07/31/2016. OMB 0651-0031
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	15646822
Filing Date	2017-07-11
First Named Inventor	Daniel J. Wendt
Art Unit	1629
Examiner Name	N/A
Attorney Docket Number	135576-010627/REI/CON

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
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	Filing Date	2017-07-11
	First Named Inventor	Daniel J. Wendt
	Art Unit	1629
	Examiner Name	N/A
	Attorney Docket Number	135576-010627/REI/CON

	1	MURTHY et al., Gi-1/Gi-2-dependent signaling by single-transmembrane natriuretic peptide clearance receptor, Am. J. Phvsiol. Gastrointest. Liver Physiol., 278:G974-80 (2000).	
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	3	NAKAO et al., Molecular biology and biochemistry of the natriuretic peptide system. I: Natriuretic peptides, J. Hypertens., 10:907-12 (1992).	
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	12	PAGANO et al., Cytoplasmic domain of natriuretic peptide receptor C constitutes Gi activator sequences that inhibit adenylyl cyclase activity, J. Biol. Chem., 276:22064-70 (2001).	
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	23	SUDOH et al., C-type natriuretic peptide (CNP): a new member of natriuretic peptide family identified in porcine brain, Biochem. Biophys. Res. Commun., 168:863-70 (1990).	
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	39	International Search Report and Written Opinion for International Application No. PCT/US2010/035586, dated November 22, 2011.	

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EXAMINER SIGNATURE

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

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Signature	/Leslie Serunian/	Date (YYYY-MM-DD)	2017-10-11
Name/Print	Leslie Serunian	Registration Number	35,353

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9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronic Acknowledgement Receipt

EFS ID:	30620912
Application Number:	15646822
International Application Number:	
Confirmation Number:	7727
Title of Invention:	Variants of C-Type Natriuretic Peptide
First Named Inventor/Applicant Name:	Daniel J. Wendt
Customer Number:	90849
Filer:	Leslie A. Serunian
Filer Authorized By:	
Attorney Docket Number:	30610/44556C1-R2
Receipt Date:	11-OCT-2017
Filing Date:	11-JUL-2017
Time Stamp:	13:06:10
Application Type:	Utility under 35 USC 111(a)

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1	Transmittal Letter	010627IDSTransmittalLetter.pdf	108599 b2afc51c4927f0f80be6e9d9e80e51d7bfb05dff	no	2
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A U.S. Patent Number Citation or a U.S. Publication Number Citation is required in the Information Disclosure Statement (IDS) form for autoloading of data into USPTO systems. You may remove the form to add the required data in order to correct the Informational Message if you are citing U.S. References. If you chose not to include U.S. References, the image of the form will be processed and be made available within the Image File Wrapper (IFW) system. However, no data will be extracted from this form. Any additional data such as Foreign Patent Documents or Non Patent Literature will be manually reviewed and keyed into USPTO systems.

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This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

<u>New Applications Under 35 U.S.C. 111</u>
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.
<u>National Stage of an International Application under 35 U.S.C. 371</u>
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.
<u>New International Application Filed with the USPTO as a Receiving Office</u>
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Daniel J. Wendt et al.

Application No.: 15/646,822	Confirmation No.: 7727
Filed: July 11, 2017	Art Unit: 1629
For: *VARIANTS OF C-TYPE NATRIURETIC PEPTIDE*	Examiner: To be assigned

INFORMATION DISCLOSURE STATEMENT LETTER

SUBMITTED VIA EFS-Web
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Commissioner:

Pursuant to 37 C.F.R. §§1.56, 1.97 and 1.98, the references listed on the attached Form PTO/SB/08a are provided for consideration by the U.S. Patent and Trademark Office ("the Office") in the above-identified patent application. Applicants respectfully request that the information provided in this IDS be expressly considered by the Office during the prosecution of the instant application and that the references be made of record therein and appear among the "References Cited" on any patent to issue therefrom.

In accordance with 37 C.F.R. §1.98(a)(2)(ii), Applicants have not submitted copies of issued U.S. patents and published U.S. patent applications. In accordance with 37 C.F.R. §1.98(d)(1) and (2), copies of documents and information which were previously cited by or submitted to the Office in prior applications U.S. Serial No. 14/604,262, filed January 23, 2015; U.S. Serial No. 13/466,672, filed May 8, 2012 (now U.S. Patent No. 8,598,121), and U.S. Serial No. 12/784,117, filed May 20, 2010 (now U.S. Patent No. 8,198,242), and which are relied upon

in the instant application for an earlier filing date under 35 U.S.C. §120, are not submitted herewith.

In accordance with 37 C.F.R. §1.97(g), the filing of this Information Disclosure Statement shall not be construed to mean that a search has been made or that no other material information as defined in 37 C.F.R. §1.56(a) exists.

In accordance with 37 C.F.R. §1.97(h), the filing of this Information Disclosure Statement shall not be construed to be an admission that any patent, publication, or other information referred to herein is "prior art" for this invention unless specifically designated as such.

Applicants submit that the Information Disclosure Statement is in compliance with 37 C.F.R. §1.98 and respectfully request that the Examiner consider the references listed on the attached Form PTO SB/08a.

AUTHORIZATION

No fee is believed to be due for the filing of this Information Disclosure Statement. However, the Commissioner is hereby authorized to charge any deficiency in the fee(s) filed, asserted to be filed, or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to Deposit Account No. 50-2678, under Order No. 135576-010627/REI/CON.

Dated: October 11, 2017

Respectfully submitted,
Greenberg Traurig, LLP

By: /Leslie Serunian/
Leslie Serunian
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12-11-17 OK TO ENTER 1/23/18

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No.: 15/646,822

Filed: 07-11-2017

CON REI of 14/604,262 filed 1-23-2015 REI of 13/466,672 filed 5-8-2012, now US 8,598,121, CON of 12/784,117 filed 5-20-2010, which claims benefit of 61/254,563 filed 10-23-2009 and 61/180,112, filed 5-20-2009)

Title: Variants of C-Type Natriuretic Peptide

Applicant: BioMarin Pharmaceutical Inc.

Confirmation No.: 7727

Art Unit: 1629

Examiner: Not yet assigned

CON REISSUE APPLICATION UNDERGOING PREEXAM PROCESSING

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450
Attention Director Art Unit 3991

Protest under 37 CFR 1.291

Ascendis Pharma A/S, of Tuborg Boulevard 5, 2900 Hellerup, Denmark, as real party in interest, submits the following protest against the above-captioned application under 37 CFR 1.291. This is the first protest submitted by Ascendis Pharma in this application. A protest can be submitted any time up to allowance in a reissue case so the protest is timely filed.

All claims of the present reissue application should be rejected under 35 USC 251 for attempting to correct an uncorrectable error, that is, a failure to file a divisional application on nonelected subject matter following a restriction requirement in the underlying patent being reissued (US 8,598,121). Claims 21 and 22 should also be rejected for lack of written description under 35 USC 112, first paragraph.

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Listing of documents and brief statement of relevance

This protest relies on the following documents (Exhibits A-I), for which copies are provided.

A. Claims pending in 13/466,672, filed May 8, 2012, now US 8,598,121 at the date of issuance of a restriction requirement: This document is relevant to establish the claims and sequences subject to a restriction requirement in the original prosecution.

B. Restriction requirement dated February 13, 2013 from 13/466,672: This document defines the groups and species of the restriction requirement.

C. Response to restriction requirement dated April 12, 2013 from 13/466,672: This document identifies which group and sequence were elected (Group I and SEQ ID NO:185).

D. Interview summary dated July 30, 2013 from 13/466,672: This document discloses a modification of the restriction requirement in which certain groups and sequences (SEQ ID NOS. 179, 180 and 190) were rejoined, and all other non-elected sequences were cancelled.

E. Granted claims from 13/466,672: This document shows that the granted claims were consistent with the interview summary.

F. Claims now pending in the present application: This document shows that claims directed in part to non-elected SEQ ID NOS. from 13/466,672 are now being pursued.

G. *Ex part Joachim Werres,* Appeal 2006-2966 (BPAI 2007), *Ex parte Morrison et al.,* Appeal 2012-12266 (PTAB 2013), affirmed (2014-1050 Fed. Cir. 2014), Ex *Parte Solomon,* Appeal 2012-2100 (BPAI 2012), affirmed (2012-1689 Fed. Cir. 2013) and *Ex parte Wu et al.,* Appeal 2016-0348 (PTAB 2017) are exemplary Board decisions sustaining rejections of claims presented in a reissue application under 35 USC 251 due to failure to file a divisional application on non-elected subject matter.

H. PTO Briefs from Federal Circuit appeals in *Ex parte Morrison* and *Ex parte Solomon* from Exhibit G. These briefs provide a useful summary of the *Orita, Weiler, Doyle* line

of cases limiting pursuit of non-elected claims in a divisional application.

I. Tsuberry et al., J. Biol. Chem. 279, 38118-38124 (2004)) is an exemplary reference showing that linkage of peptide drugs to PEG via a hydrolysable linkage was an art-known technique for improving half-life without compromising efficacy.

Analysis

MPEP 1412.01 provides as follows:

> **I. FAILURE TO TIMELY FILE A DIVISIONAL APPLICATION PRIOR TO ISSUANCE OF ORIGINAL PATENT**
>
> Where a restriction (or an election of species) requirement was made in an application and applicant permitted the elected invention to issue as a patent without filing a divisional application on the non-elected invention(s), the non-elected invention(s) cannot be recovered by filing a reissue application. A reissue applicant's failure to timely file a divisional application covering the non-elected invention(s) in response to a restriction (or an election of species) requirement is not considered to be error causing a patent granted on the elected claims to be partially inoperative by reason of claiming less than the applicant had a right to claim. Accordingly, this is not correctable by reissue of the original patent under 35 U.S.C. 251. *In re Watkinson,* 900 F.2d 230, 14 USPQ2d 1407 (Fed. Cir. 1990); *In re Orita,* 550 F.2d 1277, 1280, 193 USPQ 145, 148 (CCPA 1977); See also *In re Mead,* 581 F.2d 251, 198 USPQ 412 (CCPA 1978). In this situation, the reissue claims should be rejected under 35 U.S.C. 251 for lack of defect in the original patent and lack of error in obtaining the original patent. Compare with *In re Doyle,* 293 F.3d 1355, 63 USPQ2d 1161 (Fed. Cir. 2002) where the court permitted the patentee to file a reissue application to present a so-called linking claim, a claim broad enough to read on or link the invention elected (and patented) together with the invention not elected. The non-elected invention(s) were inadvertently not filed as a divisional application.

The rationale for this rule is that the error does not lie in the patent sought to be reissued in which the divisional claims could never have been pursued but in the failure to file a divisional application.

Patentees claimed exactly what they had a right to claim in the

patent, no more nor less, and appellants' failure to timely re-file does not change this fact.

In re Doyle, 293 F3d. 1355 (Fed. Cir. 2002)

Here, a restriction requirement in the underlying patent being reissued US 8,598,121 required the following elections of groups and species.

I. Claims 1-4, 30, 45 and 46, drawn to a variant of C-type natriuretic peptide (CNP), classified in class 530, subclass 300.

II. Claim 7, drawn to a method of treating a bone-related disorder or skeletal dysplasia, classified in class 514, subclass 12.4.

III. Claim 9, drawn to a method of treating a vascular smooth muscle disorder, classified in class 514, subclass 12.4.

IV. Claims 10, 16, 18, 22-24, 31, 35, drawn to a method for recombinant production of a CNP variant and a host cell used in said method, classified in class 435, subclass 69.1/325.

V. Claims 39 and 41, drawn to a method of treating a CNP-responsive condition or disorder, classified in class 514, subclass 12.4.

VI. Claim 44, drawn to a method of increasing long bone growth, classified in class 514, subclass 12.4.

Species Election #1

This application contains claims directed to the following patentably distinct species: sequences as disclosed in claims 1, 9, 10, 30, 31, 39, and 46. The species are independent or distinct because each sequence is a distinct separate polypeptide. In addition, these species are not obvious variants of each other based on the current record.

Applicant is required under 35 U.S.C. 121 to elect a single disclosed species, or a single grouping of patentably indistinct species, for prosecution on the merits to which the claims shall be restricted if no generic claim is finally held to be allowable. Currently, no claim is generic.

The applicant elected group I and SEQ ID NO:185, a C-type natriuretic peptide of 53 amino acids (CNP53) for continued prosecution.

Subsequently, the Examiner indicated that the case would be allowed subject to rejoinder of certain sequences based on CNP53 (i.e., SEQ ID NO:179, 190 and 180), and rejoinder of method claims recited in independent claims 7, 10 and 44, corresponding to Groups II, IV and VI (likewise limited to SEQ ID NOS. 185, 179, 190 and 180) with cancellation of non-elected sequences (see interview summary of July 30, 2013 reproduced below).

> *Examiner indicated SEQ ID NO:185, the elected species is free of the prior art. Examiner indicated will search other sequences based on CNP53, SEQ ID NOs:179,190 and 180. These have been found free of the art. Examiner will rejoin and examine appropriate method claims:7, 10 and dependents and 44. Atty Neville authorized cancellation of non-elected sequences and claims to non-elected sequences.*.

Consistent with this interview summary, US 8,598,121 issued with claims to a CNP variant peptide, a pharmaceutical composition comprising the variant, a method of treating a bone related disorder or skeletal dysplasia with such a variant and a method of increasing long bone growth with such a variant, with in all claims the CNP variant being limited to a peptide having the sequence of SEQ ID NO:185, 179, 190 or 180. Analogous claims in which the peptide included the sequence of numerous other non-elected SEQ ID NOS. were cancelled and not pursued by way of a divisional application.

Instead of filing claims to non-elected sequences in divisional applications of US 8,598,121, such claims have been reintroduced in the present reissue application. The independent claims currently pending are directed to a large Markush group of sequences, which includes both the elected SEQ ID NOS:185, 179, 190 and 180 from the underlying patent as well as numerous other SEQ ID NOS., many of which were present at the time of the original restriction requirement in the parent patent and were subsequently cancelled. For example, at least SEQ ID NOS. 36, 75, 144, 146-179, 183-185, 188-190 and 193 of the present Markush group were present at the time of the restriction requirement in the original prosecution.

The inclusion of both elected and non-elected sequences in the claims does not

circumvent the prohibition on pursuing non-elected subject matter under the *In re Doyle* exception referred to in MPEP 1412.01. In *In re Doyle*, a patent issued on an elected species claim and a reissue was filed with a linking claim generic to both the elected species and a non-elected species. Such a claim was never presented in the original prosecution, but the court found that it could and should have been and failure to present it was an error correctable by reissue.

But the present situation is distinct from *In re Doyle*. In contrast to the situation in *Doyle* when a linking claim was never presented in the original prosecution, claims of the type now presented (i.e., listing both elected and nonelected sequences) were presented in the original prosecution, and amended to cancel nonelected species as a condition of allowability. Thus, in contrast to the situation in *Doyle*, the applicant here acquiesced in the restriction requirement by pursuing claims in the original prosecution of the same type as are now being presented in a reissue and amending the claims to remove nonelected subject matter.

A similar issue was presented in *Ex parte Joachim Werres,* Appeal 2006-2966 (BPAI 2007). The applicant in that case limited the claims to an elected species in the original prosecution and then tried to pursue broader claim including both the elected species and nonelected species recited in the alternative in a reissue. The applicant argued that error arose in allowing the original patent to issue without a generic claim. But the Board found that the applicant "deliberately agreed to an Examiner's amendment that limited or narrowed generic claim 17 to the elected species only" (Decision at p. 8). Such is precisely what occurred in the present case. The Board in *Werres* explained its decision as follows:

> A correctable 251 error does not include these deliberate decisions by Appellant whereby the '177 patent was allowed to issue with selected species claims only (i.e., without a generic claim). *Orita,* 550 F.2d at 1280, 193 USPQ at 148. These deliberate choices do not comprise error correctable by reissue regardless of the propriety of the election requirement or claim amendment made by the Examiner. *Watkinson,* 900 F.2d at 231-232, 14 USPQ2d at 1408-09. There is a distinction between a 251 error and a deliberate choice subsequently found to be disadvantageous. *Serenkin,* 479 F.3d at 1364, 81 USPQ2d at 2015.

Werres at p. 8.

The present case is further distinguished from *Doyle* in that the purportedly linking claim presented merely lists elected and many unelected species in the alternative rather than presenting a true generic claim. It is inconceivable that the applicant will be allowed to continue to pursue the claims in their current form directed to a vast number of distinct sequences. Either as a result of a further restriction requirement or rejection due to an improper Markush group, the present claims will almost certainly have to be amended to be directed to a single sequence or group of patentably indistinct sequences (as they were in the original prosecution). In such case, the claims will presumably be directed to entirely unelected subject matter, leaving no similarity in the present situation to that in *In re Doyle*.

That a few of the CNP sequences recited in the present claims were not claimed at the time of the restriction requirement in the original prosecution should not change the analysis. The applicant was required to elect a single *disclosed* sequence of CNP variant, so the election applied to all disclosed sequences of CNP variants in the application. If the applicant had included any additional sequences to the many already present in the claims of the parent case, they would have been treated in exactly the same way. That is, the applicant would have been required to cancel them to obtain allowance of the elected sequences (SEQ ID NOS. 179, 180, 185 and 190). Applicants could no more have obtained allowance of unelected sequences never present in the claims than unelected sequences that were present in the claims and cancelled in the original prosecution. Attempting to pursue such claims does not therefore represent correcting an error in claim scope of the original patent but an error in failure to file a divisional application to obtain a further patent on a distinct invention as delineated in a restriction requirement.

That there are differences in the form of the claims now pending from those present in the original prosecution also does not avoid the prohibition on pursuing non-elected subject matter of MPEP 1412.01. Specifically whereas original claim 1 was directed to a variant of a CNP peptide of specified sequence, present claim 15 and dependents are directed to a macromolecule in which such a peptide is linked through a hydrolysable linker to a polymer.

Likewise whereas original claim 2 was directed to a pharmaceutical composition comprising a CNP variant of claim 1 and a pharmaceutical acceptable, excipient, carrier or diluent, present claim 18 and dependents are directed to a sustained release composition in which a polymer is coupled to a CNP variant by a hydrolysable linker. Similar distinctions are featured in present method claims (independent claims 23 and 27 and their dependents) compared with corresponding claim 7 from the original prosecution.

The additional recitation that CNP peptides are linked to a polymer via a hydrolysable linker does not confer any patentable distinction over corresponding claims present in the original prosecution. It has been known for many years that linkage of peptides to PEG via a hydrolysable linkage provides a means to extend half-life without compromising activity (see, e.g., Tsuberry et al., J. Biol. Chem. 279, 38118-38124 (2004)). Characterizing a CNP peptide linked to a polymer as a macromolecule is merely a difference in form without any limiting effect. The recitation of "sustained release" in the preamble of claim 18 does not appear to be of limiting effect beyond the linkage of a CNP peptide to a polymer by a hydrolysable linkage recited in the body of the claim, which itself provides for sustained release. But even if the preamble is considered further limiting of the claim, a sustained release formulation is a well-known type of pharmaceutical composition and not patentably distinct from the pharmaceutical composition of claim 2 in the original prosecution. Claims of corresponding scope to those now present could have been presented in dependent form within the claim groupings of the original restriction requirement (e.g., The CNP variant of claim 1 linked to a polymer by a hydrolysable linkage forming a macromolecule, or the pharmaceutical composition of claim 2, which is a sustained-release composition). Thus, the additional recitation of linkage via a hydrolysable linker to a polymer or of a sustained release formulation do not change the analysis or escape the prohibition against pursuing non-elected subject matter.

The issue of claims differing in form but without patentable distinction between the original prosecution and reissue was considered by *Ex parte Morrison et al.,* Appeal 2012-12266 (BPAI 2013). An original non-elected group of claims was directed to a method of providing a

tow assembly, and the newly presented claim was directed to a tow assembly per se. The Board found there were no substantial differences between the claims even though directed to distinct statutory categories. Because the claims were "simply not patentably distinct" the Board sustained the rejection under 35 USC 251.

The applicant may allege that present claims 21 and 22 have no corresponding claims in the original prosecution. Claim 21 is directed to a method of stimulating cGMP production in a cell expressing natriuretic peptide receptor B (NPR-B) comprising contacting the cell expressing NPR-B with a CNP variant. Claim 22 is directed to a method of overcoming cell growth arrest induces by a constitutively active mutant fibroblast growth factor receptor 3 (FGFR3) comprising contacting a cell expressing the constitutively active FGFR-3 with a CNP variant. Although claims 21 and 22 may appear to be directed to methods performed on isolated cells patentably distinct from the treatment methods pursued in the original prosecution, under a broadest reasonable interpretation, the cells contacted in claims 21 and 22 can be construed as being in a patient with the contacting occurring in the method of treatment. Claims 21 and 22 can thus be construed as methods of treatment encompassing either claim 7 or claim 9 from the original prosecution with the functional results of stimulating cGMP production in a cell expressing natriuretic peptide receptor B (NPR-B) or overcoming cell growth arrest induced by a constitutively active mutant fibroblast growth factor receptor 3 (FGFR3) occurring as an inherent result of treatment and of no further limiting effect. Thus, claims 21 and 22 are not necessarily patentably distinct from claims 7 and 9 in the original prosecution.

But even if claims 21 and 22 were considered patentably distinct from claims pending in the original prosecution, they would have been treated in the same way as other method claims that were present in the original prosecution. That is, they would have been placed in a restriction group separate from the elected group of CNP variant peptides, subject to rejoinder on allowance of the elected group if amended to define the CNP variant peptide in the same manner as the elected group. In other words, if such claims were present in the original prosecution, they would have either have to have been amended to be limited to the elected CNP variants or cancelled (the choice presented for the method claims that were present).

These claims could not have been pursued to allowance with non-elected sequence in the original prosecution. Thus, insofar as claims 21 and 22 are directed to non-elected species, their absence from the original patent does not represent any error in the original prosecution, but rather an uncorrectable failure to file a divisional application on nonelected subject matter.

Several cases have applied the prohibition against pursuing non-elected claims in a reissue to claims that were not pursued in the original prosecution but would have been subject to restriction had they been pursued. In *in re Weiler,* 790 F2d 1576 (Fed. Cir. 1986), the applicant accepted issuance of an original patent with claims to a single elected invention a method of quantitative analysis of limonin, which was one of three inventions delineated in a restriction requirement. Weiler then filed a reissue application claiming inter alia a method of developing citrus fruit strains low in limonin or antibodies against limonin. The court considered these claims patentably distinct from any of the groups in the original prosecution. The court sustained the rejection of these claims as follows:

> By acquiescing in the examiner's restriction requirement and failing to file divisional applications on the subject matter of non-elected claims, Weiler foreclosed (because that was not error) his right to claim that subject matter. If it was not error to forego divisional applications on subject matter to which claims had been made in the original prosecution, it cannot on the present record have been error to forego divisional applications on subject matter which claims have never been made.

The Board has also addressed several cases in which it was arguable whether newly presented claims in a reissue case would have been placed in a non-elected group from the original prosecution or in a new non-elected group and found this of no consequence to the lack of correctable error under 35 USC 251. For example, *Ex Parte Solomon,* Appeal 2012-2100 (BPAI 2012) considered a claim directed to a method of treating a beta-amyloid disease when the original prosecution contained a non-elected group III of claims directed to treating a protein aggregation disease. The Board found that the claims presented in the reissue if presented in the original prosecution would have fallen either in non-elected group III or a new group, and sustained the rejection. The Board explained:

> Consonant with *Orita*, should Appellant "prevail, the copendency requirement would become meaningless, for should an applicant fail to file a divisional application while maintain copendency as required by section 120, he could simply revert to section 251 in order to cure his mistake." *Orita,* 550 F.2d at 1281. Here, permitting appellant to correct the failure to file divisional applications by reissue would defeat the copendency requirement of 35 U.S.C. 120, the result found undesirable by the CCPA in *Orita.*
>
> *Ex parte Solomon* at pp. 10-11.

Likewise in *Ex parte Wu et al.,* Appeal 2016-0348 (PTAB 2017), the Board considered a reissue case including method claims that if presented in the original prosecution would have been grouped with either unelected Group II also directed to methods or a new group distinct from the elected Group I. The Board found that "Appellants are not entitled to obtain method claims per se, regardless of scope, because they conceded by their acquiescence in the restriction requirement that they were not entitled to claim method claims in the original application." Here likewise, the applicant conceded by its acquiescence in the restriction requirement in the original prosecution that it was not entitled to claim non-elected sequences as CNP peptides, compositions or methods of use and should not be allowed to pursue such in a reissue either.

A useful summary of case law discussed in this protest is provided by the PTO's briefs in the Federal Circuit appeals of *Ex parte Morrison* and *Ex parte Solomon*. In short:

Orita, *Weiler*, and *Doyle* hold that when an applicant makes a deliberate choice in response to a restriction requirement to prosecute a single invention and fails to file divisional application(s) to the remaining, nonelected inventions—whether originally claimed or not—error under § 251 does not exist. These cases are based on the rationale that once an applicant acquiesces to a restriction requirement, any invention distinct from the one elected and prosecuted to allowance can only be pursued in a timely-filed divisional application.

PTO Brief in *Ex parte Solomon* (2012-1689 Fed. Cir. 2012) at p. 18

This reissue case is in its essence an attempt to pursue non-elected subject matter not fitting the narrow exception of *in re Doyle*. As indicated in MPEP 1412.01, a reissue applicant's failure to timely file a divisional application covering the non-elected invention(s) in response to a restriction (or an election of species) requirement is not considered to be an error correctable by reissue of the original patent under 35 U.S.C. 251. In this situation, the reissue claims should be rejected under 35 U.S.C. 251 for lack of defect in the original patent and lack of error in obtaining the original patent.

Claims 21 and 22 should also be rejected under 35 USC 112, first paragraph for lack of written description in the original patent specification. While there is no *in haec verba* requirement, newly added claim limitations must be supported in the specification through express, implicit, or inherent disclosure. If a representative number of adequately described species are not disclosed for a genus, the claim to that genus must be rejected as lacking adequate written description (MPEP 2163). Here, the original patent does not describe claims 21 and 22 *in haec verba* but, if at all, only by exemplification. The exemplification is in the

context of methods of screening CNP peptide in cell cultures in vitro to check the CNP peptides have the desired activity (see col. 51, lines 45-66, Example 5 and Example 7). There is no exemplification or other description of stimulating cGMP production in a cell expressing natriuretic peptide receptor B (NPR-B) comprising contacting the cell expressing NPR-B or overcoming cell growth arrest induced by a constitutively active mutant fibroblast growth factor receptor 3 (FGFR3) in the context of a treatment method. Because of the lack of *in haec verba* description and exemplification of only one species, the specification fails to provide written description throughout the genus encompassed by these claims under a broadest reasonable interpretation. The claims should therefore be rejected for lack of written description.

Please charge any fee occasioned by this protest to deposit account 16-0605.

Respectfully submitted

/Joe Liebeschuetz/

Joe Liebeschuetz
Reg. No. 37, 505
On behalf of Ascendis Pharma A/S.

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I certify under 37 CFR 1.248 a copy of this protest has been sent by Express Mail (Express Mail Label No. EF 14159178 US) to the attorneys of record Marshall, Gerstein & Borun LLP, 233 South Wacker Drive, 6300 Willis Tower, Chicago IL 60606, on December 7, 2017.
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Application Number	15/646,822
Filing Date	07-11-2017
First Named Inventor	Daniel J. Wendt
Art Unit	1629
Examiner Name	Unassigned
Practitioner Docket No.	065588-495115

Enclosed is a paper filed under 37 CFR 1.291 **that requires a processing fee (37 CFR 1.17(i)(1) or (2)).**

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☐ Applicant asserts small entity status. See 37 CFR 1.27.

☐ Applicant certifies micro entity status. See 37 CFR 1.29.
Form PTO/SB/15A or B or equivalent must either be enclosed or have been submitted previously.

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Undiscounted Fee $140 (Fee Code 1053) Small Entity Fee $70 (Fee Code 2053) Micro Entity Fee $35 (Fee Code 3053)

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§ 1.28(c)(3) - for processing a non-itemized fee deficiency based on an error in small entity status.
§ 1.29(k)(3) - for processing a non-itemized fee deficiency based on an error in micro entity status.
§ 1.41(b) - for supplying the name or names of the inventor or joint inventors in an application without either an application data sheet or the inventor's oath or declaration, except in provisional applications.
§ 1.48 - for correcting inventorship, except in provisional applications.
§ 1.52(d) - for processing a nonprovisional application filed with a specification in a language other than English.
§ 1.53(c)(3) - to convert a provisional application filed under § 1.53(c) into a nonprovisional application under § 1.53(b).
§ 1.71(g)(2) - for processing a belated amendment under § 1.71(g).
§ 1.102(e) - for requesting prioritized examination of an application.
§ 1.103(b) - for requesting limited suspension of action, continued prosecution application for a design patent (§ 1.53(d)).
§ 1.103(c) - for requesting limited suspension of action, request for continued examination (§ 1.114).
§ 1.103(d) - for requesting deferred examination of an application.
§ 1.291(c)(5) - for processing a second or subsequent protest by the same real party in interest.
§ 3.81 - for a patent to issue to assignee, assignment submitted after payment of the issue fee.

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SUBMITTED BY

Signature	/Joe Liebeschuetz/	Registration No. (Attorney/Agent) 37505	Telephone 650-838-2038
Name (Print/Type)	Joe Liebeschuetz		Date 12/07/2017

This collection of information is required by 37 CFR 1.17. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 5 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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§ 1.71(g)(2) - for processing a belated amendment under § 1.71(g).
§ 1.102(e) - for requesting prioritized examination of an application.
§ 1.103(b) - for requesting limited suspension of action, continued prosecution application for a design patent (§ 1.53(d)).
§ 1.103(c) - for requesting limited suspension of action, request for continued examination (§ 1.114).
§ 1.103(d) - for requesting deferred examination of an application.
§ 1.291(c)(5) - for processing a second or subsequent protest by the same real party in interest.
§ 3.81 - for a patent to issue to assignee, assignment submitted after payment of the issue fee.

Processing Fees Under 37 CFR 1.17(i)(2):

Fee $130 (small and micro entity discounts are not available for the 37 CFR 1.17(i)(2) processing fee)

For papers filed under:
§ 1.217 - for processing a redacted copy of a paper submitted in the file of an application in which a redacted copy was submitted for the patent application publication. **FEE CODE 1808**
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SUBMITTED BY

Signature	/Joe Liebeschuetz/	Registration No. (Attorney/Agent) 37505	Telephone 650-838-2038
Name (Print/Type)	Joe Liebeschuetz		Date 12/07/2017

This collection of information is required by 37 CFR 1.17. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 5 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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OPAP AKUMA-2 DEC 08 2017 PATENT & TRADEMARK OFFICE

Modified PTO/SB/08A (01-08)

Substitute for form 1449A/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 2

Complete if Known	
Application Number	15/646,822
Filing Date	July 11, 2017
First Named Inventor	Daniel J. Wendt
Art Unit	1629
Examiner Name	Not applicable
Attorney Docket Number	065588-495115

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3] Number[4] Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T[6]
						☐

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. [1] Applicant's unique citation designation number (optional). [2] Kind Codes of U.S. Patent Documents at www.uspto.gov or MPEP 901.04. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST. 16 if possible. [6] Applicant is to place a check mark here if English language Translation is attached.

Substitute for form 1449B/PTO				Complete if Known	
INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	15/646,822
				Filing Date	July 11, 2017
				First Named Inventor	Daniel J. Wendt
				Art Unit	1629
(Use as many sheets as necessary)				Eaminer Name	Not applicable
Sheet	2	of	2	Attorney Docket Number	065588-495115

NON PATENT LITERATURE DOCUMENTS			
Examiner Initials *	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T [2]
	A	US Application 13/466,672 claims pending as of the mailing of the Restriction Requirement on February 13, 2013.	
	B	US Application 13/466,672 Restriction Requirement mailed February 13, 2013.	
	C	US Application 13/466,672 Response to Restriction Requirement submitted April 12, 2013.	
	D	US Application 13/466,672 Examiner-Initiated Interview Summary mailed July 30, 2013.	
	E	US Patent 8,598,121 B2 claims as granted on December 3, 2013.	
	F	US Application 15/332,793 claims as amended in a Preliminary Amendment submitted October 24, 2016.	
	G1	*Ex parte Joachim Werres,* Appeal 2006-2966 (BPAI 2007).	☐
	G2	*Ex parte Morrison et al.,* Appeal 2012-012266 (PTAB 2013) affirmed (2014-1050 Fed. Cir. 2014).	
	G3	Ex *Parte Solomon*, Appeal 2012-002100 (BPAI 2012), affirmed (2012-1689 Fed. Cir. 2013)	
	G4	*Ex parte Wu et al.*, Appeal 2016-0348 (PTAB 2017).	
	H1	PTO Brief from Federal Circuit appeals in *Ex parte Morrison (2012-1050 Fed. Cir. 2014).*	☐
	H2	PTO Brief from Federal Circuit appeals in *Ex parte Solomon (2012-1689 Fed. Cir. 2013).*	
	I	TSUBERRY et al., "Prolonging the Action of Protein and Peptide Drugs by a Novel Approach of Reversible Polyethylene Glycol Modification", J. Biol. Chem., Vol. 279, No. 37, pp 38118-3824 (2004).	☐

Exhibit A

Application No.: 13/466,672 Docket No.: 30610/44556C1

AMENDMENT TO THE CLAIMS

1. (Currently amended) A variant of C-type natriuretic peptide (CNP) selected from the group consisting of:

GDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP53) (SEQ ID NO: 179);

PDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP53) (SEQ ID NO: 185);

MDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP53) (SEQ ID NO: 190)

DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP-53(M48N)] (SEQ ID NO: 180);

LRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-52) (SEQ ID NO: 146);

RVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-51) (SEQ ID NO: 147);

VDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-50) (SEQ ID NO: 148);

DTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-49) (SEQ ID NO: 149);

TKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-48) (SEQ ID NO: 150);

KSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-47) (SEQ ID NO: 151);

SRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-46) (SEQ ID NO: 152);

RAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-45) (SEQ ID NO: 153);

AAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-44) (SEQ ID NO: 154);

AWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-43) (SEQ ID NO: 155);

WARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-42) (SEQ ID NO: 156);

ARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-41) (SEQ ID NO: 157);

RLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-40) (SEQ ID NO: 158);

LLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-39) (SEQ ID NO: 159);

LQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-38) (SEQ ID NO: 160);

QEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-37) (SEQ ID NO: 60);

EHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-36) (SEQ ID NO: 161);

HPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-35) (SEQ ID NO: 162);

PNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-34) (SEQ ID NO: 163);

NARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-33) (SEQ ID NO: 164);

ARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-32) (SEQ ID NO: 165);

RKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-31) (SEQ ID NO: 166);

KYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-30) (SEQ ID NO: 167);

YKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-29) (SEQ ID NO: 168);

KGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-28) (SEQ ID NO: 169);

GANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-27) (SEQ ID NO: 170);

ANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-26) (SEQ ID NO: 171);

NKKGLSKGCFGLKLDRIGSMSGLGC (CNP-25) (SEQ ID NO: 172);

KKGLSKGCFGLKLDRIGSMSGLGC (CNP-24) (SEQ ID NO: 173);

KGLSKGCFGLKLDRIGSMSGLGC (CNP-23) (SEQ ID NO: 174);

LSKGCFGLKLDRIGSMSGLGC (CNP-21) (SEQ ID NO: 175);

SKGCFGLKLDRIGSMSGLGC (CNP-20) (SEQ ID NO: 176);

KGCFGLKLDRIGSMSGLGC (CNP-19) (SEQ ID NO: 177);

GCFGLKLDRIGSMSGLGC (CNP-18) (SEQ ID NO: 178);

~~QEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP37(M32N)] (SEQ ID NO: 182);~~

~~PQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP37) (SEQ ID NO: 186);~~

~~MQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP37) (SEQ ID NO: 192);~~

GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP37) (SEQ ID NO: 75);

~~GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [Gly-CNP37(M32N)]) (SEQ ID NO: 181);~~

~~PGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-Gly-CNP37)) (SEQ ID NO: 145);~~

~~MGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-Gly-CNP37)) (SEQ ID NO: 191);~~

GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (HSA-CNP27) (SEQ ID NO: 144);

GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSNSGLGC [HSA-CNP27(M22N)) (SEQ ID NO: 183)];

PGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-HSA-CNP27)) (SEQ ID NO: 188);

MGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-HSA-CNP27)) (SEQ ID NO: 193);

GANRRGLSRGCFGLKLDRIGSMSGLGC [CNP27(K4,5,9R)] (SEQ ID NO: 36);

GANRRGLSRGCFGLKLDRIGSNSGLGC [CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);

PGANRRGLSRGCFGLKLDRIGSMSGLGC [Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189);

MGANRRGLSRGCFGLKLDRIGSMSGLGC [Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194);

PEG1K-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-CNP27(K4,5,9R)] (SEQ ID NO: 36);

PEG1K-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEG1K-CNP27(K4,5,9R, M22N)]) (SEQ ID NO. 184);

PEG1K-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189);

PEG1K-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194);

PEO12-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-CNP27(K4,5,9R)] (SEQ ID NO: 36);

PEO12-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEO12-CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);

PEO12-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189);

PEO12-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194);

PEO24-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-CNP27(K4,5,9R)] (SEQ ID NO: 36);

PEO24-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEO24-CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);

PEO24-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189); and

PEO24-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194), wherein the CNP variant can be conjugated to a moiety selected from a hydrophobic acid and an amino acid sequence from 1 to 20 amino acids useful in bone targeting.

2. (Currently amended) A pharmaceutical composition comprising a CNP variant of claim 1, and a pharmaceutically acceptable excipient, carrier or diluent.

3. (Original) The composition of claim 2, which is a lyophilized formulation prepared from a formulation that comprises a citric acid/citrate buffer or an acetic acid/acetate buffer having a pH from about 4 to about 6.

4. (Currently amended) The composition of claim 3, wherein the lyophilized formulation is prepared from a formulation that further comprises (a) an isotonicity-adjusting agent or a bulking agent or (b) an antioxidant ~~selected from the group consisting of mannitol, sucrose, sorbitol, and combinations thereof~~.

5-6. (Cancel)

7. (Currently amended) A method of treating a bone-related disorder or skeletal dysplasia, comprising administering a CNP variant to a subject in need thereof, wherein the CNP variant is a CNP variant according to claim 1, and wherein the bone-related disorder or skeletal dysplasia is selected from the group consisting of ~~osteoarthritis, hypophosphatemic rickets,~~ achondroplasia, hypochondroplasia, short stature, dwarfism~~, osteochondrodysplasias, thanatophoric dysplasia, osteogenesis imperfecta, achondrogenesis, chondrodysplasia punctata,~~ and homozygous achondroplasia~~, chondrodysplasia punctata, camptomelic dysplasia, congenital lethal hypophosphatasia, perinatal lethal type of osteogenesis imperfecta, short rib polydactyly syndromes, hypochondroplasia, rhizomelic type of chondrodysplasia punctata, Jansen type metaphyseal dysplasia, spondyloepiphyseal dysplasia congenita, atelosteogenesis, diastrophic dysplasia, congenital short femur, Langer type mesomelic dysplasia, Nievergelt type mesomelic dysplasia, Robinow syndrome, Reinhardt syndrome, acrodysostosis, peripheral dysostosis, Kniest dysplasia, fibrochondrogenesis, Roberts syndrome, acromesomelic dysplasia, micromelia, Morquio syndrome, Kniest syndrome, metatrophic dysplasia, and spondyloepimetaphyseal dysplasia~~.

8. (Cancel)

9. (Currently amended) A method of treating a vascular smooth muscle disorder, comprising administering a CNP variant to a subject, wherein the CNP variant is a CNP variant according to claim 1, and wherein the vascular smooth muscle disorder is selected from the group consisting of hypertension, restenosis, arteriosclerosis, acute decompensated heart failure, congestive heart failure, cardiac edema, nephredema, hepatic edema, acute renal insufficiency, and chronic renal insufficiency, wherein the CNP variant is selected from the group consisting of

GDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP53) (SEQ ID NO: 179);

PDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP53) (SEQ ID NO: 185);

MDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP53) (SEQ ID NO: 190)

DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP-53(M48N)] (SEQ ID NO: 180);

LRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-52) (SEQ ID NO: 146);

RVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-51) (SEQ ID NO: 147);

VDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-50) (SEQ ID NO: 148);

DTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-49) (SEQ ID NO: 149);

TKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-48) (SEQ ID NO: 150);

KSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-47) (SEQ ID NO: 151);

SRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-46) (SEQ ID NO: 152);

RAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-45) (SEQ ID NO: 153);

AAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-44) (SEQ ID NO: 154);

AWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-43) (SEQ ID NO: 155);

WARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-42) (SEQ ID NO: 156);

ARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-41) (SEQ ID NO: 157);

RLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-40) (SEQ ID NO: 158);

LLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-39) (SEQ ID NO: 159);

LQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-38) (SEQ ID NO: 160);

QEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-37) (SEQ ID NO: 60);
EHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-36) (SEQ ID NO: 161);
HPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-35) (SEQ ID NO: 162);
PNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-34) (SEQ ID NO: 163);
NARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-33) (SEQ ID NO: 164);
ARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-32) (SEQ ID NO: 165);
RKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-31) (SEQ ID NO: 166);
KYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-30) (SEQ ID NO: 167);
YKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-29) (SEQ ID NO: 168);
KGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-28) (SEQ ID NO: 169);
GANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-27) (SEQ ID NO: 170);
ANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-26) (SEQ ID NO: 171);
NKKGLSKGCFGLKLDRIGSMSGLGC (CNP-25) (SEQ ID NO: 172);
KKGLSKGCFGLKLDRIGSMSGLGC (CNP-24) (SEQ ID NO: 173);
KGLSKGCFGLKLDRIGSMSGLGC (CNP-23) (SEQ ID NO: 174);
LSKGCFGLKLDRIGSMSGLGC (CNP-21) (SEQ ID NO: 175);
SKGCFGLKLDRIGSMSGLGC (CNP-20) (SEQ ID NO: 176);
KGCFGLKLDRIGSMSGLGC (CNP-19) (SEQ ID NO: 177);
GCFGLKLDRIGSMSGLGC (CNP-18) (SEQ ID NO: 178);
QEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP37(M32N)] (SEQ ID NO: 182);
PQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP37) (SEQ ID NO: 186);
MQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP37) (SEQ ID NO: 192);
GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP37) (SEQ ID NO: 75);
GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [Gly-CNP37(M32N)]) (SEQ ID NO: 181);
PGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-Gly-CNP37)) (SEQ ID NO: 145);

MGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-Gly-CNP37)) (SEQ ID NO: 191);

GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (HSA-CNP27) (SEQ ID NO: 144);

GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSNSGLGC [HSA-CNP27(M22N)) (SEQ ID NO: 183)];

PGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-HSA-CNP27)) (SEQ ID NO: 188);

MGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-HSA-CNP27)) (SEQ ID NO: 193);

GANRRGLSRGCFGLKLDRIGSMSGLGC [CNP27(K4,5,9R)] (SEQ ID NO: 36);

GANRRGLSRGCFGLKLDRIGSNSGLGC [CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);

PGANRRGLSRGCFGLKLDRIGSMSGLGC [Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189);

MGANRRGLSRGCFGLKLDRIGSMSGLGC [Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194);

PEG1K-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-CNP27(K4,5,9R)] (SEQ ID NO: 36);

PEG1K-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEG1K-CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);

PEG1K-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189);

PEG1K-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194);

PEO12-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-CNP27(K4,5,9R)] (SEQ ID NO: 36);

PEO12-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEO12-CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);

PEO12-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189);

PEO12-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194);

PEO24-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-CNP27(K4,5,9R)] (SEQ ID NO: 36);

PEO24-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEO24-CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);
PEO24-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189); and
PEO24-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194), wherein the CNP variant can be conjugated to a moiety selected from a hydrophobic acid and an amino acid sequence from 1 to 20 amino acids useful in bone targeting.

10. (Currently amended) A method for recombinant production of a CNP variant, comprising culturing in a medium a host cell comprising a first polynucleotide encoding a CNP variant polypeptide linked to a second polynucleotide encoding a cleavable peptide or protein under conditions that result in expression of a fusion polypeptide encoded by the polynucleotides, wherein the fusion polypeptide comprises the CNP variant polypeptide directly linked to the cleavable peptide or protein or indirectly linked thereto via a linker, wherein the CNP variant is selected from the group consisting of:

R-CNP22(K4R) (Analog AZ) (SEQ ID NO: 41);
ER-CNP22(K4R) (Analog BA) (SEQ ID NO: 39);
GANPR-CNP22(K4R) (Analog CI) (SEQ ID NO: 37);
GANQQ-CNP22(K4R) (Analog CH) (SEQ ID NO: 69);
AAWARLLQEHPNA-CNP22 (Analog CA) (SEQ ID NO: 61);
AAWARLLQEHPNAR-CNP22 (Analog CB)(SEQ ID NO: 62);
DLRVDTKSRAAWAR-CNP22 (Analog CC) (SEQ ID NO: 63);
GQPREPQVYTLPPS-CNP22 (Analog CF) (SEQ ID NO: 79);
GERAFKAWAVARLSQ-CNP22 (Analog CE) (SEQ ID NO: 81);
GHHSHEQHPHGANQQ-CNP22 (Analog CQ) (SEQ ID NO: 76);
GQEHPNARKYKGANPK-CNP22 (Analog CS) (SEQ ID NO: 71);
GQEHPNARKYKGANQK-CNP22 (Analog CT) (SEQ ID NO: 130);
GAHHPHEHDTHGANQQ-CNP22 (Analog CR) (SEQ ID NO: 77);
GQTHSSGTQSGANQQ-CNP22(K4R) (Analog CN) (SEQ ID NO: 87);
SPKMVQGSG-CNP17-KVLRRH (Analog CD) (SEQ ID NO: 68);
PNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-34);

GANRRGLSRGCFGLKLDRIGSMSGLGC [CNP27(K4,5,9R)] (SEQ ID NO: 36);
GANRRGLSRGCFGLKLDRIGSNSGLGC [CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);
PGANRRGLSRGCFGLKLDRIGSMSGLGC [Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189);
MGANRRGLSRGCFGLKLDRIGSMSGLGC [Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194);
GDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP53)) (SEQ ID NO: 179);
PDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP53)) (SEQ ID NO: 185);
MDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP53)) (SEQ ID NO: 190);
DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP-53(M48N)]) (SEQ ID NO: 180);
QEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-37) (SEQ ID NO: 60);
GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP37) (SEQ ID NO: 75);
GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (HSA-CNP27) (SEQ ID NO: 144);
GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSNSGLGC [HSA-CNP27(M22N)]) (SEQ ID NO: 183);
PGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-HSA-CNP27)) (SEQ ID NO: 188);
MGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-HSA-CNP27)) (SEQ ID NO: 193);

LRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-52) (SEQ ID NO: 146);

RVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-51) (SEQ ID NO: 147);
VDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-50) (SEQ ID NO: 148);
DTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-49) (SEQ ID NO: 149);

TKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-48) (SEQ ID NO: 150);
KSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-47) (SEQ ID NO: 151);
SRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-46) (SEQ ID NO: 152);
RAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-45) (SEQ ID NO: 153);
AAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-44) (SEQ ID NO: 154);
AWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-43) (SEQ ID NO: 155);
WARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-42) (SEQ ID NO: 156);
ARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-41) (SEQ ID NO: 157);
RLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-40) (SEQ ID NO: 158);
LLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-39) (SEQ ID NO: 159);
LQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-38) (SEQ ID NO: 160);
EHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-36) (SEQ ID NO: 161);
HPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-35) (SEQ ID NO: 162);
PNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-34) (SEQ ID NO: 163);
NARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-33) (SEQ ID NO: 164);
ARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-32) (SEQ ID NO: 165);
RKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-31) (SEQ ID NO: 166);
KYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-30) (SEQ ID NO: 167);
YKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-29) (SEQ ID NO: 168);
KGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-28) (SEQ ID NO: 169);
GANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-27) (SEQ ID NO: 170);

ANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-26) (SEQ ID NO: 171);
NKKGLSKGCFGLKLDRIGSMSGLGC (CNP-25) (SEQ ID NO: 172);
KKGLSKGCFGLKLDRIGSMSGLGC (CNP-24) (SEQ ID NO: 173);
KGLSKGCFGLKLDRIGSMSGLGC (CNP-23) (SEQ ID NO: 174);
LSKGCFGLKLDRIGSMSGLGC (CNP-21) (SEQ ID NO: 175);
SKGCFGLKLDRIGSMSGLGC (CNP-20) (SEQ ID NO: 176);
KGCFGLKLDRIGSMSGLGC (CNP-19) (SEQ ID NO: 177); and
GCFGLKLDRIGSMSGLGC (CNP-18) (SEQ ID NO: 178).

11-15. (Cancel)

16. (Original) The method of claim 10, wherein the cleavable peptide or protein is selected from the group consisting of histidine tags, human transcription factor TAF12, TAF12 fragments, TAF12 histone fold domain, mutants of TAF12 and fragments thereof, TAF12(C/A), TAF12(D/E), TAF12(4D/4E), TAF12(6D/6E), TAF12(10D/10E), TAF12(C/A & D/E), TAF12(C/A & 4D/4E), TAF12(C/A & 6D/6E), TAF12(C/A & 10D/10E), ketosteroid isomerase, maltose-binding protein, ß-galactosidase, glutathione-S-transferase, thioredoxin, chitin-binding domain, BMP-2, BMP-2 mutants, BMP-2(C/A), and mutants and fragments thereof.

17. (Cancel)

18. (Original) The method of claim 10, wherein the host cell is bacterial.

19-21. (Cancel)

22. (Original) The method of claim 10, wherein the fusion polypeptide is expressed as a soluble protein or as an inclusion body.

23. (Original) The method of claim10, further comprising isolating the expressed fusion polypeptide from the host cell or culture medium.

24. (Original) The method of claim 23, further comprising contacting the isolated fusion polypeptide with a cleaving agent selected from the group consisting of formic acid,

cyanogen bromide (CNBr), hydroxylamine, protein self cleavage, Factor Xa, enterokinase, ProTEV, and SUMO protease.

25-29. (Cancel)

30. (Currently amended) A CNP variant produced according to the method of claim 10, wherein the CNP variant is selected from the group consisting of:
R-CNP22(K4R) (Analog AZ) (SEQ ID NO: 41);
ER-CNP22(K4R) (Analog BA) (SEQ ID NO: 39);
GANPR-CNP22(K4R) (Analog CI) (SEQ ID NO: 37);
GANQQ-CNP22(K4R) (Analog CH) (SEQ ID NO: 69);
AAWARLLQEHPNA-CNP22 (Analog CA) (SEQ ID NO: 61);
AAWARLLQEHPNAR-CNP22 (Analog CB)(SEQ ID NO: 62);
DLRVDTKSRAAWAR-CNP22 (Analog CC) (SEQ ID NO: 63);
GQPREPQVYTLPPS-CNP22 (Analog CF) (SEQ ID NO: 79);
GERAFKAWAVARLSQ-CNP22 (Analog CE) (SEQ ID NO: 81);
GHHSHEQHPHGANQQ-CNP22 (Analog CQ) (SEQ ID NO: 76);
GQEHPNARKYKGANPK-CNP22 (Analog CS) (SEQ ID NO: 71);
GQEHPNARKYKGANQK-CNP22 (Analog CT) (SEQ ID NO: 130);
GAHHPHEHDTHGANQQ-CNP22 (Analog CR) (SEQ ID NO: 77);
GQTHSSGTQSGANQQ-CNP22(K4R) (Analog CN) (SEQ ID NO: 87);
SPKMVQGSG-CNP17-KVLRRH (Analog CD) (SEQ ID NO: 68);
PNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-34);
GANRRGLSRGCFGLKLDRIGSMSGLGC [CNP27(K4,5,9R)] (SEQ ID NO: 36);
GANRRGLSRGCFGLKLDRIGSNSGLGC [CNP27(K4,5,9R, M22N)];
PGANRRGLSRGCFGLKLDRIGSMSGLGC [Pro-CNP27(K4,5,9R)];
MGANRRGLSRGCFGLKLDRIGSMSGLGC [Met-CNP27(K4,5,9R)];
GDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP53)) (SEQ ID NO: 179);
PDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP53)) (SEQ ID NO: 185);
MDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP53) (SEQ ID NO: 190);

DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP-53(M48N)] (SEQ ID NO: 180);

QEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-37) (SEQ ID NO: 60);

QEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP37(M32N)] (SEQ ID NO: 182);

PQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP37) (SEQ ID NO: 186);

MQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP37) (SEQ ID NO: 192);

GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP37) (SEQ ID NO: 75);

GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [Gly-CNP37(M32N)] (SEQ ID NO: 181);

~~PGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-Gly-CNP37) (SEQ ID NO: 145);~~

MGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-Gly-CNP37) (SEQ ID NO: 191);

GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (HSA-CNP27) (SEQ ID NO: 144);

GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSNSGLGC [HSA-CNP27(M22N)] (SEQ ID NO: 183);

PGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-HSA-CNP27) (SEQ ID NO: 188); and

MGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-HSA-CNP27) (SEQ ID NO: 193)[[;]].

31. (Currently amended) A host cell comprising an expression vector, the vector comprising a first polynucleotide encoding a CNP variant polypeptide linked to a second polynucleotide encoding a cleavable peptide or protein, wherein the CNP variant is selected from the group consisting of

GDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP53) (SEQ ID NO: 179);

PDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP53) (SEQ ID NO: 185);

MDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP53) (SEQ ID NO: 190)

DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP-53(M48N)] (SEQ ID NO: 180);

LRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-52) (SEQ ID NO: 146);

RVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-51) (SEQ ID NO: 147);

VDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-50) (SEQ ID NO: 148);

DTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-49) (SEQ ID NO: 149);

TKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-48) (SEQ ID NO: 150);

KSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-47) (SEQ ID NO: 151);

SRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-46) (SEQ ID NO: 152);

RAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-45) (SEQ ID NO: 153);

AAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-44) (SEQ ID NO: 154);

AWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-43) (SEQ ID NO: 155);

WARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-42) (SEQ ID NO: 156);

ARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-41) (SEQ ID NO: 157);

RLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-40) (SEQ ID NO: 158);

LLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-39) (SEQ ID NO: 159);
LQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-38) (SEQ ID NO: 160);
QEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-37) (SEQ ID NO: 60);
EHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-36) (SEQ ID NO: 161);
HPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-35) (SEQ ID NO: 162);
PNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-34) (SEQ ID NO: 163);
NARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-33) (SEQ ID NO: 164);
ARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-32) (SEQ ID NO: 165);
RKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-31) (SEQ ID NO: 166);
KYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-30) (SEQ ID NO: 167);
YKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-29) (SEQ ID NO: 168);
KGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-28) (SEQ ID NO: 169);
GANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-27) (SEQ ID NO: 170);
ANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-26) (SEQ ID NO: 171);
NKKGLSKGCFGLKLDRIGSMSGLGC (CNP-25) (SEQ ID NO: 172);
KKGLSKGCFGLKLDRIGSMSGLGC (CNP-24) (SEQ ID NO: 173);
KGLSKGCFGLKLDRIGSMSGLGC (CNP-23) (SEQ ID NO: 174);
LSKGCFGLKLDRIGSMSGLGC (CNP-21) (SEQ ID NO: 175);
SKGCFGLKLDRIGSMSGLGC (CNP-20) (SEQ ID NO: 176);
KGCFGLKLDRIGSMSGLGC (CNP-19) (SEQ ID NO: 177);
GCFGLKLDRIGSMSGLGC (CNP-18) (SEQ ID NO: 178);
QEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP37(M32N)] (SEQ ID NO: 182);
PQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP37) (SEQ ID NO: 186);
MQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP37) (SEQ ID NO: 192);
GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP37) (SEQ ID NO: 75);

GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [Gly-CNP37(M32N)]) (SEQ ID NO: 181);

PGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-Gly-CNP37)) (SEQ ID NO: 145);

MGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-Gly-CNP37)) (SEQ ID NO: 191);

GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (HSA-CNP27) (SEQ ID NO: 144);

GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSNSGLGC [HSA-CNP27(M22N)) (SEQ ID NO: 183)];

PGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-HSA-CNP27)) (SEQ ID NO: 188);

MGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-HSA-CNP27)) (SEQ ID NO: 193);

GANRRGLSRGCFGLKLDRIGSMSGLGC [CNP27(K4,5,9R)] (SEQ ID NO: 36);

GANRRGLSRGCFGLKLDRIGSNSGLGC [CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);

PGANRRGLSRGCFGLKLDRIGSMSGLGC [Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189);

MGANRRGLSRGCFGLKLDRIGSMSGLGC [Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194);

PEG1K-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-CNP27(K4,5,9R)] (SEQ ID NO: 36);

PEG1K-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEG1K-CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);

PEG1K-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189);

PEG1K-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194);

PEO12-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-CNP27(K4,5,9R)] (SEQ ID NO: 36);

PEO12-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEO12-CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);

PEO12-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189);

PEO12-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194);
PEO24-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-CNP27(K4,5,9R)] (SEQ ID NO: 36);
PEO24-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEO24-CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);
PEO24-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189); and
PEO24-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194).

32-34. (Cancel)

35. (Original) The host cell of claim 31, wherein the host cell is cultured in a medium under conditions suitable for expression of a fusion polypeptide encoded by the polynucleotides, wherein the fusion polypeptide comprises the CNP variant polypeptide directly linked to the cleavable peptide or protein or indirectly linked thereto via a linker.

36-38. (Cancel)

39. (Currently amended) A method of treating a CNP-responsive condition or disorder, comprising

administering a CNP peptide or variant to a subject, and

monitoring the level of at least one bone- or cartilage-associated biomarker in the subject,

wherein an increase in the level of the at least one bone- or cartilage-associated biomarker indicates a therapeutic effect of the CNP peptide or variant on the subject or the condition or disorder, wherein the CNP variant is selected from the group consisting of

GDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP53) (SEQ ID NO: 179);
PDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP53) (SEQ ID NO: 185);

MDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP53) (SEQ ID NO: 190)

DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP-53(M48N)] (SEQ ID NO: 180);

LRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-52) (SEQ ID NO: 146);

RVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-51) (SEQ ID NO: 147);

VDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-50) (SEQ ID NO: 148);

DTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-49) (SEQ ID NO: 149);

TKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-48) (SEQ ID NO: 150);

KSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-47) (SEQ ID NO: 151);

SRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-46) (SEQ ID NO: 152);

RAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-45) (SEQ ID NO: 153);

AAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-44) (SEQ ID NO: 154);

AWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-43) (SEQ ID NO: 155);

WARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-42) (SEQ ID NO: 156);

ARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-41) (SEQ ID NO: 157);

RLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-40) (SEQ ID NO: 158);

LLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-39) (SEQ ID NO: 159);

LQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-38) (SEQ ID NO: 160);
QEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-37) (SEQ ID NO: 60);
EHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-36) (SEQ ID NO: 161);
HPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-35) (SEQ ID NO: 162);
PNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-34) (SEQ ID NO: 163);
NARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-33) (SEQ ID NO: 164);
ARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-32) (SEQ ID NO: 165);
RKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-31) (SEQ ID NO: 166);
KYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-30) (SEQ ID NO: 167);
YKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-29) (SEQ ID NO: 168);
KGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-28) (SEQ ID NO: 169);
GANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-27) (SEQ ID NO: 170);
ANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-26) (SEQ ID NO: 171);
NKKGLSKGCFGLKLDRIGSMSGLGC (CNP-25) (SEQ ID NO: 172);
KKGLSKGCFGLKLDRIGSMSGLGC (CNP-24) (SEQ ID NO: 173);
KGLSKGCFGLKLDRIGSMSGLGC (CNP-23) (SEQ ID NO: 174);
LSKGCFGLKLDRIGSMSGLGC (CNP-21) (SEQ ID NO: 175);
SKGCFGLKLDRIGSMSGLGC (CNP-20) (SEQ ID NO: 176);
KGCFGLKLDRIGSMSGLGC (CNP-19) (SEQ ID NO: 177);
GCFGLKLDRIGSMSGLGC (CNP-18) (SEQ ID NO: 178);
QEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP37(M32N)] (SEQ ID NO: 182);
PQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP37) (SEQ ID NO: 186);
MQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP37) (SEQ ID NO: 192);
GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP37) (SEQ ID NO: 75);
GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [Gly-CNP37(M32N)]) (SEQ ID NO: 181);

PGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-Gly-CNP37)) (SEQ ID NO: 145);

MGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-Gly-CNP37)) (SEQ ID NO: 191);

GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (HSA-CNP27) (SEQ ID NO: 144);

GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSNSGLGC [HSA-CNP27(M22N)) (SEQ ID NO: 183)];

PGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-HSA-CNP27)) (SEQ ID NO: 188);

MGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-HSA-CNP27)) (SEQ ID NO: 193);

GANRRGLSRGCFGLKLDRIGSMSGLGC [CNP27(K4,5,9R)] (SEQ ID NO: 36);

GANRRGLSRGCFGLKLDRIGSNSGLGC [CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);

PGANRRGLSRGCFGLKLDRIGSMSGLGC [Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189);

MGANRRGLSRGCFGLKLDRIGSMSGLGC [Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194);

PEG1K-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-CNP27(K4,5,9R)] (SEQ ID NO: 36);

PEG1K-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEG1K-CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);

PEG1K-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189);

PEG1K-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194);

PEO12-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-CNP27(K4,5,9R)] (SEQ ID NO: 36);

PEO12-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEO12-CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);

PEO12-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189);

PEO12-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194);

PEO24-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-CNP27(K4,5,9R)] (SEQ ID NO: 36);
PEO24-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEO24-CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);
PEO24-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189); and
PEO24-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194), wherein the CNP variant can be conjugated to a moiety selected from a hydrophobic acid and an amino acid sequence from 1 to 20 amino acids useful in bone targeting.

40. (Cancel)

41. (Currently amended) The method of claim ~~37 or~~ 39, wherein the at least one bone- or cartilage-associated biomarker is selected from the group consisting of CNP, cGMP, propeptides of collagen type II and fragments thereof, collagen type II and fragments thereof, osteocalcin, proliferating cell nuclear antigen (PCNA), propeptides of type I procollagen (PINP) and fragments thereof, collagen type I and fragments thereof, aggrecan chondroitin sulfate, and alkaline phosphatase.

42-43. (Cancel)

44. (New) A method of increasing long bone growth, comprising administering a CNP variant according to Claim 1 to a subject in need thereof, where the administration increases long bone growth.

45. (New) A CNP variant according to Claim 1 useful for increasing long bone growth or treating achondroplasia, hypochondroplasia, short stature, dwarfism, or homozygous achondroplasia in a subject in need thereof.

46. (New) A CNP variant where the CNP variant is selected from
QEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP37(M32N)] (SEQ ID NO: 182);

PQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP37) (SEQ ID NO: 186);

MQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP37) (SEQ ID NO: 192);

GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [Gly-CNP37(M32N)]) (SEQ ID NO: 181);

PGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-Gly-CNP37)) (SEQ ID NO: 145); and

MGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-Gly-CNP37)) (SEQ ID NO: 191),

wherein the variant is conjugated to a moiety selected from a hydrophobic acid and an amino acid sequence from 1 to 20 amino acids useful in bone targeting.

UNITED STATES PATENT AND TRADEMARK OFFICE

Exhibit B

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/466,672	05/08/2012	Daniel J. Wendt	30610/44556C1	1368

90849 7590 02/13/2013

Marshall, Gerstein & Borun LLP (Biomarin)
233 South Wacker Drive
6300 Willis Tower
Chicago, IL 60606

EXAMINER	
SHAFER, SHULAMITH H	

ART UNIT	PAPER NUMBER
1647	

NOTIFICATION DATE	DELIVERY MODE
02/13/2013	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

lbigornia@bmrn.com
mgbdocket@marshallip.com
mflores@bmrn.com

Office Action Summary	Application No.	Applicant(s)	
	13/466,672	WENDT ET AL.	
	Examiner	Art Unit	
	SHULAMITH H. SHAFER	1647	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 1 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

5)☒ Claim(s) *1-4,7,9,10,16,18,22-24,30,31,35,39,41 and 44-46* is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☐ Claim(s) _____ is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☒ Claim(s) *See Continuation Sheet* are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)

2) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.

4) ☐ Other: _____.

Continuation of Disposition of Claims: Claims subject to restriction and/or election requirement are 1-4,7,9,10,16,18,22-24,30,31,35,39,41 and 44-46.

DETAILED ACTION

Applicants' amendment of 20 August 2012 is acknowledged. Claims 1-4, 7, 9, 10, 16, 18, 22-24, 30, 31, 35, 39, 41, and 44-46 are pending in the instant application.

Election/Restrictions

Restriction to one of the following inventions is required under 35 U.S.C. 121:

I. Claims 1-4, 30, 45 and 46, drawn to a variant of C-type natriuretic peptide (CNP), classified in class 530, subclass 300.

II. Claim 7, drawn to a method of treating a bone-related disorder or skeletal dysplasia, classified in class 514, subclass 12.4.

III. Claim 9, drawn to a method of treating a vascular smooth muscle disorder, classified in class 514, subclass 12.4.

IV. Claims 10, 16, 18, 22-24, 31, 35, drawn to a method for recombinant production of a CNP variant and a host cell used in said method, classified in class 435, subclass 69.1/325.

V. Claims 39 and 41, drawn to a method of treating a CNP-responsive condition or disorder, classified in class 514, subclass 12.4.

VI. Claim 44, drawn to a method of increasing long bone growth, classified in class 514, subclass 12.4.

The inventions are distinct, each from the other because of the following reasons:

Inventions II-VI are directed to related processes. The related inventions are distinct if: (1) the inventions as claimed are either not capable of use together or can have a materially different design, mode of operation, function, or effect; (2) the inventions do not overlap in scope, i.e., are mutually exclusive; and (3) the inventions as claimed are not obvious variants. See MPEP § 806.05(j). In the instant case, the inventions as claimed are directed to methods that are distinct both physically and functionally, and are not required one for the other. Inventions II, III, V and VI are all drawn to methods of treatment, while Invention IV is drawn to a method of producing a CNP variant recombinantly. While Inventions II, III, V and VI are all directed to methods

of treatment, Invention II is directed to a method of treating a bone-related disorder or skeletal dysplasia, Invention III is drawn to a method of treating a vascular smooth muscle disorder, Invention V is drawn to a method of treating a CNP-responsive condition or disorder and monitoring the level of at least one bone- or cartilage-associated biomarker, while Invention VI is directed to a method of increasing long bone growth; thus each Invention requires a search for a distinct patient or subject population. All of these methods recite different goals, method steps and conclusion. Thus, a search of all these methods would result in an undue burden, as the searches would not be co-extensive. A search and examination of all these methods in one patent application would result in an undue burden, since the searches for the methods are not co-extensive. Furthermore, the inventions as claimed do not encompass overlapping subject matter and there is nothing of record to show them to be obvious variants.

Inventions I and II/III/V and VI are related as product and process of use. The inventions can be shown to be distinct if either or both of the following can be shown: (1) the process for using the product as claimed can be practiced with another materially different product or (2) the product as claimed can be used in a materially different process of using that product. See MPEP § 806.05(h). In the instant case the CNP variant of Invention I can be used to generate antibodies.

Inventions IV and I are related as process of making and product made. The inventions are distinct if either or both of the following can be shown: (1) that the process as claimed can be used to make another and materially different product or (2) that the product as claimed can be made by another and materially different process (MPEP § 806.05(f)). In the instant case the CNP variant of Invention I can be made by chemical synthesis.

Restriction for examination purposes as indicated is proper because all these inventions listed in this action are independent or distinct for the reasons given above

and there would be a serious search and examination burden if restriction were not required because one or more of the following reasons apply:

(a) the inventions have acquired a separate status in the art in view of their different classification;

(b) the inventions have acquired a separate status in the art due to their recognized divergent subject matter;

(c) the inventions require a different field of search (for example, searching different classes/subclasses or electronic resources, or employing different search queries);

(d) the prior art applicable to one invention would not likely be applicable to another invention;

(e) the inventions are likely to raise different non-prior art issues under 35 U.S.C. 101 and/or 35 U.S.C. 112, first paragraph.

Applicant is advised that the reply to this requirement to be complete must include (i) an election of a invention to be examined even though the requirement may be traversed (37 CFR 1.143) **and (ii) identification of the claims encompassing the elected invention**.

The election of an invention may be made with or without traverse. To reserve a right to petition, the election must be made with traverse. If the reply does not distinctly and specifically point out supposed errors in the restriction requirement, the election shall be treated as an election without traverse. Traversal must be presented at the time of election in order to be considered timely. Failure to timely traverse the requirement will result in the loss of right to petition under 37 CFR 1.144. If claims are added after the election, applicant must indicate which of these claims are readable on the elected invention.

If claims are added after the election, applicant must indicate which of these claims are readable upon the elected invention.

Should applicant traverse on the ground that the inventions are not patentably distinct, applicant should submit evidence or identify such evidence now of record showing the inventions to be obvious variants or clearly admit on the record that this is

the case. In either instance, if the examiner finds one of the inventions unpatentable over the prior art, the evidence or admission may be used in a rejection under 35 U.S.C. 103(a) of the other invention.

Species Election #1

This application contains claims directed to the following patentably distinct species: sequences as disclosed in claims 1, 9, 10, 30, 31, 39, and 46. The species are independent or distinct because each sequence is a distinct separate polypeptide. In addition, these species are not obvious variants of each other based on the current record.

Applicant is required under 35 U.S.C. 121 to elect a single disclosed species, or a single grouping of patentably indistinct species, for prosecution on the merits to which the claims shall be restricted if no generic claim is finally held to be allowable. Currently, no claim is generic.

There is a search and/or examination burden for the patentably distinct species as set forth above because at least the following reason(s) apply:

The species are independent or distinct because each sequence is a distinct separate polypeptide; a search for the recited sequences would not be coextensive.

Species Election #2

This application contains claims directed to the following patentably distinct species bone-related disorders. The species are independent or distinct because each disorder comprises a condition of unique etiology, progression and prognosis. In addition, these species are not obvious variants of each other based on the current record.

1. achondroplasia,
2. hypochondroplasia,
3. short stature,
4. dwarfism,
5. homozygous achondroplasia,

If Group II is elected, Applicant is required under 35 U.S.C. 121 to elect a single disclosed species, or a single grouping of patentably indistinct species, for prosecution on the merits to which the claims shall be restricted if no generic claim is finally held to be allowable. Currently, no claim is generic.

There is a search and/or examination burden for the patentably distinct species as set forth above because at least the following reason(s) apply:

The species are independent or distinct because each disorder comprises a condition of unique etiology, progression and prognosis; a search for prior art directed at achondroplasia, for example, would not necessarily reveal art relevant to an unspecified dwarfism.

Species Election #3

This application contains claims directed to the following patentably distinct species: vascular smooth muscle disorder. The species are independent or distinct because each species comprises a condition of unique etiology, progression and prognosis. In addition, these species are not obvious variants of each other based on the current record.

6. hypertension,
7. restenosis,
8. arteriosclerosis,
9. acute decompensated heart failure,
10. congestive heart failure,
11. cardiac edema,
12. nephredema,
13. hepatic edema,
14. acute renal insufficiency, and
15. chronic renal insufficiency.

If Group III is elected, Applicant is required under 35 U.S.C. 121 to elect a single disclosed species, or a single grouping of patentably indistinct species, for prosecution on the merits to which the claims shall be restricted if no generic claim is finally held to be allowable. Currently, no claim is generic.

There is a search and/or examination burden for the patentably distinct species as set forth above because at least the following reason(s) apply:

The species are independent or distinct because each disorder comprises a condition of unique etiology, progression and prognosis; a search for prior art directed at hypertension, for example, would not necessarily reveal art relevant to chronic renal insufficiency.

Species Election #4

This application contains claims directed to the following patentably distinct species: bone or cartilage associated biomarker. The species are independent or distinct because each biomarker is a compound of distinct structure and function. In addition, these species are not obvious variants of each other based on the current record.

16. CNP,
17. cGMP,
18. propeptides of collagen type II and fragments thereof,
19. collagen type II and fragments thereof,
20. osteocalcin,
21. proliferating cell nuclear antigen (PCNA),
22. propeptides of type I procollagen (PINP) and fragments thereof,
23. collagen type I and fragments thereof,
24. aggrecan chondroitin sulfate, and
25. alkaline phosphatase.

If Group V is elected, Applicant is required under 35 U.S.C. 121 to elect a single disclosed species, or a single grouping of patentably indistinct species, for prosecution on the merits to which the claims shall be restricted if no generic claim is finally held to be allowable. Currently, claim 39 is generic.

There is a search and/or examination burden for the patentably distinct species as set forth above because at least the following reason(s) apply:

The species are independent or distinct because each each compound has a distinct structure and function; a search for the recited compounds would not be coextensive.

Applicant is advised that the reply to this requirement to be complete must include (i) an election of a species or a grouping of patentably indistinct species to be examined even though the requirement may be traversed (37 CFR 1.143) **and (ii) identification of the claims encompassing the elected species or grouping of patentably indistinct species**, including any claims subsequently added. An argument that a claim is allowable or that all claims are generic is considered nonresponsive unless accompanied by an election.

The election may be made with or without traverse. To preserve a right to petition, the election must be made with traverse. If the reply does not distinctly and specifically point out supposed errors in the election of species requirement, the election shall be treated as an election without traverse. Traversal must be presented at the time of election in order to be considered timely. Failure to timely traverse the requirement will result in the loss of right to petition under 37 CFR 1.144. If claims are added after the election, applicant must indicate which of these claims are readable on the elected species or grouping of patentably indistinct species.

Should applicant traverse on the ground that the species, or groupings of patentably indistinct species from which election is required, are not patentably distinct, applicant should submit evidence or identify such evidence now of record showing them to be obvious variants or clearly admit on the record that this is the case. In either instance, if the examiner finds one of the species unpatentable over the prior art, the evidence or admission may be used in a rejection under 35 U.S.C. 103(a) of the other species.

Upon the allowance of a generic claim, applicant will be entitled to consideration of claims to additional species which depend from or otherwise require all the limitations of an allowable generic claim as provided by 37 CFR 1.141.

The examiner has required restriction between product and process claims. Where applicant elects claims directed to the product, and the product claims are

subsequently found allowable, withdrawn process claims that depend from or otherwise require all the limitations of the allowable product claim will be considered for rejoinder. All claims directed to a nonelected process invention must require all the limitations of an allowable product claim for that process invention to be rejoined.

In the event of rejoinder, the requirement for restriction between the product claims and the rejoined process claims will be withdrawn, and the rejoined process claims will be fully examined for patentability in accordance with 37 CFR 1.104. Thus, to be allowable, the rejoined claims must meet all criteria for patentability including the requirements of 35 U.S.C. 101, 102, 103 and 112. Until all claims to the elected product are found allowable, an otherwise proper restriction requirement between product claims and process claims may be maintained. Withdrawn process claims that are not commensurate in scope with an allowable product claim will not be rejoined. See MPEP § 821.04(b). Additionally, in order to retain the right to rejoinder in accordance with the above policy, applicant is advised that the process claims should be amended during prosecution to require the limitations of the product claims. **Failure to do so may result in a loss of the right to rejoinder**. Further, note that the prohibition against double patenting rejections of 35 U.S.C. 121 does not apply where the restriction requirement is withdrawn by the examiner before the patent issues. See MPEP § 804.01.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to SHULAMITH H. SHAFER whose telephone number is (571)272-3332. The examiner can normally be reached on Monday through Friday, 8 AM to 5 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Joanne Hama can be reached on 571-272-2911. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

SHULAMITH H. SHAFER
Primary Examiner
Art Unit 1647

/SHULAMITH H. SHAFER/
Primary Examiner, Art Unit 1647

Exhibit C

Docket No.: 30610/44556C1
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Daniel J. Wendt et al.

Application No.: 13/466,672

Filed: May 8, 2012

For: Variants of C-type Natriuretic Peptide

Confirmation No.: 1368

Art Unit: 1646

Examiner: S. Shafer

RESPONSE TO RESTRICTION REQUIREMENT

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Madam:

This paper is filed in response to an Office Action (hereinafter "the Action") mailed February 13, 2013 and setting out a Restriction Requirement in the above-mentioned application.

Amendments to the Claims begin on page 2 of this paper.

Remarks begin on page 24 of this paper.

AMENDMENT TO THE CLAIMS

1. (Currently amended) A variant of C-type natriuretic peptide (CNP) selected from the group consisting of:

GDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP53) (SEQ ID NO: 179);

PDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP53) (SEQ ID NO: 185);

MDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP53) (SEQ ID NO: 190)

DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP-53(M48N)] (SEQ ID NO: 180);

LRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-52) (SEQ ID NO: 146);

RVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-51) (SEQ ID NO: 147);

VDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-50) (SEQ ID NO: 148);

DTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-49) (SEQ ID NO: 149);

TKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-48) (SEQ ID NO: 150);

KSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-47) (SEQ ID NO: 151);

SRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-46) (SEQ ID NO: 152);

RAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-45) (SEQ ID NO: 153);

AAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-44) (SEQ ID NO: 154);

AWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-43) (SEQ ID NO: 155);

WARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-42) (SEQ ID NO: 156);

ARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-41) (SEQ ID NO: 157);

RLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-40) (SEQ ID NO: 158);

LLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-39) (SEQ ID NO: 159);

LQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-38) (SEQ ID NO: 160);

QEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-37) (SEQ ID NO: 60);

EHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-36) (SEQ ID NO: 161);

HPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-35) (SEQ ID NO: 162);

PNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-34) (SEQ ID NO: 163);

NARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-33) (SEQ ID NO: 164);

ARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-32) (SEQ ID NO: 165);

RKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-31) (SEQ ID NO: 166);

KYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-30) (SEQ ID NO: 167);

YKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-29) (SEQ ID NO: 168);

KGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-28) (SEQ ID NO: 169);

GANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-27) (SEQ ID NO: 170);

ANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-26) (SEQ ID NO: 171);

NKKGLSKGCFGLKLDRIGSMSGLGC (CNP-25) (SEQ ID NO: 172);

KKGLSKGCFGLKLDRIGSMSGLGC (CNP-24) (SEQ ID NO: 173);

KGLSKGCFGLKLDRIGSMSGLGC (CNP-23) (SEQ ID NO: 174);

LSKGCFGLKLDRIGSMSGLGC (CNP-21) (SEQ ID NO: 175);

SKGCFGLKLDRIGSMSGLGC (CNP-20) (SEQ ID NO: 176);

KGCFGLKLDRIGSMSGLGC (CNP-19) (SEQ ID NO: 177);

GCFGLKLDRIGSMSGLGC (CNP-18) (SEQ ID NO: 178);

GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP37) (SEQ ID NO: 75);

GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (HSA-CNP27) (SEQ ID NO: 144);

GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSNSGLGC [HSA-CNP27(M22N)) (SEQ ID NO: 183)];

PGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-HSA-CNP27)) (SEQ ID NO: 188);

MGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-HSA-CNP27)) (SEQ ID NO: 193);

~~GANRRGLSRGCFGLKLDRIGSMSGLGC [CNP27(K4,5,9R)] (SEQ ID NO: 36);~~

GANRRGLSRGCFGLKLDRIGSNSGLGC [CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);

PGANRRGLSRGCFGLKLDRIGSMSGLGC [Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189);

MGANRRGLSRGCFGLKLDRIGSMSGLGC [Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194);

~~PEG1K-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-CNP27(K4,5,9R)] (SEQ ID NO: 36);~~

PEG1K-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEG1K-CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);

PEG1K-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189);

PEG1K-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194);

~~PEO12-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-CNP27(K4,5,9R)] (SEQ ID NO: 36);~~

PEO12-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEO12-CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);

PEO12-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189);

PEO12-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194);

~~PEO24-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-CNP27(K4,5,9R)] (SEQ ID NO: 36);~~

PEO24-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEO24-CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);

PEO24-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189); and

PEO24-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194), wherein the CNP variant can be conjugated to a moiety selected from a hydrophobic acid and an amino acid sequence from 1 to 20 amino acids useful in bone targeting.

2. (Previously presented) A pharmaceutical composition comprising a CNP variant of claim 1, and a pharmaceutically acceptable excipient, carrier or diluent.

3. (Original) The composition of claim 2, which is a lyophilized formulation prepared from a formulation that comprises a citric acid/citrate buffer or an acetic acid/acetate buffer having a pH from about 4 to about 6.

4. (Previously presented) The composition of claim 3, wherein the lyophilized formulation is prepared from a formulation that further comprises (a) an isotonicity-adjusting agent or a bulking agent or (b) an antioxidant.

5-6. (Cancel)

7. (Previously presented) A method of treating a bone-related disorder or skeletal dysplasia, comprising administering a CNP variant to a subject in need thereof, wherein the CNP variant is a CNP variant according to claim 1, and wherein the bone-related disorder or skeletal dysplasia is selected from the group consisting of achondroplasia, hypochondroplasia, short stature, dwarfism and homozygous achondroplasia.

8. (Cancel)

9. (Currently amended) A method of treating a vascular smooth muscle disorder, comprising administering a CNP variant to a subject, wherein the CNP variant is a CNP variant according to claim 1, and wherein the vascular smooth muscle disorder is selected from the group consisting of hypertension, restenosis, arteriosclerosis, acute decompensated heart failure, congestive heart failure, cardiac edema, nephredema, hepatic edema, acute renal insufficiency, and chronic renal insufficiency, wherein the CNP variant is selected from the group consisting of

GDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP53) (SEQ ID NO: 179);

PDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP53) (SEQ ID NO: 185);

MDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP53) (SEQ ID NO: 190)

DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP-53(M48N)] (SEQ ID NO: 180);

LRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-52) (SEQ ID NO: 146);

RVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-51) (SEQ ID NO: 147);

VDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-50) (SEQ ID NO: 148);

DTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-49) (SEQ ID NO: 149);

TKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-48) (SEQ ID NO: 150);

KSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-47) (SEQ ID NO: 151);

SRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-46) (SEQ ID NO: 152);

RAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-45) (SEQ ID NO: 153);

AAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-44) (SEQ ID NO: 154);

AWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-43) (SEQ ID NO: 155);

WARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-42) (SEQ ID NO: 156);

ARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-41) (SEQ ID NO: 157);

RLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-40) (SEQ ID NO: 158);

LLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-39) (SEQ ID NO: 159);

LQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-38) (SEQ ID NO: 160);

~~QEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-37) (SEQ ID NO: 60);~~

EHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-36) (SEQ ID NO: 161);

HPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-35) (SEQ ID NO: 162);

PNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-34) (SEQ ID NO: 163);

NARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-33) (SEQ ID NO: 164);

ARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-32) (SEQ ID NO: 165);

RKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-31) (SEQ ID NO: 166);

KYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-30) (SEQ ID NO: 167);

YKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-29) (SEQ ID NO: 168);

KGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-28) (SEQ ID NO: 169);

GANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-27) (SEQ ID NO: 170);

ANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-26) (SEQ ID NO: 171);

NKKGLSKGCFGLKLDRIGSMSGLGC (CNP-25) (SEQ ID NO: 172);

KKGLSKGCFGLKLDRIGSMSGLGC (CNP-24) (SEQ ID NO: 173);

KGLSKGCFGLKLDRIGSMSGLGC (CNP-23) (SEQ ID NO: 174);

LSKGCFGLKLDRIGSMSGLGC (CNP-21) (SEQ ID NO: 175);

SKGCFGLKLDRIGSMSGLGC (CNP-20) (SEQ ID NO: 176);

KGCFGLKLDRIGSMSGLGC (CNP-19) (SEQ ID NO: 177);

GCFGLKLDRIGSMSGLGC (CNP-18) (SEQ ID NO: 178);

QEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP37(M32N)] (SEQ ID NO: 182);

PQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP37) (SEQ ID NO: 186);

MQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP37) (SEQ ID NO: 192);

GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP37) (SEQ ID NO: 75);

GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [Gly-CNP37(M32N)]) (SEQ ID NO: 181);

PGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-Gly-CNP37)) (SEQ ID NO: 145);

MGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-Gly-CNP37)) (SEQ ID NO: 191);

GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (HSA-CNP27) (SEQ ID NO: 144);

GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSNSGLGC [HSA-CNP27(M22N)) (SEQ ID NO: 183)];

PGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-HSA-CNP27)) (SEQ ID NO: 188);

MGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-HSA-CNP27)) (SEQ ID NO: 193);

~~GANRRGLSRGCFGLKLDRIGSMSGLGC [CNP27(K4,5,9R)] (SEQ ID NO: 36);~~

GANRRGLSRGCFGLKLDRIGSNSGLGC [CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);

PGANRRGLSRGCFGLKLDRIGSMSGLGC [Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189);

MGANRRGLSRGCFGLKLDRIGSMSGLGC [Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194);

~~PEG1K-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-CNP27(K4,5,9R)] (SEQ ID NO: 36);~~

PEG1K-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEG1K-CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);

PEG1K-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189);

PEG1K-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194);

~~PEO12-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-CNP27(K4,5,9R)] (SEQ ID NO: 36);~~

PEO12-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEO12-CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);

PEO12-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189);

PEO12-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194);

~~PEO24-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-CNP27(K4,5,9R)] (SEQ ID NO: 36);~~

PEO24-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEO24-CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);

PEO24-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189); and

PEO24-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194), wherein the CNP variant can be conjugated to a moiety selected from a hydrophobic acid and an amino acid sequence from 1 to 20 amino acids useful in bone targeting.

10. (Previously presented) A method for recombinant production of a CNP variant, comprising culturing in a medium a host cell comprising a first polynucleotide encoding a CNP variant polypeptide linked to a second polynucleotide encoding a cleavable peptide or protein under conditions that result in expression of a fusion polypeptide encoded by the polynucleotides, wherein the fusion polypeptide comprises the CNP variant polypeptide directly linked to the cleavable peptide or protein or indirectly linked thereto via a linker, wherein the CNP variant is selected from the group consisting of:

R-CNP22(K4R) (Analog AZ) (SEQ ID NO: 41);
ER-CNP22(K4R) (Analog BA) (SEQ ID NO: 39);
GANPR-CNP22(K4R) (Analog CI) (SEQ ID NO: 37);
GANQQ-CNP22(K4R) (Analog CH) (SEQ ID NO: 69);
AAWARLLQEHPNA-CNP22 (Analog CA) (SEQ ID NO: 61);
AAWARLLQEHPNAR-CNP22 (Analog CB)(SEQ ID NO: 62);
DLRVDTKSRAAWAR-CNP22 (Analog CC) (SEQ ID NO: 63);
GQPREPQVYTLPPS-CNP22 (Analog CF) (SEQ ID NO: 79);
GERAFKAWAVARLSQ-CNP22 (Analog CE) (SEQ ID NO: 81);
GHHSHEQHPHGANQQ-CNP22 (Analog CQ) (SEQ ID NO: 76);

GQEHPNARKYKGANPK-CNP22 (Analog CS) (SEQ ID NO: 71);
GQEHPNARKYKGANQK-CNP22 (Analog CT) (SEQ ID NO: 130);
GAHHPHEHDTHGANQQ-CNP22 (Analog CR) (SEQ ID NO: 77);
GQTHSSGTQSGANQQ-CNP22(K4R) (Analog CN) (SEQ ID NO: 87);
SPKMVQGSG-CNP17-KVLRRH (Analog CD) (SEQ ID NO: 68);
PNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-34);
GANRRGLSRGCFGLKLDRIGSMSGLGC [CNP27(K4,5,9R)] (SEQ ID NO: 36);
GANRRGLSRGCFGLKLDRIGSNSGLGC [CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);
PGANRRGLSRGCFGLKLDRIGSMSGLGC [Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189);
MGANRRGLSRGCFGLKLDRIGSMSGLGC [Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194);
GDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP53)) (SEQ ID NO: 179);
PDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP53)) (SEQ ID NO: 185);
MDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP53)) (SEQ ID NO: 190);
DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP-53(M48N)]) (SEQ ID NO: 180);
QEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-37) (SEQ ID NO: 60);
GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP37) (SEQ ID NO: 75);
GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (HSA-CNP27) (SEQ ID NO: 144);
GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSNSGLGC [HSA-CNP27(M22N)]) (SEQ ID NO: 183);
PGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-HSA-CNP27)) (SEQ ID NO: 188);
MGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-HSA-CNP27)) (SEQ ID NO: 193);

LRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-52) (SEQ ID NO: 146);

RVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-51) (SEQ ID NO: 147);

VDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-50) (SEQ ID NO: 148);

DTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-49) (SEQ ID NO: 149);

TKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-48) (SEQ ID NO: 150);

KSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-47) (SEQ ID NO: 151);

SRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-46) (SEQ ID NO: 152);

RAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-45) (SEQ ID NO: 153);

AAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-44) (SEQ ID NO: 154);

AWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-43) (SEQ ID NO: 155);

WARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-42) (SEQ ID NO: 156);

ARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-41) (SEQ ID NO: 157);

RLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-40) (SEQ ID NO: 158);

LLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-39) (SEQ ID NO: 159);

LQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-38) (SEQ ID NO: 160);

EHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-36) (SEQ ID NO: 161);

HPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-35) (SEQ ID NO: 162);

PNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-34) (SEQ ID NO: 163);

NARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-33) (SEQ ID NO: 164);

ARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-32) (SEQ ID NO: 165);
RKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-31) (SEQ ID NO: 166);
KYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-30) (SEQ ID NO: 167);
YKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-29) (SEQ ID NO: 168);
KGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-28) (SEQ ID NO: 169);
GANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-27) (SEQ ID NO: 170);
ANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-26) (SEQ ID NO: 171);
NKKGLSKGCFGLKLDRIGSMSGLGC (CNP-25) (SEQ ID NO: 172);
KKGLSKGCFGLKLDRIGSMSGLGC (CNP-24) (SEQ ID NO: 173);
KGLSKGCFGLKLDRIGSMSGLGC (CNP-23) (SEQ ID NO: 174);
LSKGCFGLKLDRIGSMSGLGC (CNP-21) (SEQ ID NO: 175);
SKGCFGLKLDRIGSMSGLGC (CNP-20) (SEQ ID NO: 176);
KGCFGLKLDRIGSMSGLGC (CNP-19) (SEQ ID NO: 177); and
GCFGLKLDRIGSMSGLGC (CNP-18) (SEQ ID NO: 178).

11-15. (Cancel)

16. (Original) The method of claim 10, wherein the cleavable peptide or protein is selected from the group consisting of histidine tags, human transcription factor TAF12, TAF12 fragments, TAF12 histone fold domain, mutants of TAF12 and fragments thereof, TAF12(C/A), TAF12(D/E), TAF12(4D/4E), TAF12(6D/6E), TAF12(10D/10E), TAF12(C/A & D/E), TAF12(C/A & 4D/4E), TAF12(C/A & 6D/6E), TAF12(C/A & 10D/10E), ketosteroid isomerase, maltose-binding protein, ß-galactosidase, glutathione-S-transferase, thioredoxin, chitin-binding domain, BMP-2, BMP-2 mutants, BMP-2(C/A), and mutants and fragments thereof.

17. (Cancel)

18. (Original) The method of claim 10, wherein the host cell is bacterial.

19-21. (Cancel)

22. (Original) The method of claim 10, wherein the fusion polypeptide is expressed as a soluble protein or as an inclusion body.

23. (Original) The method of claim10, further comprising isolating the expressed fusion polypeptide from the host cell or culture medium.

24. (Original) The method of claim 23, further comprising contacting the isolated fusion polypeptide with a cleaving agent selected from the group consisting of formic acid, cyanogen bromide (CNBr), hydroxylamine, protein self cleavage, Factor Xa, enterokinase, ProTEV, and SUMO protease.

25-29. (Cancel)

30. (Currently amended) A CNP variant produced according to the method of claim 10, wherein the CNP variant is selected from the group consisting of:
~~R-CNP22(K4R) (Analog AZ) (SEQ ID NO: 41);~~
~~ER-CNP22(K4R) (Analog BA) (SEQ ID NO: 39);~~
~~GANPR-CNP22(K4R) (Analog CI) (SEQ ID NO: 37);~~
~~GANQQ-CNP22(K4R) (Analog CH) (SEQ ID NO: 69);~~
~~AAWARLLQEHPNA-CNP22 (Analog CA) (SEQ ID NO: 61);~~
~~AAWARLLQEHPNAR-CNP22 (Analog CB)(SEQ ID NO: 62);~~
~~DLRVDTKSRAAWAR-CNP22 (Analog CC) (SEQ ID NO: 63);~~
~~GQPREPQVYTLPPS-CNP22 (Analog CF) (SEQ ID NO: 79);~~
~~GERAFKAWAVARLSQ-CNP22 (Analog CE) (SEQ ID NO: 81);~~
~~GHHSHEQHPHGANQQ-CNP22 (Analog CQ) (SEQ ID NO: 76);~~
~~GQEHPNARKYKGANPK-CNP22 (Analog CS) (SEQ ID NO: 71);~~
~~GQEHPNARKYKGANQK-CNP22 (Analog CT) (SEQ ID NO: 130);~~
~~GAHHPHEHDTHGANQQ-CNP22 (Analog CR) (SEQ ID NO: 77);~~
~~GQTHSSGTQSGANQQ-CNP22(K4R) (Analog CN) (SEQ ID NO: 87);~~
~~SPKMVQGSG-CNP17-KVLRRH (Analog CD) (SEQ ID NO: 68);~~
PNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-34);
~~GANRRGLSRGCFGLKLDRIGSMSGLGC [CNP27(K4,5,9R)] (SEQ ID NO: 36);~~
GANRRGLSRGCFGLKLDRIGSNSGLGC [CNP27(K4,5,9R, M22N)];
PGANRRGLSRGCFGLKLDRIGSMSGLGC [Pro-CNP27(K4,5,9R)];
MGANRRGLSRGCFGLKLDRIGSMSGLGC [Met-CNP27(K4,5,9R)];

GDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP53)) (SEQ ID NO: 179);

PDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP53)) (SEQ ID NO: 185);

MDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP53) (SEQ ID NO: 190);

DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP-53(M48N)] (SEQ ID NO: 180);

~~QEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-37) (SEQ ID NO: 60);~~

QEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP37(M32N)] (SEQ ID NO: 182);

PQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP37) (SEQ ID NO: 186);

MQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP37) (SEQ ID NO: 192);

~~GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP37) (SEQ ID NO: 75);~~

GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [Gly-CNP37(M32N)] (SEQ ID NO: 181);

MGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-Gly-CNP37) (SEQ ID NO: 191);

GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (HSA-CNP27) (SEQ ID NO: 144);

GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSNSGLGC [HSA-CNP27(M22N)] (SEQ ID NO: 183);

PGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-HSA-CNP27) (SEQ ID NO: 188); and

MGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-HSA-CNP27) (SEQ ID NO: 193).

31. (Currently amended) A host cell comprising an expression vector, the vector comprising a first polynucleotide encoding a CNP variant polypeptide linked to a second

polynucleotide encoding a cleavable peptide or protein, wherein the CNP variant is selected from the group consisting of

GDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP53) (SEQ ID NO: 179);

PDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP53) (SEQ ID NO: 185);

MDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP53) (SEQ ID NO: 190)

DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP-53(M48N)] (SEQ ID NO: 180);

LRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-52) (SEQ ID NO: 146);

RVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-51) (SEQ ID NO: 147);

VDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-50) (SEQ ID NO: 148);

DTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-49) (SEQ ID NO: 149);

TKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-48) (SEQ ID NO: 150);

KSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-47) (SEQ ID NO: 151);

SRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-46) (SEQ ID NO: 152);

RAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-45) (SEQ ID NO: 153);

AAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-44) (SEQ ID NO: 154);

AWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-43) (SEQ ID NO: 155);

WARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-42) (SEQ ID NO: 156);

ARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-41) (SEQ ID NO: 157);

RLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-40) (SEQ ID NO: 158);

LLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-39) (SEQ ID NO: 159);

LQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-38) (SEQ ID NO: 160);

~~QEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-37) (SEQ ID NO: 60);~~

EHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-36) (SEQ ID NO: 161);

HPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-35) (SEQ ID NO: 162);

PNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-34) (SEQ ID NO: 163);

NARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-33) (SEQ ID NO: 164);

ARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-32) (SEQ ID NO: 165);

RKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-31) (SEQ ID NO: 166);

KYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-30) (SEQ ID NO: 167);

YKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-29) (SEQ ID NO: 168);

KGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-28) (SEQ ID NO: 169);

GANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-27) (SEQ ID NO: 170);

ANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-26) (SEQ ID NO: 171);

NKKGLSKGCFGLKLDRIGSMSGLGC (CNP-25) (SEQ ID NO: 172);

KKGLSKGCFGLKLDRIGSMSGLGC (CNP-24) (SEQ ID NO: 173);

KGLSKGCFGLKLDRIGSMSGLGC (CNP-23) (SEQ ID NO: 174);

LSKGCFGLKLDRIGSMSGLGC (CNP-21) (SEQ ID NO: 175);

SKGCFGLKLDRIGSMSGLGC (CNP-20) (SEQ ID NO: 176);

KGCFGLKLDRIGSMSGLGC (CNP-19) (SEQ ID NO: 177);

GCFGLKLDRIGSMSGLGC (CNP-18) (SEQ ID NO: 178);

QEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP37(M32N)] (SEQ ID NO: 182);

PQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP37) (SEQ ID NO: 186);

MQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP37) (SEQ ID NO: 192);

~~GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP37) (SEQ ID NO: 75);~~

GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [Gly-CNP37(M32N)]) (SEQ ID NO: 181);

PGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-Gly-CNP37)) (SEQ ID NO: 145);

MGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-Gly-CNP37)) (SEQ ID NO: 191);

GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (HSA-CNP27) (SEQ ID NO: 144);

GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSNSGLGC [HSA-CNP27(M22N)) (SEQ ID NO: 183)];

PGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-HSA-CNP27)) (SEQ ID NO: 188);

MGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-HSA-CNP27)) (SEQ ID NO: 193);

~~GANRRGLSRGCFGLKLDRIGSMSGLGC [CNP27(K4,5,9R)] (SEQ ID NO: 36);~~

GANRRGLSRGCFGLKLDRIGSNSGLGC [CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);

PGANRRGLSRGCFGLKLDRIGSMSGLGC [Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189);

MGANRRGLSRGCFGLKLDRIGSMSGLGC [Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194);

~~PEG1K-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-CNP27(K4,5,9R)] (SEQ ID NO: 36);~~

~~PEG1K-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEG1K-CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);~~

~~PEG1K-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189);~~

~~PEG1K-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194);~~

~~PEO12-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-CNP27(K4,5,9R)] (SEQ ID NO: 36);~~

~~PEO12-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEO12-CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);~~

~~PEO12-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189);~~

~~PEO12-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194);~~

~~PEO24-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-CNP27(K4,5,9R)] (SEQ ID NO: 36);~~

~~PEO24-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEO24-CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);~~

~~PEO24-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189); and~~

~~PEO24-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194)~~.

32-34. (Cancel)

35. (Original) The host cell of claim 31, wherein the host cell is cultured in a medium under conditions suitable for expression of a fusion polypeptide encoded by the polynucleotides, wherein the fusion polypeptide comprises the CNP variant polypeptide directly linked to the cleavable peptide or protein or indirectly linked thereto via a linker.

36-38. (Cancel)

39. (Currently amended) A method of treating a CNP-responsive condition or disorder, comprising

administering a CNP peptide or variant to a subject, and

monitoring the level of at least one bone- or cartilage-associated biomarker in the subject,

wherein an increase in the level of the at least one bone- or cartilage-associated biomarker indicates a therapeutic effect of the CNP peptide or variant on the subject or the condition or disorder, wherein the CNP variant is selected from the group consisting of

GDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP53) (SEQ ID NO: 179);

PDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP53) (SEQ ID NO: 185);

MDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP53) (SEQ ID NO: 190)

DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP-53(M48N)] (SEQ ID NO: 180);

LRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-52) (SEQ ID NO: 146);

RVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-51) (SEQ ID NO: 147);

VDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-50) (SEQ ID NO: 148);

DTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-49) (SEQ ID NO: 149);

TKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-48) (SEQ ID NO: 150);

KSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-47) (SEQ ID NO: 151);

SRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-46) (SEQ ID NO: 152);

RAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-45) (SEQ ID NO: 153);

AAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-44) (SEQ ID NO: 154);

AWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-43) (SEQ ID NO: 155);

WARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-42) (SEQ ID NO: 156);

ARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-41) (SEQ ID NO: 157);

RLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-40) (SEQ ID NO: 158);

LLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-39) (SEQ ID NO: 159);

LQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-38) (SEQ ID NO: 160);

QEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-37) (SEQ ID NO: 60);

EHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-36) (SEQ ID NO: 161);

HPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-35) (SEQ ID NO: 162);

PNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-34) (SEQ ID NO: 163);

NARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-33) (SEQ ID NO: 164);

ARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-32) (SEQ ID NO: 165);

RKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-31) (SEQ ID NO: 166);

KYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-30) (SEQ ID NO: 167);

YKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-29) (SEQ ID NO: 168);

KGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-28) (SEQ ID NO: 169);

GANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-27) (SEQ ID NO: 170);

ANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-26) (SEQ ID NO: 171);

NKKGLSKGCFGLKLDRIGSMSGLGC (CNP-25) (SEQ ID NO: 172);

KKGLSKGCFGLKLDRIGSMSGLGC (CNP-24) (SEQ ID NO: 173);

KGLSKGCFGLKLDRIGSMSGLGC (CNP-23) (SEQ ID NO: 174);

LSKGCFGLKLDRIGSMSGLGC (CNP-21) (SEQ ID NO: 175);

SKGCFGLKLDRIGSMSGLGC (CNP-20) (SEQ ID NO: 176);

KGCFGLKLDRIGSMSGLGC (CNP-19) (SEQ ID NO: 177);

GCFGLKLDRIGSMSGLGC (CNP-18) (SEQ ID NO: 178);

QEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP37(M32N)] (SEQ ID NO: 182);

PQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP37) (SEQ ID NO: 186);

MQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP37) (SEQ ID NO: 192);

GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP37) (SEQ ID NO: 75);

GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [Gly-CNP37(M32N)]) (SEQ ID NO: 181);

PGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-Gly-CNP37)) (SEQ ID NO: 145);

MGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-Gly-CNP37)) (SEQ ID NO: 191);

GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (HSA-CNP27) (SEQ ID NO: 144);

GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSNSGLGC [HSA-CNP27(M22N)) (SEQ ID NO: 183)];

PGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-HSA-CNP27)) (SEQ ID NO: 188);

MGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-HSA-CNP27)) (SEQ ID NO: 193);

~~GANRRGLSRGCFGLKLDRIGSMSGLGC [CNP27(K4,5,9R)] (SEQ ID NO: 36);~~

GANRRGLSRGCFGLKLDRIGSNSGLGC [CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);

PGANRRGLSRGCFGLKLDRIGSMSGLGC [Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189);

MGANRRGLSRGCFGLKLDRIGSMSGLGC [Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194);

~~PEG1K-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-CNP27(K4,5,9R)] (SEQ ID NO: 36);~~

PEG1K-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEG1K-CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);

PEG1K-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189);

PEG1K-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194);

~~PEO12-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-CNP27(K4,5,9R)] (SEQ ID NO: 36);~~

PEO12-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEO12-CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);

PEO12-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189);

PEO12-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194);

~~PEO24-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-CNP27(K4,5,9R)] (SEQ ID NO: 36);~~

PEO24-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEO24-CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);

PEO24-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189); and

PEO24-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194), wherein the CNP variant can be conjugated to a moiety selected from a hydrophobic acid and an amino acid sequence from 1 to 20 amino acids useful in bone targeting.

40. (Cancel)

41. (Previously presented) The method of claim 39, wherein the at least one bone- or cartilage-associated biomarker is selected from the group consisting of CNP, cGMP, propeptides of collagen type II and fragments thereof, collagen type II and fragments thereof, osteocalcin, proliferating cell nuclear antigen (PCNA), propeptides of type I procollagen (PINP) and fragments thereof, collagen type I and fragments thereof, aggrecan chondroitin sulfate, and alkaline phosphatase.

42-43. (Cancel)

44. (Previously presented) A method of increasing long bone growth, comprising administering a CNP variant according to Claim 1 to a subject in need thereof, where the administration increases long bone growth.

45. (Previously presented) A CNP variant according to Claim 1 useful for increasing long bone growth or treating achondroplasia, hypochondroplasia, short stature, dwarfism, or homozygous achondroplasia in a subject in need thereof.

46. (Previously presented) A CNP variant where the CNP variant is selected from
QEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP37(M32N)] (SEQ ID NO: 182);
PQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP37) (SEQ ID NO: 186);
MQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP37) (SEQ ID NO: 192);
GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [Gly-CNP37(M32N)]) (SEQ ID NO: 181);
PGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-Gly-CNP37)) (SEQ ID NO: 145); and
MGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-Gly-CNP37)) (SEQ ID NO: 191),
wherein the variant is conjugated to a moiety selected from a hydrophobic acid and an amino acid sequence from 1 to 20 amino acids useful in bone targeting.

REMARKS

In the Action, the examiner required restriction of pending claims 1-4, 7, 9, 10, 16, 18, 22-24, 30-31, 35, 39, 41 and 44-46 into Groups I-VI. Applicants hereby provisionally elect Group I (claims 1-4, 30, 45 and 46). In addition, Applicants elect the species Pro-CNP53 (SEQ ID NO: 185) for continued examination.

Applicants respectfully note that claim 45 should be in Group VI as the claim is directed to a composition for use in increasing long bone growth. Claims 1-4, 30 and 46 read on the elected subject matter.

Applicants request rejoinder of the non-elected method claims (Groups II-VI) upon notification of allowance of the elected composition claims.

Amendments to the Claims

Claims 1, 9, 30, 31 and 39 are amended recite particular CNP variants. The amendments include no new matter.

Conclusion

Applicants submit that the present application is in condition for allowance and respectfully request notification of the same.

This paper is accompanied by a Petition for One-Month Extension of Time and the requisite fee. The Director is hereby authorized to charge any deficiency in the fees filed to our Deposit Account No. 13-2855, under Order No. 30610/44556C1.

Dated: April 12, 2013

Respectfully submitted,

By/Katherine L. Neville/
Katherine L. Neville
Registration No.: 53,379
MARSHALL, GERSTEIN & BORUN LLP
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PTO/SB/22 (03-13)
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PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a)	Docket Number (Optional) 30610/44556C1
Application Number 13/466,672-Conf. #1368	Filed May 8, 2012
For Variants of C-Type Natriuretic Peptide	
Art Unit 1647	Examiner Shulamith H. Shafer

This is a request under the provisions of 37 CFR 1.136(a) to extend the period for filing a reply in the above-identified application.

The requested extension and fee are as follows (check time period desired and enter the appropriate fee below):

	Fee	Small Entity Fee	Micro Entity Fee	
[x] One month (37 CFR 1.17(a)(1))	$200	$100	$50	$ 200.00
[ ] Two months (37 CFR 1.17(a)(2))	$600	$300	$150	$
[ ] Three months (37 CFR 1.17(a)(3))	$1,400	$700	$350	$
[ ] Four months (37 CFR 1.17(a)(4))	$2,200	$1,100	$550	$
[ ] Five months (37 CFR 1.17(a)(5))	$3,000	$1,500	$750	$

[ ] Applicant asserts small entity status. See 37 CFR 1.27.

[ ] Applicant certifies micro entity status. See 37 CFR 1.29.
Form PTO/SB/15A or B or equivalent must either be enclosed or have been submitted previously.

[ ] A check in the amount of the fee is enclosed.

[x] Payment by credit card.

[ ] The Director has already been authorized to charge fees in this application to a Deposit Account.

[x] The Director is hereby authorized to charge any fees which may be required, or credit any overpayment, to Deposit Account Number 13-2855 .

[x] Payment made via EFS-Web.

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

I am the

[ ] applicant/inventor.

[ ] assignee of record of the entire interest. See 37 CFR 3.71. 37 CFR 3.73(b) statement is enclosed (Form PTO/SB/96).

[x] attorney or agent of record. Registration number 53,379 .

[ ] attorney or agent acting under 37 CFR 1.34. Registration number _____ .

/Katherine L. Neville/	April 12, 2013
Signature	Date
Katherine L. Neville	(312) 474-6300
Typed or printed name	Telephone Number

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. Submit multiple forms if more than one signature is required, see below*.

[ ] *Total of 1 forms are submitted.

2220551

Electronic Patent Application Fee Transmittal

Application Number:	13466672
Filing Date:	08-May-2012
Title of Invention:	Variants of C-type Natriuretic Peptide
First Named Inventor/Applicant Name:	Daniel J. Wendt
Filer:	Katherine Lynn Neville/Tiffany Sevcik
Attorney Docket Number:	30610/44556C1

Filed as Large Entity

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Extension - 1 month with $0 paid	1251	1	200	200

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
			Total in USD ($)	**200**

Electronic Acknowledgement Receipt

EFS ID:	15504947
Application Number:	13466672
International Application Number:	
Confirmation Number:	1368
Title of Invention:	Variants of C-type Natriuretic Peptide
First Named Inventor/Applicant Name:	Daniel J. Wendt
Customer Number:	90849
Filer:	Katherine Lynn Neville
Filer Authorized By:	
Attorney Docket Number:	30610/44556C1
Receipt Date:	12-APR-2013
Filing Date:	08-MAY-2012
Time Stamp:	14:58:22
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Credit Card
Payment was successfully received in RAM	$200
RAM confirmation Number	1439
Deposit Account	132855
Authorized User	NEVILLE, KATHERINE L.
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		44556C1_response_to_restriction_1.pdf	61433 6fa7b7f729f6b130ed15349a29c1afe1513c0542	yes	24
	Multipart Description/PDF files in .zip description				
	Document Description		Start	End	
	Response to Election / Restriction Filed		1	1	
	Claims		2	23	
	Applicant Arguments/Remarks Made in an Amendment		24	24	
Warnings:					
Information:					
2	Extension of Time	One_Month_Request_for_Extension_of_Time_Under_37_CFR_1136a_2.pdf	21846 f0c89eaeea901ef9fa5fbe5e514bc0ccbd2281ca	no	1
Warnings:					
Information:					
3	Fee Worksheet (SB06)	fee-info.pdf	30284 078a9801ebb9e9137ed03e55c506e965ca9fedc8	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	113563		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Exhibit D

Examiner-Initiated Interview Summary	**Application No.** 13/466,672	**Applicant(s)** WENDT ET AL.	
	Examiner SHULAMITH H. SHAFER	**Art Unit** 1647	

All participants (applicant, applicant's representative, PTO personnel):

(1) *SHULAMITH H. SHAFER*. (3)_____.

(2) *Atty K.L.Neville*. (4)_____.

Date of Interview: *23 July 2013*.

Type: ☒ Telephonic ☐ Video Conference
☐ Personal [copy given to: ☐ applicant ☐ applicant's representative]

Exhibit shown or demonstration conducted: ☐ Yes ☒ No.
If Yes, brief description: _____.

Issues Discussed ☐101 ☐112 ☐102 ☐103 ☒Others
(For each of the checked box(es) above, please describe below the issue and detailed description of the discussion)

Claim(s) discussed: *1-4,10,16,18,22-24,30,44 and 45*.

Identification of prior art discussed: *N/A*.

Substance of Interview
(For each issue discussed, provide a detailed description and indicate if agreement was reached. Some topics may include: identification or clarification of a reference or a portion thereof, claim interpretation, proposed amendments, arguments of any applied references etc...)

Examiner indicated SEQ ID NO:185, the elected species is free of the prior art. Examiner indicated will search other sequences based on CNP53, SEQ ID NOs:179,190 and 180. These have been found free of the art. Examiner will rejoin and examine appropriate method claims:7, 10 and dependents and 44. Atty Neville authorized cancellation of non-elected sequences and claims to non-elected sequences..

Applicant recordation instructions: It is not necessary for applicant to provide a separate record of the substance of interview.

Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

☐ Attachment

/SHULAMITH H. SHAFER/ Primary Examiner, Art Unit 1647	

Exhibit E

US008598121B2

(12) **United States Patent**
Wendt et al.

(10) **Patent No.: US 8,598,121 B2**
(45) **Date of Patent: Dec. 3, 2013**

(54) **VARIANTS OF C-TYPE NATRIURETIC PEPTIDE**

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Related U.S. Application Data

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(51) **Int. Cl.**

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(52) **U.S. Cl.**

USPC **514/12.4**; 514/16.7; 514/21.3; 435/69.4; 435/69.7; 435/70.1; 530/324

(58) **Field of Classification Search**

None

See application file for complete search history.

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(57) **ABSTRACT**

The present disclosure provides variants of C-type natriuretic peptide (CNP), pharmaceutical compositions comprising CNP variants, and methods of making CNP variants. The CNP variants are useful as therapeutic agents for the treatment of diseases responsive to CNP, including but not limited to bone-related disorders, such as skeletal dysplasias (e.g., achondroplasia), and vascular smooth muscle disorders (e.g., restenosis and arteriosclerosis).

14 Claims, 61 Drawing Sheets

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What is claimed is:

1. A variant of C-type natriuretic peptide (CNP) selected from the group consisting of:

(SEQ ID NO: 179)
GDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMS GLGC (Gly-CNP53);

(SEQ ID NO: 185)
PDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMS GLGC (Pro-CNP53);

(SEQ ID NO: 190)
MDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMS GLGC (Met-CNP53)

(SEQ ID NO: 180)
DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNS GLGC [CNP-53(M48N)].

2. A pharmaceutical composition comprising a CNP variant of claim 1, and a pharmaceutically acceptable excipient, carrier or diluent.

3. The composition of claim 2, which is a lyophilized formulation prepared from a formulation that comprises a citric acid/citrate buffer or an acetic acid/acetate buffer having a pH from about 4 to about 6.

4. The composition of claim 3, wherein the lyophilized formulation is prepared from a formulation that further comprises (a) an isotonicity-adjusting agent or a bulking agent or (b) an antioxidant.

5. A method of treating a bone-related disorder or skeletal dysplasia, comprising administering a CNP variant to a subject in need thereof, wherein the CNP variant is a CNP variant according to claim 1, and wherein the bone-related disorder or skeletal dysplasia is selected from the group consisting of achondroplasia, hypochondroplasia, short stature, dwarfism and homozygous achondroplasia.

6. A method for recombinant production of a CNP variant, comprising culturing in a medium a host cell comprising a first polynucleotide encoding a CNP variant polypeptide linked to a second polynucleotide encoding a cleavable peptide or protein under conditions that result in expression of a fusion polypeptide encoded by the polynucleotides, wherein the fusion polypeptide comprises the CNP variant polypeptide directly linked to the cleavable peptide or protein or indirectly linked thereto via a linker, wherein the CNP variant is selected from the group consisting of:

(SEQ ID NO: 179)
GDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMS GLGC (Gly-CNP53);

(SEQ ID NO: 185)
PDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMS GLGC (Pro-CNP53);

(SEQ ID NO: 190)
MDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMS GLGC (Met-CNP53);

(SEQ ID NO: 180)
DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNS GLGC [CNP-53(M48N)].

7. The method of claim 6, wherein the cleavable peptide or protein is selected from the group consisting of histidine tags, human transcription factor TAF12, TAF12 fragments, TAF12 histone fold domain, mutants of TAF12 and fragments thereof, TAF12(C/A), TAF12(D/E), TAF12(4D/4E), TAF12 (6D/6E), TAF12(10D/10E), TAF12(C/A & D/E), TAF12 (C/A & 4D/4E), TAF12(C/A & 6D/6E), TAF12(C/A & 10D/10E), ketosteroid isomerase, maltose-binding protein, β-galactosidase, glutathione-S-transferase, thioredoxin, chitin-binding domain, BMP-2, BMP-2 mutants, BMP-2(C/A), and mutants and fragments thereof.

8. The method of claim 6, wherein the host cell is bacterial.

9. The method of claim 6, wherein the fusion polypeptide is expressed as a soluble protein or as an inclusion body.

10. The method of claim 6, further comprising isolating the expressed fusion polypeptide from the host cell or culture medium.

11. The method of claim 10, further comprising contacting the isolated fusion polypeptide with a cleaving agent selected from the group consisting of formic acid, cyanogen bromide (CNBr), hydroxylamine, protein self cleavage, Factor Xa, enterokinase, ProTEV, and SUMO protease.

12. A CNP variant produced according to the method of claim 6, wherein the CNP variant is selected from the group consisting of:

(SEQ ID NO: 179)
GDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMS
GLGC (Gly-CNP53));

(SEQ ID NO: 185)
PDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMS
GLGC (Pro-CNP53));

(SEQ ID NO: 190)
MDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMS
GLGC (Met-CNP53));

(SEQ ID NO: 180)
DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSG
LGC [CNP-53(M48N)]).

13. A method of increasing long bone growth, comprising administering a CNP variant according to claim 1 to a subject in need thereof, where the administration increases long bone growth.

14. A CNP variant according to claim 1 useful for increasing long bone growth or treating achondroplasia, hypochondroplasia, short stature, dwarfism, or homozygous achondroplasia in a subject in need thereof.

* * * * *

AMENDMENTS TO THE CLAIMS

1-43. (Canceled)

44. (New) A variant of C-type natriuretic peptide (CNP) comprising the amino acid sequence LQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (SEQ ID NO: 160).

45. (New) The CNP variant of claim 44 further comprising a synthetic polymeric group.

46. (New) The CNP variant of claim 45 wherein the synthetic polymeric group comprises a hydrophilic polymeric moiety.

47. (New) The CNP variant of claim 46 wherein the hydrophilic polymeric moiety comprises polyethylene glycol (PEG).

48. (New) The CNP variant of claim of 46 wherein the hydrophilic polymeric moiety is coupled to the amino acid sequence through a stable linkage.

49. (New) The CNP variant of claim of 46 wherein the hydrophilic polymeric moiety is coupled to the amino acid sequence through a hydrolysable linkage.

50. (New) A pharmaceutical composition comprising a variant of C-type natriuretic peptide (CNP) consisting of the amino acid sequence LQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (SEQ ID NO: 160) as the active agent.

51. (New) The pharmaceutical composition of claim 50 further comprising a pharmaceutically acceptable excipient, carrier, or diluent.

52. (New) A method of treating a bone-related disorder or skeletal dysplasia, comprising administering the pharmaceutical composition according to claim 50 to a subject, wherein the administering treats the bone-related disorder or skeletal dysplasia.

53. (New) The method of claim 52 wherein the bone-related disorder or skeletal dysplasia is achondroplasia and wherein administration treats achondroplasia.

54. (New) A method of increasing long bone growth comprising administering the pharmaceutical composition according to claim 50 to a subject in need thereof, wherein the administering increases long bone growth.

55. (New) A method of increasing long bone growth comprising contacting chondrocytes of a subject in need thereof with an effective amount of a CNP variant having the amino acid sequence LQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (SEQ ID NO: 160).

56. (New) The method of claim 55 wherein the chondrocytes are located within a bone growth plate of the subject.

57. (New) The method of claim 55 wherein the CNP variant binds to and activates natriuretic peptide receptor B (NPR-B) on the chondrocytes.

58. (New) A method of treating a bone-related disorder or skeletal dysplasia comprising contacting chondrocytes of a subject in need thereof with an effective amount of a CNP variant having the amino acid sequence LQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (SEQ ID NO: 160).

59. (New) The method of claim 58 wherein the chondrocytes are located within a bone growth plate of the subject.

60. (New) The method of claim 58 wherein the CNP variant binds to and activates natriuretic peptide receptor B (NPR-B) on the chondrocytes.

61. (New) The method of claim 58 wherein the bone-related disorder or skeletal dysplasia is achondroplasia.

EXHIBIT G

The opinion in support of the decision being entered today is *not* binding precedent of the Board.

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

Ex parte JOACHIM WERRES

Appeal 2006-2966
Application 09/148,152[1]
Technology Center

Decided: July 3, 2007

Before BRADLEY R. GARRIS, Administrative Patent Judge,
FRED E. MCKELVEY, Senior Administrative Patent Judge, and
ALLEN R. MACDONALD, *Administrative Patent Judge.*

GARRIS, *Administrative Patent Judge.*

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[1] Application filed September 3, 1998, for reissue of U.S. Patent No. 5,656,177, which issued August 12, 1997 from Application No. 08/352,097, filed November 30, 1994.

DECISION ON APPEAL

Statement of the Case

This is an appeal under 35 U.S.C. § 134 from the Examiner's final rejection of claims 1-39 in the above-identified reissue application. We have jurisdiction under 35 U.S.C. § 6(b).

We AFFIRM.

Appellant claims a method of decreasing microbial growth and preventing slime formation in an aqueous medium which comprises incorporating into the aqueous medium an effective microbial growth retarding and slime formation preventing amount of an oil-in-water emulsion. Reissue claim 1, which corresponds to Patent claim 1, defines the method in terms of the species elected without traverse during patent prosecution. Reissue claim 10 defines the method in terms which are generic to the elected species. These claims adequately represent the claimed subject matter on appeal and are reproduced below:

1. A method of decreasing microbial growth and preventing slime formation in an aqueous medium comprising the step of incorporating in said aqueous medium an effective microbial growth retarding and slime formation preventing amount of an oil-in-water emulsion comprising an oil phase and at least one emulsifier for the oil phase, wherein the oil phase comprises saturated or unsaturated, open-chain, normal or isomeric hydrocarbons containing 8 to 30 carbon atom [sic]; and wherein said emulsion is free of toxic microbiocides.

10. A method of decreasing microbial growth and preventing slime formation in an aqueous medium comprising the step of incorporating in said aqueous medium an effective microbial growth retarding and slime formation preventing amount of an oil-in-water emulsion comprising an oil phase and at least one emulsifier for the oil phase, wherein the oil phase comprises at least one substance selected from the group consisting of:

(i) saturated or unsaturated, open-chain or cyclic, normal or isomeric hydrocarbons containing 8 to 30 carbon atoms;

(ii) saturated or unsaturated fatty alcohols, saturated or unsaturated fatty acids, fatty acid monoalkyl esters, fatty acid amides, or monoalkyl amides of saturated or unsaturated fatty acids, wherein the fatty alcohol, fatty acid or fatty acid residue contains 8 to 30 carbon atoms;

(iii) monoesters or polyesters of saturated or unsaturated fatty acids containing 4 to 30 carbon atoms and monoalcohols or polyols except for polyethylene glycols;

(iv) polyamides of saturated or unsaturated fatty acids containing 8 to 30 carbon atoms and aliphatic polyamines containing 2 to 6 nitrogen atoms;

(v) acyclic, monocyclic or bicyclic terpenes; and

(vi) polyoxyalkylene compounds formed from alkylene oxides and at least one substance selected from the group consisting of C_{12}-C_{18} fatty alcohols, C_{12}-C_{18} fatty acids and glycerides of C_{12}-C_{18} fatty acids;

wherein when the aqueous medium is a water piping or a cooling or heating cycle or an industrial process water as a water carrying system and a substance of group (v) is used, said substance of group (v) is always used in admixture with at least one substance of the groups (i) – (iv) or (vi), and wherein said emulsion is free of toxic microbiocides.

Claims 1-39 are rejected under 35 U.S.C. § 251 as being based upon a defective reissue declaration. According to the Examiner, the declaration is defective "because none of the errors which are relied upon to support the reissue application are errors upon which a reissue can be based" (Final Office Action, mailed Oct. 7, 1999, 2).

Claims 10-39 are rejected under 35 U.S.C. § 251 "as being an improper recapture of claimed subject matter deliberately canceled in the application for the patent upon which the present reissue is based" (*id.* at 3).

The Issues

(1) Regarding claims 1-39, is the Reissue Declaration of record defective for failing to establish an error that is correctable by reissue under 35 U.S.C. § 251?

(2) Do claims 10-39 violate the recapture rule by reciting subject matter that was deliberately surrendered in an effort to obtain allowance of the '177 patent claims?

The Reissue Declaration

The Reissue Declaration, filed November 16, 1998, presents two errors in support of reissue. These errors and the undisputed facts which engendered the errors are copied below from pages 1-2 of the Declaration.

1. Through error and without deceptive intent, the patent was allowed to issue without a generic claim. The facts are as follows:

> a. On October 4, 1995, the Examiner issued an Action making two election of species requirements, the first being a choice from among 19 defined "species" of compounds and the second being a choice from among six defined "species" of uses;
>
> b. On January 3, 1996, a provisional election was made;
>
> c. On June 28, 1996, an Amendment was filed in response to an Office Action dated April 9, 1996, inserting a number of claims, including a generic claim numbered "17".

d. A final rejection issued September 23, 1996, and a response was filed December 23, 1996.

e. On January 17, 1997, the Examiner faxed a proposed amendment to my attorney, proposing to amend Claim 17 to limit it only to the elected species. *That proposal was erroneously agreed to.* [Emphasis added.] The Examiner then entered an Examiner's Amendment making the proposed changes and allowed the claims. The patent issued on August 12, 1997.

f. Since the Examiner found the elected "species" allowable, the generic claims were then *per se* allowable unless and until the Examiner would conduct a further search of the remaining "species". Considering the number of permutations of the denoted "species" (19 x 6 = 114), it was error to have agreed to the cancellation of the generic claims [sic].

2. Through error and without deceptive intent, references cited in a Search Report dated July 14, 1994, from the German Patent Office in a corresponding German application were not brought to the attention of the U.S. Examiner. The facts, as established by a Declaration of Dr. Gerald Kloepsch accompanying this Reissue Declaration, are as follows:

a. The Search Report from the German Patent Office was mailed July 14, 1994, and received July 21, 1994. As was Dr. Kloepsch's practice, he reported the German Search Report to his client. As was the usual practice in his office, since no response to a Search Report is necessary, the Search Report was merely placed in the respective file, to wait for an Action on the merits.

b. The application Dr. Kloepsch prepared for filing in other countries was more detailed and of a different scope then [sic] that of the German application. It was his opinion at that time that the references cited in the German Search Report were not material to the scope of the coverage to be obtained in the other countries.

c. U.S. Application serial no. 08/352,097 was filed on November 30, 1994, about four months after receipt of the German Search Report. At that time, the issuance of the German Search report was forgotten.

d. During the prosecution of the U.S. application, Dr. Kloepsch sent to his U.S. associates the Search Report of the PCT Application identical in scope with the U.S. application. The references of the PCT Search Report were brought to the U.S. Examiner's attention in an IDS of January 3, 1996. But, apparently, Dr. Kloepsch still did not remember the German Search report so the references cited in it were not brought to the attention of the U.S. Examiner.

e. At least some of the references cited in the German Search Report may be considered material to the patentability of the generic claims of the U.S. application [i.e., the subject reissue application].

Principles of Law

Regarding Issue (1):

Under 35 U.S.C. § 251 (1952), reissue is permissible "[w]henever any patent is, through error without any deceptive intention, deemed totally or partly inoperative or invalid, by reason of a defective specification or drawing, or by reason of the patentee claiming more or less than he had a right to claim in the patent."

The deliberate action on an inventor or attorney during prosecution generally fails to qualify as a correctible error under § 251. *In re Serenkin*, 479 F.3d 1359, 1362, 81 USPQ2d 2011, 2014 (Fed. Cir. 2007). There is no correctable § 251 error when an inventor or attorney deliberately agrees with an Examiner's requirement that non-elected claims be prosecuted in a divisional application rather than in the application containing the elected

claims. *In re Orita*, 550 F.2d 1277, 1280, 193 USPQ 145, 148 (CCPA 1977). This is so regardless of the propriety of the requirement. *In re Watkinson*, 900 F.2d 230, 231-32, 14 USPQ2d 1407, 1408-09 (Fed. Cir. 1990).

Regarding Issue (2):

The recapture rule prevents a patentee from regaining through reissue the subject matter that the patentee/applicant surrendered (e.g., via claim amendment) in an effort to obtain allowance of the original patent claims. *In re Clement*, 131 F.3d 1464, 1468, 45 USPQ2d 1161, 1164 (Fed. Cir. 1997). However, the recapture rule does not apply in the absence of evidence that the applicant's amendment was an admission that the scope of the amended claim was not in fact patentable. *Id.* In determining whether surrender of subject matter has occurred, the proper inquiry is whether an objective observer viewing the prosecution history would conclude that the purpose of the applicant's amendment (or argument) was to overcome the prior art and secure the patent. *Kim v. Conagra Foods, Inc.*, 465 F.3d 1312, 1323, 80 USPQ2d 1495, 1502 (Fed. Cir. 2006).

Analysis of The Defective Reissue Declaration Rejection

Reissue Declaration Error 1

In the Reissue Declaration, the Appellant states that, through error and without deceptive intent, the '177 patent was allowed to issue without a generic claim (Declaration 1, Item 1). The undisputed facts presented in support of this statement reveal that the asserted "error" was occasioned by deliberate actions on the part of Appellant (or his attorney).

These deliberate actions included agreement with the Examiner's requirement for an election of species to be prosecuted in the '177 patent application with non-elected species to be prosecuted in divisional applications (id. at Items 1.a. and 1.b.).[2] Similarly, Appellant (or his attorney) deliberately agreed to an Examiner's amendment that limited or narrowed generic claim 17 to the elected species only (id. at Item 1.e.).

A correctable § 251 error does not include these deliberate decisions by Appellant whereby the '177 patent was allowed to issue with elected species claims only (i.e., without a generic claim). *Orita,* 550 F.2d at 1280, 193 USPQ at 148. These deliberate choices do not comprise error correctable by reissue regardless of the propriety of the election requirement or claim amendment made by the Examiner. *Watkinson*, 900 F.2d at 231-32, 14 USPQ2d at 1408-09. There is a distinction between a § 251 error and a deliberate choice subsequently found to be disadvantageous. *Serenkin*, 479 F.3d at 1364, 81 USPQ2d at 2015.

Appellant cites *Ex parte Holt*, 214 USPQ 381 (Bd. App. 1982), "as standing for the proposition that failure to take other remedial action, such as filing a divisional or continuation application, should not adversely affect an applicant's right to reissue" (Supple. Appeal Brief, filed May 22, 2007, 1; *see also* Revised Appeal Brief, filed April 25, 2000, (hereinafter Br.) 10). *Holt* involved an improper rejection of a Markush claim under 35 U.S.C. § 121. The Board found that applicant's failure to recognize the impropriety

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[2] It might be presumed that no such divisional applications were filed and that such non-filing occurred by inadvertence and without deceptive intent as in *Orita*, 550 F.2d 1277, 193 USPQ 145. However, the record is silent on these matters.

of the rejection was a correctable error under § 251 and that applicant's failure to file a timely divisional application in response to the improper rejection should not foreclose § 251 relief.

Interestingly, *Holt* was similarly relied on by the appellant in *Watkinson*. As explained in *Watkinson*, 900 F.2d at 232, 14 USPQ2d at 1409, there is a legally significant difference between the rejection in *Holt* and a restriction requirement. That is, a restriction requirement is "proper" under 35 U.S.C. § 121, whereas the rejection of a Markush claim is not. *Id.* Moreover, the merit of a restriction requirement is not reviewable on appeal since the requirement is a matter within an Examiner's discretion and not tantamount to a rejection of claims. *Id.*, 900 F.2d at 233, 14 USPQ2d at 1409. For these reasons, the court in *Watkinson* found *Holt* inapplicable to the facts of that appeal.

The facts of the *Watkinson* appeal parallel the facts of the subject appeal. The applicants in both cases acquiesced in a restriction or election requirement, canceled (or permitted cancellation of) the non-elected claims and accepted issuance of a patent containing elected claims only. Therefore, we likewise find *Holt* inapplicable to the facts of this appeal.

In an apparent attempt to analogize the facts of this appeal to those of *Holt*, Appellant argues that the election of species requirement "results in 114 permutations of combinations of 'species' [, and] [t]he result is a *de facto* rejection of Claim 17 resulting in a denial of generic coverage such that Applicant could not be made whole through the filing of multiple applications to each species" (Br. 12). Thus, Appellant argues that the "cancellation" of generic claim 17 (i.e., amendment of claim 17 to limit it to

elected species only) was not in response to an election of species requirement but rather was in response to a *de facto* rejection of a generic claim (Br. 11).

This argument is unpersuasive. The Examiner's election of species requirement can not be properly considered a *de facto* rejection which is analogous to the improper Markush rejection in *Holt.* Indeed, as expressly stated in *Watkinson*, 900 F.2d at 233, 14 USPQ2d at 1409, "a restriction requirement is a matter within the discretion of the Examiner and not tantamount to a rejection of claims."

As further support for his position, Appellant cites *In re Doyle*, 293 F.3d 1355, 1358, 63 USPQ2d 1161, 1163 (Fed. Cir. 2002), wherein the court determined that failure to present a so-called linking claim (i.e., a generic claim broad enough to read on or link two or more groups of claims subject to a restriction requirement) is an error correctable by reissue. (Further Supple. Br., filed July 1, 2002, 1-2; Reply to Supple. Examiner's Answer, filed July 11, 2006, 3-4). According to Appellant, "[t]he facts of the present case fall into the situation described by the court in *Doyle* [since] [t]he new claims in the instant reissue application are generic, or "linking," claims which are broader than any nonelected claims and could have been prosecuted with the elected claims" (Further Supple. Br. 2). As a matter of clarification, we point out that the "new claims" referred to by Appellant are newly added reissue claims 10-39 and that Appellant characterizes independent reissue claim 10 as a generic claim corresponding to generic claim 17 of the '177 patent application (Br. 6-7 and 23-24 (Appendix II)).

The Appellant is incorrect in stating that "[t]he facts of the present case fall into the situation described by the court in Doyle" (Further Supple. Br. 2). As a basis for its holding in *Doyle*, the court repeatedly emphasized that the generic or linking claims of Dr. Doyle's reissue application had never been submitted for prosecution in the original patent application. *See Doyle*, 293 F.3d at 1358, 63 USPQ at 1164 ("Dr. Doyle simply seeks a broadening reissue of his '595 patent to cover material that he invented and disclosed, but inadvertently *failed to claim* in his issued patent;" emphasis added) and 293 F.3d at 1360, 63 USPQ at 1165 ("the applicant [Dr. Doyle] never asserted the reissue claims or anything similar to them in his original application").

In contrast, Appellant submitted generic claim 17 for prosecution in the '177 patent application and deliberately authorized an amendment by the Examiner of the claim to limit or narrow its coverage to the elected species only. By now seeking reissue of appealed generic claim 10 which corresponds to former generic claim 17, the Appellant clearly is attempting to retract his deliberate decision made during prosecution of the '177 patent application. This deliberate decision is not an error that is correctable under § 251. *Serenkin*, 479 F.3d at 1362-63, 81 USPQ2d at 2014.

For the above-stated reasons, the facts presented in the Reissue Declaration do not establish that issuance of the '177 patent without a generic claim was due to an error which can be corrected by reissue under 35 U.S.C. § 251.

Reissue Declaration Error 2

As an additional reissue error, Appellant states: "Through error and without deceptive intent, references cited in a Search Report dated July 14, 1994, from the German Patent Office in a corresponding German application were not brought to the attention of the US Examiner" (Decl. 2, Item 2). Concerning the relevancy of these references, Appellant states only that "[a]t least some of the references cited in the German Search Report may be considered material to the patentability of the generic claims of the US application [i.e., the subject reissue application]" (Decl. 2, Item 2.e). Indeed, Appellant acknowledges that these references would not raise a substantial new question of patentability of the patented claims but urges that they would have been relevant to generic claim 17 of the original '177 patent application and are relevant to generic claim 10 of this reissue application (Br. 6).

As previously explained, § 251 permits the reissue of defective patents "[w]henever any patent is, through error without any deceptive intention, deemed wholly or partly inoperative or invalid, by reason of a defective specification or drawing, or by reason of the patentee claiming more or less than he had a right to claim in the patent" (1952). Here, the failure to bring the above-noted references to the attention of the US Examiner has not rendered the '177 patent defective in any perceptible way. As the Appellant has acknowledged, these references do not raise a substantial new question of patentability of the '177 patent claims. Furthermore, it is simply irrelevant to Appellant's request for reissue under

§ 251 that these references may be pertinent to other claims such as former generic claim 17 and current generic reissue claim 10.

As support for his belief that the above discussed failure is correctable by reissue, Appellant cites *In re Harita*, 847 F.2d 801, 6 USPQ2d 1930 (Fed. Cir. 1988). However, unlike the circumstances before us, the references cited in the reissue application of *Harita* rendered at least some of Harita's patent claims invalid. *Id.*, 847 F.2d at 802, 6 USPQ2d at 1930. Although the reissue claims patentably distinguished over this prior art, they were subjected to an "inequitable conduct" rejection which was reversed. *Harita*, 847 F.2d at 802, 6 USPQ2d at 1930. Because these facts are completely dissimilar to those before us, the Appellant's position is not supported by *Harita*.

Summary of Analysis of Defective Reissue Declaration Rejection

For the above-stated reasons, the Reissue Declaration of record is defective in that it fails to present any error which is correctable by reissue under 35 U.S.C. § 251.

Analysis of The Recapture Rejection

This rejection of newly added reissue claims 10-39 is based on the Examiner's determination that generic reissue claim 10 is an attempt by Appellant to recapture subject matter which was surrendered when Appellant authorized the Examiner to amend generic claim 17 of the '177 patent application to narrow its coverage to the elected species only. However, there is no evidence that Appellant authorized this narrowing

amendment in order to avoid a prior art (or any other kind of) rejection, and the Examiner does not argue otherwise. Instead, the Examiner believes his recapture rejection is proper simply because Appellant authorized the Examiner to amend claim 17 so as to remove from its coverage subject matter generic to the elected species.

The Examiner's theory for this rejection is not well-taken. As explained earlier, the recapture rule does not apply in the absence of evidence that a claim amendment was an admission that the scope of the unamended claim was not in fact patentable. *Clement,* 131 F.3d at 1468, 45 USPQ2d at 1164. Here, there is no evidence that Appellant's authorization of the Examiner's amendment was an admission that the scope of generic claim 17 was not patentable. Additionally, the Examiner points to nothing and we find nothing in the prosecution history of the '177 patent application which would lead an objective observer to conclude that the purpose of the claim 17 amendment was to overcome prior art and secure the patent. In the absence of such prosecution history, no basis exists for determining that the subject matter defined by the reissue claims had been surrendered during prosecution of the original application. *Kim v. Conagra Foods, Inc.*, 465 F.3d at 1323, 80 USPQ2d at 1502.

Conclusions of Law

(1) With respect to claims 1-39, the Appellant has failed to establish on this record any error which is correctable by reissue under 35 U.S.C. § 251.

(2) With respect to claims 10-39, the Examiner has failed to establish on this record that these claims recapture subject matter which was surrendered by Appellant during prosecution of the '177 patent application.

Order

The rejection of claims 1-39 under 35 U.S.C. § 251 based upon a defective reissue declaration is AFFIRMED.

The rejection of claims 10-39 under 35 U.S.C. § 251 based on recapture is REVERSED.

No time period for taking any subsequent action in connection with this appeal may be extended under 37 C.F.R. § 1.136(a)(iv)(effective Sept. 13, 2004).

AFFIRMED

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UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

Ex parte EDWARD BEVERLY MORRISON,
PETR VALENTINOVICH KUZMIN, and PAVEL TISHIN

Appeal 2012-012266
Application 12/827,650
Patent 7,157,914 B2
Technology Center 2800

Before MAHSHID D. SAADAT, DENISE M. POTHIER,
and JEFFREY S. SMITH, *Administrative Patent Judges*.

SAADAT, *Administrative Patent Judge*.

DECISION ON APPEAL[1]

Appellants appeal under 35 U.S.C. § 134(a) from the rejection of claims 28-36 as lacking an error correctable by reissue. We have jurisdiction under 35 U.S.C. § 6(b).

We affirm.

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[1] An Oral Hearing for this appeal was held on February 4, 2013.

STATEMENT OF THE CASE

Introduction

Appellants' invention relates to a Time Domain Electromagnetic (TDEM) surveying method having improved sensor resolution. (*See* Spec. col. 3, ll. 25-26).

Exemplary Claim

> 28. A method for providing a tow assembly for an electromagnetic airborne survey system at an assembly location, comprising:
>
> providing a plurality of hollow transmitter support frame sections;
>
> providing a transmitter coil for generating a primary magnetic field;
>
> providing connector cables and a tow cable; and
>
> assembling the transmitter support frame sections and the transmitter coil and the cables to provide a tow assembly having a flexible hollow transmitter support frame that is circular or approximates a circle having the transmitter coil disposed within the hollow transmitter support frame and formed from serially connecting the transmitter support frame sections, with the connector cables each having a first end connected to a respective location on a circumference of the transmitter support flame and a second end connected to a first end of die tow cable.

The Rejection

Claims 28-36 stand rejected under 35 U.S.C. § 251 for lack of defect or error in the original patent and as not being an error correctable by reissue. (*See* Ans. 5-6).

FINDINGS OF FACT

Prosecution History

1. Appellants filed U.S. Application No. 10/716,813 ("813 Application") on Nov. 20, 2003.

2. On May 8, 2006, the Examiner required restriction to three patentably distinct groups of claims as follows:

> I. Claims 1, 3-10, 12, 13, 15-28, 32, and 33, drawn to a method and apparatus using a tow assembly, the assembly comprising a flexible frame, the receiver flexibly connected to a receiver support frame and a dual mode gain amplifier, classified in class 324, subclass 330.
>
> II. Claim 29, drawn to a computer program comprising a computer readable medium and instructions to operate a general electromagnetic antenna assembly, classified in class 702, subclass 5.
>
> III. Claims 30, 31, 34, and 35, drawn to trailing airborne surveying systems having specific antenna structures, classified in class 343, subclass 707.

3. On August 18, 2006, Appellants filed a response to the Examiner's Restriction Requirement and elected to prosecute the claims of group I. Appellants did indicate that "[t]his election/restriction is made by the Applicant without prejudice to the right to file one or more divisional applications comprising the subject matter of species (II) or (III)." However, no such divisional application was filed.

4. On January 2, 2007, Application 10/716,813 issued as U.S. Patent 7,157,914 ("914 Patent") including claims 1, 3-10, 12, 13, 15-28, 32, and 33 (renumbered as claims 1-27).

5. On December 23, 2008, Appellants filed reissue U.S. Application No. 12/343,321 ("Reissue Application").

6. In U.S. Application No. 12/343,321, claims 1-27 were claims 1, 3-10, 12, 13, 15-28, 32, and 33 of 813 Application and newly added claims 28-33 were directed to a method for providing a tow assembly.

7. On December 10, 2009, the Examiner required restriction to one of the following two inventions:

> I. Claims 1-27, drawn to a method and apparatus for airborne electromagnetic surveying and circuitry therefor, classified in class 324, subclass 330.
>
> II. Claim 28-33, drawn to an antenna for a tow assembly, classified in class 343, subclass 707.

8. On March 10, 2010, Appellants filed a response to the Examiner's Restriction Requirement and elected to prosecute the claims of group I.

9. On June 30, 2010, Appellants filed (as a divisional of Application 12/343,321) reissue U.S. Application No. 12/827,650 containing claims 28-36 alleging error as follows:

> in overlooking embodiments of my invention which pertain to a method for providing a tow assembly for an electromagnetic airborne survey system at an assembly location, comprising: providing a plurality of transmitter support frame sections, a transmitter coil for generating a primary magnetic field, connector cables and a tow cable and assembling the transmitter support frame sections and the transmitter coil and the cables to provide a tow assembly having a transmitter support frame that is circular or approximates a circle bearing the transmitter coil and formed from serially connecting the transmitter support flame sections, with the connector cables each having a first end connected to a respective location on a circumference of the transmitter support frame and a second end connected to a first end of the tow cable.

10. On August 24, 2011, the Examiner finally rejected claims 28-36 under 35 U.S.C. § 251 as being improper for reissue.

11. On February 23, 2012, Appellants filed an appeal of the Examiner's final rejection.

The Examiner's Rejection

12. The Examiner found that a restriction requirement had been made during prosecution of application for the 914 Patent between Groups I, II, and III, where Appellants elected without traverse to prosecute the invention of claims Group I including claims 1, 3-10, 12, 13, 15-28, 32, and 33 of the 813 Application and "never filed any divisional applications covering such non-elected species" (Ans. 5).

13. The Examiner found further that

> The claims of the present divisional reissue application read on or are broader versions of the claims in Group III (claims 30, 31, 34 and 35) of the '813 application and thus cover the primary features of the Group, i.e., the specific features of the antenna structures of the towed assembly. Alternatively, *if these divisional reissue claims were presented in the '813 application during prosecution thereof, they would also have been subject to the same restriction by either being placed in Group III or in a separate group* for similar reasons for restriction from Group I. Thus, the present claims should have been presented in a divisional application to the parent application.

(Ans. 5-6) (Emphasis added).

14. The Examiner concluded that "failure of Applicants to file a divisional application during the pendency of the '813 application (which later issued to the '914 patent) does not amount to an error correctable by reissue" (Ans. 6).

Issues on Appeal

1. Is the failure to present claims 28-36 during the prosecution of the 914 Patent an error that can be corrected by reissue?

2. Are appealed claims 28-36 of reissue U.S. Application No. 12/827,650 substantially identical to claims of the non-elected groups identified in the Examiner's restriction requirement during the prosecution of the 914 Patent?

PRINCIPLES OF LAW

The section of the patent statute providing for correction of errors in a patent by reissue of the patent reads, in relevant part:

> Whenever any patent is, through error, deemed wholly or partly inoperative or invalid . . . by reason of the patentee claiming more or less than he had a right to claim in the patent, the Director shall, on the surrender of such patent and the payment of the fee required by law, reissue the patent for the invention disclosed in the original patent.

35 U.S.C. § 251, first paragraph.

"[T]he whole purpose of the [reissue] statute, so far as claims are concerned, is to permit limitations to be added to claims that are too broad or to be taken from claims that are too narrow." *In re Weiler*, 790 F.2d 1576, 1580 (Fed. Cir. 1986) (quoting *In re Handel*, 312 F.2d 943,948 (CCPA 1963)).

The "*Orita* doctrine" precludes applicants from obtaining by reissue claims which, because of a requirement for restriction in which they had acquiesced, they could not claim in their patent. *In re Orita,* 550 F.2d 1277, 1280 (CCPA 1977).

When applicants acquiesce in the Examiner's requirement for restriction, such action manifestly is not "error" causing patentee to claim "less than he had a right to claim in the patent" in the language of 35 U.S.C. § 251. *Id.* 550 F.2d at 1280.

The so-called *Orita* doctrine precludes reissue applicants from obtaining substantially identical claims to those of non-elected groups identified in an Examiner's restriction requirement when such claims could not have been prosecuted in the application from which they were restricted. *In re Doyle,* 293 F.3d 1355, 1359 (Fed. Cir. 2002).

ANALYSIS

We have reviewed the Examiner's rejection in light of Appellants' contentions that the Examiner has erred. We disagree with Appellants' conclusions. We agree with the Examiner that with respect to claims 28-36 on appeal, the patent resulting from the prosecution of the 813 Application was error-free.

Appellants argue that the Examiner erred in failing to recognize that the present claims "to the 'method for providing a tow assembly,' which were never part of the subject matter claimed during the prosecution of the original '914 patent . . . [and] were never held to be patentably distinct during the prosecution of the '813 application" (App. Br. 5). Appellants conclude that, contrary to the decision in *Weiler*, 790 F.2d at 1579, failure to file a divisional application to the non-elected Groups in the original '813 application has no bearing on the present reissue claims which were "not 'non-elected' in that application" (*id.*).

According to Appellants (App. Br. 11), "in the instant reissue application, claims to an overlooked invention were never filed in the original application," and therefore, relate to a situation different from that of *Orita*, 550, F.2d at 1280 (the failure to file a timely divisional application to non-elected subject matter is not error correctable by reissue). Moreover,

Appellants argue that "*Orita* does not address the situation in which an invention that was fully disclosed in an original application for patent . . . was overlooked by the applicant through error without deceptive intent" (Reply Br. 11).

We disagree. The critical issue is the relation of the subject matter of newly presented claims 28-36 to the subject matter covered by Groups I - III of the restriction requirement of the 813 Application. Contrary to Appellants' arguments that appealed claims 28-36 "could never be a part of any restriction requirement promulgated in that original application, because there were no claims to that invention" (*id.*), appealed claims 28-36 are substantially identical claims to those of nonelected Group III (claims 30, 31, 34, and 35) identified in the Examiner's restriction requirement of the 813 Application. As stated above, the reissue claims asserted in *In re Orita* did not just read on nonelected subject matter, but rather were "substantially identical to those non-elected in [the] application." *Orita*, 550 F.2d at 1280. Therefore, presently appealed claims 28-36 could not have been prosecuted in the 813 Application from which their invention (Group III, including claims 30, 31, 34, and 35) was restricted.

In the following table, we compare the subject matter of claim 28 of the present reissue Application to the subject matter of claim 34 of the 813 Application, grouped in the non-elected Group III. We note the nominal recitation of "a method for providing" followed by steps of "providing" and "assembling" in claim 28 of the present application, but see no substantial difference with respect to the scope and the structural features between the claims.

	Reissue Application No. 12/827,650 Claim 28	Application No. 10/716,813 Non-Elected Claim 34
1	A method for providing a *tow assembly for* an electromagnetic *airborne survey system* at an assembly location, comprising:	A *tow assembly apparatus for airborne* time domain electromagnetic *surveying* comprising: . . . the transmitter section can be assembled and disassembled . . .
2	providing *a plurality of hollow transmitter support frame sections*; . . . to provide *a tow assembly having a flexible hollow transmitter support frame . . .*	(a) A *flexible support frame* including: (i) *A transmitter section* having a central axis and *consisting of a plurality of interconnectable transmitter section frame members*, such that the transmitter section can be assembled and disassembled, and the transmitter section frame members define a polygonal profile; and
3	providing *a transmitter coil* for generating a primary magnetic field;	Wherein the transmitter section houses an elastically suspended *transmitter coil*
4	providing *connector cables* and a tow cable;	Wherein the transmitter section and the receiver section arc connected by means of *a plurality of connecting cables*
5	with *the connector cables each having a first end connected to a respective location on a circumference of the transmitter support frame and a second end connected to a first end of the tow cable.*	Wherein *the tow assembly apparatus is connected to the aircraft by means of at least one cable means connected to the transmitter section at a plurality of points*;

A comparison of the subject matter of method claim 28 of the present reissue Application 12/827,650 to the subject matter of apparatus claim 34 of the 813 Application leads us to a similar invention in that there is no substantial difference between the claims even though they are directed to different statutory categories. In this case, these claims are simply not patentably distinct. Under *Orita*, of course, there is no correctable error in failing to prosecute divisional applications on inventions of the non-elected groups identified by the Examiner in the original restriction requirement.

We also disagree with Appellants that failure to file a divisional application to the non-elected Groups in the original 813 Application has no bearing on the present reissue claims which were "not 'non-elected' in that application" (App. Br. 5). That is, had they been presented in the original application, appealed claims 28-36 would also have been subjected to the same restriction by either being placed in Group III or in a separate group for similar reasons for restriction from Group I. Consistent with the decision in *Weiler*, 790 F.2d at 1579, even if the present reissue claims were "not 'non-elected'" in the 813 Application, we agree with the Examiner that failing to file divisional applications on the subject matter to which the *claims had never been presented*, similar to failing to file divisional applications on subject matter to which *claims had been made in the original application* would not have been "error" as set forth in § 251 (*see* Ans. 10).

We conclude that as a matter of law, the "Orita doctrine" precludes Appellants from obtaining by reissue the claims on appeal.

CONCLUSION

The Examiner did not err in rejecting claims 28-36 as being improper for reissue.

No time period for taking any subsequent action in connection with this appeal may be extended under 37 C.F.R. § 1.136(a)(1)(iv).

AFFIRMED

ELD

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

Ex parte BEKA SOLOMON

Appeal 2012-002100
Application 11/358,951
Technology Center 1600

Before DONALD E. ADAMS, DEMETRA J. MILLS, and
JEFFREY N. FREDMAN, *Administrative Patent Judges*.

FREDMAN, *Administrative Patent Judge*.

DECISION ON APPEAL

This is an appeal under 35 U.S.C. § 134 involving claims to a method of inhibiting aggregation of soluble β-amyloid in a human. The Examiner rejected the claims as lacking an error correctable by reissue, as obvious, as claiming new matter and as failing to satisfy the written description requirement. We have jurisdiction under 35 U.S.C. § 6(b). We affirm.

Statement of the Case

Background

"Aggregated amyloid β-protein (βA4) is a major constituent of the abnormal extracellular amyloid plaque that characterizes the brains of victims of Alzheimer's disease (AD)" (Spec. col. 3, ll. 8-10). The Specification teaches that "a method is provided of selecting anti-aggregation molecule such as a monoclonal antibody" (Spec. col. 3, ll. 42-43). The Specification teaches that after "the identification of the anti-aggregation molecules has been completed; it is possible to utilize two or more to prevent or reverse aggregation" (Spec. col. 5, ll. 40-42).

The Claims

Claims 5, 7-9, 17, 19-21, and 31-38 are on appeal. Claim 5 is representative and reads as follows:

> 5. A method of inhibiting aggregation of soluble β-amyloid in a human subject, comprising causing an effective amount of an anti-β-amyloid antibody, or an antigen-binding fragment thereof, to come into contact with the soluble β-amyloid, said antibody or fragment being effective to bind β-amyloid and to inhibit aggregation of the soluble β-amyloid in said subject to an extent at least as great as that obtainable with antibody AMY-33, thereby inhibiting aggregation of the soluble β-amyloid, wherein said anti-β-amyloid antibody is one that is obtainable using an immunogen consisting of a peptide consisting of residues 1-28 of β-amyloid, and said antigen binding fragment is an antigen binding fragment of said antibody, and wherein said antibody or fragment is not conjugated with a detectable moiety.

The issues

A. The Examiner rejected claims 5, 7-9, 17, 19-21, and 31-38 under 35 U.S.C. § 251 for lack of defect or error in the original patent and as not being an error correctable by reissue (Ans. 5-7).

B. The Examiner rejected claims 5, 7-9, 17, 19-21 and 31-38 under 35 U.S.C. § 103(a) as obvious over Walker,[1] Hanan,[2] Bacskai,[3] Friden,[4] and Becker[5] (Ans. 8-14).

C. The Examiner rejected claims 17, 19-21 and 35-38 under 35 U.S.C. § 103(a) as obvious over Becker, Cordell,[6] Spillantini,[7] and Kirschner[8] (Ans. 14-22).

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[1] Walker et al., *Labelling of Cerebral Amyloid In Vivo with a Monoclonal Antibody*, 53 J. NEUROPATHOLOGY AND EXPERIMENTAL NEUROLOGY 377-383 (1994).
[2] Eilat Hanan and Beka Solomon, *Inhibitory effect of monoclonal antibodies on Alzheimer's β-amyloid peptide aggregation*, 3 INT. J. EXP. CLIN. INVEST. 130-133 (1996).
[3] Bacskai et al., *Imaging of amyloid-β deposits in brains of living mice permits direct observation of clearance of plaques with immunotherapy*, 7 NATURE MEDICINE 369-372 (2001).
[4] Friden, P., US 5,182,107, issued Jan. 26, 1993.
[5] Becker et al., EP 0 613 007 A2, published Aug. 31, 1994.
[6] Cordell, B., *β-Amyloid Formation as a Potential Therapeutic Target for Alzheimer's Disease*, 34 ANNU. REV. PHARMACAL. TOXICOL. 69-89 (1994).
[7] Spillantini et al., *Different configurational states of β-amyloid and their distributions relative to plaques and tangles in Alzheimer disease*, 87 PROC. NAT'L ACAD. SCI. USA 3947-3951 (1990).
[8] Kirschner et al., *Synthetic peptide homologous to β protein from Alzheimer disease forms amyloid-like fibrils in vitro*, 84 PROC. NAT'L ACAD. SCI. USA 6953-6957 (1987).

D. The Examiner rejected claims 5, 7-9, and 31-34 under 35 U.S.C. § 112, first paragraph, new matter, as failing to satisfy the written description requirement (Ans. 22-24).

E. The Examiner rejected claims 5, 7-9, and 31-34 under 35 U.S.C. § 112, first paragraph, as failing to satisfy the written description requirement (Ans. 24-28).

A. 35 U.S.C. § 251 for lack of defect or error

The Examiner finds that:

> Appellant's failure to timely file divisional application(s) for non-elected claims from the original application is not an error correctable by reissue under 35 U.S.C. 251, since appellant, by acquiescing in restriction requirement, canceling non-elected claims at allowance (Paper No. 16, mailed 03/03/1997), and allowing the patent to issue on remaining claims, lost her opportunity to challenge propriety of the restriction requirement, and since holding otherwise would permit appellant to undermine copendency requirements of 35 U.S.C. 120 and 121 by using reissue statute to avoid restriction requirement.

(Ans. 6.)

Appellant contends that the "present claims are not the same as or substantially identical to the claim in the original patent application that was cancelled subsequent to a restriction requirement" (App. Br. 15).

Appellant contends that

> the present claims are not of "substantially similar scope" as claim 24 of the original application, as is required by the above-quoted portion of Doyle to invoke the Orita Doctrine. The present claims all require that the aggregating protein be β-amyloid, while claim 24 is generic to any aggregated protein. The examiner has conceded, in the restriction

> requirement of September 20, 2006, in this case that every species within the scope of the above-quoted claim 24 is patentably distinct from every other species within the scope of that claim

(App. Br. 16).

The issue with respect to this rejection is: Does the evidence of record support the Examiner's conclusion that there is a lack of defect or error in the original patent and where the asserted error is not an error correctable by reissue?

Findings of Fact

The following findings of fact ("FF") are supported by a preponderance of the evidence of record.

1. The Examiner restricted the original claims of US application 08/358,786 into three groups, Group I, drawn to selection of anti-aggregation molecules, Group II, drawn to treating a protein aggregation disease using an expression vector encoding an anti-aggregation molecule, and Group III, drawn to treating a protein aggregation disease with a monoclonal antibody (*see* US 08/358,786, Restriction, filed Nov. 12, 1995).

2. Appellant elected Group I (*see* US 08/358,786, Election, filed Feb. 8, 1996).

3. Group III comprised claim 24, which reads as follows:

> The method of treating a protein aggregation disease including the steps of preparing at least one human monoclonal antibody that binds to an aggregated protein which is the cause of a disease and which reverses aggregation allowing bioactivity, and administering the monoclonal antibody.

4. Originally filed reissue claim 5 reads as follows:

> A method of preventing or reducing aggregation of a soluble aggregating protein in a subject, comprising causing an effective amount of an anti-aggregation molecule, wherein said anti-aggregation molecule is effective to prevent or reduce aggregation of the soluble aggregating protein in said subject, to come into contact with the soluble aggregating protein, thereby preventing or reducing aggregation of the soluble aggregating protein.

5. Originally filed reissue claim 7 reads as follows:

> A method in accordance with claim 5, wherein said anti-aggregation molecule is a monoclonal antibody.

Principles of Law

"The board held that, under the circumstances of this case, failure to file a timely divisional application cannot be corrected by reissue. . . . We are in substantial agreement with the board's analysis of this case." *In re Orita*, 550 F.2d 1277, 1279-1280 (CCPA 1977).

Analysis

In the instant case, Appellant stated that the error was that "all the parties involved, i.e., Applicant, Assignee, and the attorney . . . were concentrating their efforts on the patenting of the presently claimed method of selecting an anti-aggregation molecule having the chaperone-like activity of anti-aggregation that issued in the 5,688,651 patent as claims 1-4, the only claims in the patent" (Reissue Dec. 2, Feb. 22, 2006). Appellant further stated that "[i]t was only recently that the Applicant/Patentee and the Assignee realized that the concepts fully disclosed in the specification directed to . . . methods of disaggregating an aggregate of or preventing the

aggregation of an aggregating protein . . . were not claimed *per se*, and further they could be and should be now so claimed" (Reissue Dec 2-3, Feb. 22, 2006).

In the instant reissue, Appellant filed new claims 5-28 drawn to either a "method of preventing or reducing aggregation of a soluble aggregating protein in a subject" as in originally filed claim 5 or a "method of disaggregating an aggregate of a soluble aggregating protein in a subject" as in originally filed claim 17 (*see* Prelim. Amdt., filed Feb. 22, 2006; FF 4). In response to an Examiner's restriction requirement in the reissue, Appellant elected the narrower claims under examination limited to a "method of inhibiting aggregation of soluble β-amyloid in a human subject" (Claim 5, Claims Appx., App. Br. 82).

In the originally filed application, US 08/358,786, Appellant elected Group I drawn to methods of selecting anti-aggregation molecules without traverse (FF 1-2). Appellant did not elect claims drawn to treatment, as both of the non-elected Groups II and III in US 08/358,786 were drawn to methods of treating protein aggregation diseases (FF 1).

We agree with the Examiner that whether we consider the originally filed reissue claims or the narrower elected and currently pending reissue claims, all of these claims are drawn to methods of *treating* protein aggregation diseases. All of these claims are substantially similar to the claims 7-24 which were not elected in US 08/358,786. Therefore, these claims would all fall into either nonelected Group II or III of the original restriction, which were both drawn to types of treatment. None of these

claims would fall within the originally elected Group I of *selecting* anti-aggregation molecules.

This fact pattern precisely matches that in *Orita*, where the Applicants "had forgotten to timely file a divisional application covering the originally non-elected subject matter. Appellants further averred that their failure to file the divisional application was an error without any deceptive intention." *Orita*, 550 F.2d at 1279. Here, while Appellant does not directly state that the error was a failure to file divisional applications, the subject matter of the reissue claims was present in claims 7-24 of the originally filed US 08/358,786 application and was non-elected as Groups II and III of that application (FF 1-3).

Appellant contends that

> the present claims are not of "substantially similar scope" as claim 24 of the original application, as is required by the above-quoted portion of Doyle to invoke the Orita Doctrine. The present claims all require that the aggregating protein be β-amyloid, while claim 24 is generic to any aggregated protein. The examiner has conceded, in the restriction requirement of September 20, 2006, in this case that every species within the scope of the above-quoted claim 24 is patentably distinct from every other species within the scope of that claim

(App. Br. 16).

We are not persuaded. The originally filed reissue claims were of substantially the same scope as that of claim 24 of US 08/358,786, differing only in that claim 24 was limited to monoclonal antibodies as the anti-aggregation molecule, a limitation found in claim 7 of the originally filed reissue claims (*see* FF 3-5).

We also are not persuaded by Appellant's reliance on *Doyle*. *Doyle* found that the case involved "a matter of first impression: whether failure to present a so-called linking claim, a claim broad enough to read on—or link—two or more groups of claims subject to a restriction requirement, is an error correctable by reissue" laid out by the Court of Customs and Patent Appeals. *In re Doyle*, 293 F.3d 1355, 1358 (Fed. Cir. 2002).

In *Doyle*, the Court found that

> it was crucial that the applicant explicitly agreed to the requirement of independent prosecution of the disputed claims (or claims substantially similar to the disputed claims) in a divisional, and not as a part of the application directed towards the elected group. When the applicants returned in reissue seeking to add the disputed claims, the examiner, the Board, and this court rightly held them to the terms of their original agreements. The case is different where, as here, the applicant never asserted the reissue claims or anything similar to them in his original application, and also never agreed to prosecute the reissue claims in a divisional application.

Doyle, 293 F.3d at 1360. The instant facts track much more closely to *Orita* than to *Doyle*. In *Doyle*, there was a linking claim that was broader than all of the original claims and encompassed both groups. *Id.* at 1358. In this case however, no linking claim was filed. Instead, Appellant agreed to the restriction requirement, and then, in the reissue, introduced new claims which would have properly been placed into the non-elected Group III, and not into the elected Group I which was examined and issued. Unlike the new claims in *Doyle*, the newly introduced claims 5-28 filed in US reissue 09/441,140 were not so broad that they would serve as linking claims between Groups I and III, but were treatment claims which would clearly

fall within non-elected Group III. The newly introduced claims 5-28 filed in US reissue 09/441,140 did not encompass nor relate to methods of selecting anti-aggregation molecules of the elected Group I which was prosecuted to issuance as US 5,688,651.

Appellant also contends that "present claims 5, 17, 31 and 35 are not identical to claim 24 as had been deleted from the original application, nor are they of 'substantially similar scope' to that nonelected claim" (App. Br. 17).

We are not persuaded. While there is no doubt that the instant claims, as amended, are narrower than the originally filed claim 24 which was not limited to treatment of β-amyloid disease, we do not find that Appellant has overcome the *Orita* doctrine for two reasons. First, the asserted error permitting reissue identified in Appellant's declaration was drawn to the originally filed claims 5-28, where claim 7, for example, is of substantially identical scope to originally filed claim 24 in US 08/358,786. Thus, the originally identified error is not susceptible to cure by reissue under the *Orita* doctrine.

Second, even the narrower claims now in prosecution, if presented to the Examiner in the original US 08/358,786 application, would minimally have been restricted into either the original Group III or a different Group other than the elected Group I. Unlike the situation in *Doyle*, where the new claims were broader than the groups elected, in this case, the claims are narrower and would have properly fallen either into Group III or into further restriction Groups as in the Restriction requirement in 11/358,951 mailed Sep. 20, 2006. Consonant with *Orita*, should Appellant "prevail, the

copendency requirement would become meaningless, for should an applicant fail to file a divisional application while maintaining copendency as required by section 120, he could simply revert to section 251 in order to cure his mistake." *Orita*, 550 F.2d at 1281. Here, permitting Appellant to correct the failure to file divisional applications by reissue would defeat the copendency requirement of 35 U.S.C. § 120, the result found undesirable by the CCPA in *Orita*.

Conclusion of Law

The evidence of record supports the Examiner's conclusion that there is a lack of defect or error in the original patent and where the asserted error is not an error correctable by reissue.

B. 35 U.S.C. § 103(a) over Walker, Hanan, Bacskai, Friden, and Becker

The Examiner finds that "Walker's method of *in vivo* administration meets the instantly recited step of causing an effective amount of anti-β-amyloid antibody, or an antigen-binding fragment thereof, to come into contact with β-amyloid" (Ans. 9). The Examiner finds that "[a]dministration of the antibody to a subject would be expected to inherently result in inhibition of soluble β-amyloid aggregation and/or disaggregation of aggregated β-amyloid, as evidenced by Bacskai et al. who demonstrate that *in vivo* administration of the 10D5 antibody . . . results in reduction of brain amyloid deposits in aged PDAPP transgenic mice" (*id.* at 10).

The Examiner finds it obvious "to genetically engineer the 10D5 monoclonal antibody to create a less immunogenic antibody molecule, such as a single chain antibody, for use as a delivery vehicle in therapeutic

applications in humans as suggested by Walker et al. and in view of both Friden and Becker et al" (*id.* at 12).

Appellant contends that "the present invention is based on the discovery that the 'naked' antibody (i.e., without a linked drug) has therapeutic properties: reduction of the amyloid plaque and inhibition of the aggregation of soluble β-amyloid" (App. Br. 34). Appellant contends that "the newly discovered fact that the naked antibody can itself inhibit aggregation of soluble β-amyloid and/or cause disaggregation of aggregated β-amyloid confers on the antibody a 'direct' therapeutic capacity which is far more significant than the mere binding property" (*id.* at 39).

The issues with respect to this rejection are:

(i) Does the evidence of record support the Examiner's conclusion that Walker, Hanan, Bacskai, Friden, and Becker render claim 5 obvious?

(ii) If so, has Appellant presented evidence of secondary considerations, that when weighed with the evidence of obviousness, is sufficient to support a conclusion of non-obviousness?

Findings of Fact

6. Walker teaches that for "all studies, we used monoclonal antibody (MAb) 10D5, a murine IgG_1 kappa light chain (whole IgG and/or Fab fragments) to amino acids 1-16 of Aβ" (Walker 377, col. 2).

7. Walker teaches that "[a]ntibody 10D5 or nonimmune IgG (in sterile saline) was injected directly into the CSF . . . . In the two experimental squirrel monkeys. 450 μg (3 μg/μl) of Fab fragments were injected" (Walker 378, col. 1).

8. Walker teaches that "β-Amyloid in untreated, fresh-frozen tissue sections was robustly labeled by antibody 10D5, whereas normal (non Aβ-immune) mouse IgG did not bind to native Aβ in this tissue (data not shown). The 10D5 whole IgG and Fab fragments showed comparable immunostaining *in vitro*" (Walker 379, col. 1).

9. Walker teaches that:

> our studies in aged nonhuman primates demonstrate that it is possible to label Aβ selectively *in vivo* by a monoclonal antibody infused into the CSF. It will be necessary to increase the sensitivity of the method and to determine the safety of the procedure before this approach can be assessed for efficacy in a clinical setting. In particular, the specific etiology of the leukocytic reaction to intracisternal injection requires further study. However, *in vivo* labeling has considerable potential for delivering therapeutic agents that could prevent or reverse Aβ deposition in the brains of patients with cerebrovascular amyloidosis or Alzheimer's disease. Furthermore, this strategy eventually might be useful in diagnostic and experimental approaches to β-amyloidogenesis.

(Walker 382, col. 1.)

10. Hanan teaches that "[a]ggregation of β-amyloid was monitored using a commercially available monoclonal antibody, called mAb AMY 33, raised against the peptide 1-28 of β-amyloid (Zymed, San Francisco, CA) and another four monoclonal antibodies called 6C6 and 10D5, raised against peptide 1-28 of β-amyloid" (Hanan 131, col. 1).

11. Bacskai teaches "clearance of amyloid-β deposits after initial treatment of the cortex with direct application of 10D5. . . . These data indicate that clearance of amyloid-β after exposure to 10D5 is a specific

response to anti-amyloid-β antibodies rather than a nonspecific response to injury" (Bacskai 371, col. 1).

12. Friden teaches that "[a]nti-amyloid antibodies or antibody fragments immunoreactively bind with amyloid plaques that are characteristic of Alzheimer's Disease. For clinical diagnostic detection purposes, a detectable group such as gadolinium or technitium is often attached to the anti-amyloid antibody" (Friden, col. 22, ll. 61-67).

13. Friden teaches that the "conjugate was localized to the brain vasculature in a manner similar to that for OX-26 alone, indicating that the attachment of this anti-amyloid antibody to OX-26 did not affect its ability to target to brain capillary endothelial cells in vivo" (Friden, col. 23, ll. 18-22).

14. Becker teaches that the "antibodies of the present invention are especially preferred in the diagnosis and/or treatment of Alzheimer's disease in mammals, preferably humans" (Becker, col. 7, ll. 49-52).

15. Becker teaches that the

> Winter technology involves the replacement of complementarity determining regions (CDRs) of a human antibody with the CDRs of a murine monoclonal antibody thereby converting the specificity of the human antibody to the specificity of the murine antibody which was the source of the CDR regions. This "CDR grafting" technology affords a molecule containing minimal murine sequence and thus is less immunogenic.

(Becker, col. 7, ll. 2-10.)

16. The Specification teaches that the "present invention provides a method of selecting monoclonal antibodies, genetically engineered antibody

fragments and small peptides which mimic binding sites of the antibodies and which prevent aggregation and yet do not inhibit bioactivity" (Spec. col. 4, ll. 62-66).

17. The Specification teaches in a "preferred embodiment the human monoclonal antibody that binds to an aggregating protein and which prevents aggregation is utilized. In a further preferred embodiment the monoclonal antibody is an anti-β-amyloid and is designated AMY-33 which recognizes amino acids 1-28 of β-amyloid" (Spec. col. 6, ll. 21-26).

18. The Specification teaches, in Example 2, that "[b]inding of mAb AMY-33 to βA4 prevents self-aggregation of the β-amyloid, probably by recognizing the sequence 25-28 located in the proposed aggregation fragment comprising the amino acids between 25-28" (Spec. col. 16, ll. 5-8).

Principles of Law

"In proceedings before the Patent and Trademark Office, the Examiner bears the burden of establishing a prima facie case of obviousness based upon the prior art." *In re Fritch*, 972 F.2d 1260, 1265 (Fed. Cir. 1992).

"[R]ejections on obviousness grounds cannot be sustained by mere conclusory statements; instead, there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness." *KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. 398, 418 (2007).

Analysis

Prima facie obviousness

Walker and Becker provide some suggestion, however bare, to treat β-amyloid plaques in Alzheimer's disease with monoclonal antibodies (FF 6-9, 14, 15). However, none of the prior art references suggest inhibiting

aggregation. In particular, neither Walker, Becker, nor Friden teach the use of any particular level of inhibition of aggregation where the level is "to an extent at least as great as that obtainable with antibody AMY-33" as required by claim 5. There is also no teaching of a method "of disaggregating an aggregate of soluble β-amyloid in a human subject" as required by claim 17 in Walker, Becker or Friden.

While the postfiling date Hanan and Bacskai evidence that 10D5 inherently had the capacity of inhibiting aggregation of soluble β-amyloid in a human subject (FF 10-11), these references cannot properly be relied upon to provide a reason or motivation to administer the antibodies of Walker, Becker or Friden to a patient. Nor can these post filing date references provide a reasonable expectation of success in such an administration.

While we agree with the Examiner that Walker and Becker provide some suggestion to treat patients with anti-β-amyloid antibodies, the Examiner has not established that the Becker antibody or the Walker murine 10D5, even when humanized, would have reasonably been expected to be safe for patients and capable of treating β-amyloid aggregation. The essence of the rejection is based on an improper obvious to try argument, since there is no evidence that the combination had a reasonable expectation of success in treating Alzheimer's disease. Walker is not concerned with safety, since the animals in Walker are sacrificed (FF 6-9). While Becker may be more success oriented, Becker does not identify an antibody which would fall within the scope of the claim nor does Becker provide any evidence of any successful treatment of β-amyloid aggregation. Therefore, in the absence of

any suggestion of a reasonable expectation of success, we see the invention as obvious to try at best, in view of the combined teachings of Walker, Becker and Friden. That is not a proper standard of obviousness. *In re O'Farrell*, 853 F.2d 894, 904 (Fed. Cir. 1988).

Secondary Considerations

Appellant contends that "even if, *arguendo*, the examiner has established a *prima facie* case of obviousness, the evidence of record is sufficient to rebut such *prima facie* case and establish the patentability of the present invention." (App. Br. 32.)

The Examiner finds that "Appellant's argument is not persuasive because the evidence of unexpected results is not commensurate in scope with the entire range of the claimed invention" (Ans. 43). The Examiner also finds that several antibodies cited did not function (*id.* at 44).

We find that Appellant has the better position. In this case, the functional attribute of the antibody to have anti-aggregating or disaggregating properties for therapeutic use to treat Alzheimer's disease is an unexpected result which overcomes the prima facie case of obviousness. We conclude that Appellant's unexpected result is reasonably commensurate with the scope of the claim which is limited to antibodies to amino acids 1-28 of β-amyloid and which inhibit aggregation at least as well as AMY-33.

To the extent the Examiner may be concerned that the claims may embrace possibly inoperative antibody embodiments, we point to *Atlas Powder Co. v. E.I. du Pont de Nemours & Co.*, 750 F.2d 1569, 1576 (Fed. Cir. 1984), where the court stated that "[e]ven if some of the claimed combinations were inoperative, the claims are not necessarily invalid. 'It is

not a function of the claims to specifically exclude ... possible inoperative substances....'"

Conclusion of Law

(i) The evidence of record does not support the Examiner's conclusion that Walker, Hanan, Bacskai, Friden, and Becker render claim 5 obvious.

(ii) Appellant has presented evidence of secondary considerations, that when weighed with the evidence of obviousness, is sufficient to support a conclusion of non-obviousness.

C. 35 U.S.C. § 103(a) over Becker, Cordell, Spillantini, and Kirschner

The Examiner finds that Becker teaches "therapeutic use of anti-β-amyloid antibodies for the treatment of humans having Alzheimer's disease" (Ans. 15). The Examiner finds that "antibodies specific for β-amyloid peptide in the β-sheet conformation are 'functional as direct therapeutic and diagnostic aids'" (*id.* at 16). The Examiner finds that Cordell teaches "compounds that bind β-amyloid and block its ability to seed further molecular addition would be of therapeutic advantage . . . . Figure 4 on page 82 exemplifies this therapeutic approach, wherein compounds that bind to and interrupt β-amyloid protein aggregation are indicated as therapeutic targets" (*id.* at 16-17). The Examiner finds that Spillantini teaches that "antibodies that recognize epitopes within β-amyloid 1-28 are capable of staining β-amyloid proteins that are in a more soluble state and have not yet formed dense core aggregates" (*id.* at 18). The Examiner finds that Kirschner teaches that "the N-terminal region of β-amyloid (i.e., residues 1-28), and in particular the region comprising residues 18-28, is crucial for β-

sheet fibril formation and subsequent aggregation of β-amyloid peptide" (Ans. 18).

The Examiner finds it obvious that "the ordinarily skilled artisan would have recognized the value of inhibitory antibodies directed to β-amyloid 1-28, and in particular residues 14-28 of β-amyloid, as therapeutic agents for diseases associated with abnormal β-amyloid aggregation and deposition, such as Alzheimer's disease" (Ans. 20).

Appellant contends that "the examiner has pointed to no reference of record that suggests that such antibodies would be useful as a therapeutic because of an expectation that they would disaggregate plaque" (App. Br. 47-48). Appellant contends that the "fact that an antibody may bind to amyloid plaque would not necessarily create an expectation, at the time the present invention was made, that such binding will automatically cause disaggregation of the plaque. Indeed, even today it is known that not all antibodies that bind to amyloid plaque will cause disaggregation" (*id.* at 47).

The issue with respect to this rejection is: Does the evidence of record support the Examiner's conclusion that Becker, Cordell, Spillantini, and Kirschner render claim 17 obvious?

Findings of Fact

19. The Examiner finds that "Cordell adds, compounds that bind β-amyloid and block its ability to seed further molecular addition would be of therapeutic advantage" (Ans. 16).

20. Spillantini teaches:

> that antisera against peptides in β-amyloid can be obtained that react predominantly with one of a number of different objects-namely, amyloid plaques without cores, amyloid

> plaques with cores, or tangle-bearing bodies and cells. This suggests that the β-amyloid sequence exists in different configurations and/or in different states of aggregation in amyloid plaques and neurofibrillary tangle-bearing cells.

(Spillantini 3949, col. 2.)

21. Kirschner teaches that "[w]e have demonstrated that a synthetic peptide identical in sequence to the first 28 amino acids of the 4-kDa amyloid protein can assemble *in vitro* into fibrils and bundles having very similar structural and antigenic features as those observed *in situ*" (Kirschner 6957, col. 1).

Analysis

Prima facie obviousness

Becker teaches treating β-amyloid plaques in Alzheimer's disease with monoclonal antibodies (FF 14, 15). The Examiner finds that "Cordell adds, compounds that bind β-amyloid and block its ability to seed further molecular addition would be of therapeutic advantage" (Ans. 16; FF 19). Spillantini and Kirschner teach antibodies which can bind β-amyloid (Ans. 20-21).

Even if we agree with the Examiner that the prior art teaches treatment of β-amyloid plaques with antibodies, none of the prior art references suggest disaggregating an aggregate of β-amyloid using an antibody, either *in vivo* or *in vitro*. There is no evidence of record that the prior art recognized that the anti-β-amyloid antibodies would disaggregate β-amyloid plaques.

Conclusion of Law

The evidence of record does not support the Examiner's conclusion that Becker, Cordell, Spillantini, and Kirschner render claim 17 obvious.

D. 35 U.S.C. § 112, first paragraph, new matter

The Examiner finds that there "is no *verbatim* support in the specification as originally filed for anti-β-amyloid antibodies which inhibit aggregation of β-amyloid to a particular specified degree, nor does this language flow naturally from the disclosure as originally filed" (Ans. 23). The Examiner finds that the "specification as filed thus does not support the genus of antibodies that is described in terms of meeting or exceeding the ability of the antibody AMY-33 to inhibit β-amyloid aggregation" (*id.*).

Appellant contends that the "concept of using any antibody that binds to β-amyloid and prevents aggregation is present in the present specification as filed, for example, at column 16, line 21-26" (App. Br. 59). Appellant contends that "one of ordinary skill in the art would certainly have understood that, once one raises and tests other antibodies for these properties, those that behave even better than the antibodies shown in the assay of Figure 7A might be found" (*id.* at 59-60).

The issue with respect to this rejection is: Does the evidence of record support the Examiner's finding that the limitation "to inhibit aggregation of the soluble β-amyloid in said subject to an extent at least as great as that obtainable with antibody AMY-33" in claim 5 represents new matter?

Findings of Fact

22. The Specification teaches that "the formation of the immuno-complexes with selected, highly specific monoclonal antibodies, should provide a general and convenient method to prevent aggregation of the proteins without affecting their biological properties" (Spec. col. 16, ll. 17-21).

Principles of Law

"[I]t is the specification itself that must demonstrate possession. And while the description requirement does not demand any particular form of disclosure, ... or that the specification recite the claimed invention *in haec verba*, a description that merely renders the invention obvious does not satisfy the requirement" *Ariad Pharmaceuticals, Inc. v. Eli Lilly and Co.*, 598 F.3d 1336, 1352 (Fed. Cir. 2010).

Analysis

While the Examiner is correct that the specific language of the claims was not disclosed *ipsis verbis* in the Specification (Ans. 23), *ipsis verbis* support is not required. *Fujikawa v. Wattanasin*, 93 F.3d 1559, 1570 (Fed. Cir. 1996).

The Specification teaches that the antibody AMY-33 functions to prevent self-aggregation of β-amyloid (FF 18), and teaches that AMY-33 is preferred monoclonal antibody from the class of all monoclonal antibodies which bind aggregating proteins (FF 17-18, 22).

We agree with Appellant that the person of ordinary skill, in reading the Specification, would have found the Specification demonstrating possession of monoclonal antibodies or fragments thereof which are

generated by an immunogen that is amino acids 1-28 of β-amyloid. The ordinary artisan would also find that the Specification teaches that the monoclonal antibodies have an activity which effectively functions to prevent self-aggregation of β-amyloid, where the standard for determining that function is the known AMY-33 antibody disclosed by the Specification (FF 17-18, 22). We therefore conclude that the claim limitation is supported by the Specification as filed.

Conclusion of Law

The evidence of record support the Examiner's finding that the limitation "to inhibit aggregation of the soluble β-amyloid in said subject to an extent at least as great as that obtainable with antibody AMY-33" in claim 5 represents new matter.

E. 35 U.S.C. § 112, first paragraph, written description

The Examiner finds that "present claims are drawn to the use of a genus of antibody molecules having a specific functional property, for which appellant has only disclosed a single species within the genus" (Ans. 24). The Examiner finds that "there is insufficient guidance and direction of the genus of antibodies broadly encompassed by the claimed invention" (*id.*).

The Examiner finds that

> the specification does not provide adequate written description of the claimed genus nor guidance as to which of myriad of antibody molecules that are encompassed by the claimed invention would be capable of inhibiting β-amyloid aggregation to a level that meets or exceeds the ability of the single disclosed species of AMY-33 to inhibit aggregation

(*id.* at 27).

Appellant contends that "the inventor was fully in possession of the concept of raising monoclonal antibodies in the standard manner and using those that bind to the immunogen in the same assays in which the commercial antibodies were used in the remainder of the specification" (App. Br. 65-66).

Appellant contends that the "claims do not recite the antibodies merely by means of a functional statement but rather recite specific properties that the antibodies possess. These properties include binding to β-amyloid and either inhibiting aggregation of soluble β-amyloid or causing disaggregation of aggregated β-amyloid" (*id.* at 68).

The issue with respect to this rejection is: Does the evidence of record support the Examiner's conclusion that the disclosure of the Specification failed to demonstrate possession and descriptive support for Claim 5?

Principles of Law

> [T]he hallmark of written description is disclosure.... [T]he test requires an objective inquiry into the four corners of the specification from the perspective of a person of ordinary skill in the art. Based on that inquiry, the specification must describe an invention understandable to that skilled artisan and show that the inventor actually invented the invention claimed.

Ariad Pharmaceuticals, Inc. v. Eli Lilly and Co., 598 F.3d at 1351.

Analysis

We agree with the Examiner that the Specification does not adequately describe the antibody or a fragment which "binds beta-amyloid and inhibits aggregation of soluble beta-amyloid to an extent at least as great as that obtainable with antibody AMY-33" of claim 5 as broadly claimed.

It is well settled that:

> the inventor cannot lay claim to that subject matter unless he can provide a description of the compound sufficient to distinguish infringing compounds from non-infringing compounds, or infringing methods from non-infringing methods. As the district court observed, "[t]he claimed method depends upon finding a compound that selectively inhibits PGHS-2 activity. Without such a compound, it is impossible to practice the claimed method of treatment."

University Of Rochester v. G.D. Searle & Co., 358 F.3d 916, 926 (Fed. Cir. 2004).

Claim 5, as interpreted in light of the Specification, is broadly drawn to any method of using an antibody, where the antibody "binds beta-amyloid and inhibits aggregation of soluble beta-amyloid to an extent at least as great as that obtainable with antibody AMY-33." The Specification therefore must adequately describe that genus of compounds.

The written description requirement can be met by disclosing "complete or partial structure, other physical and/or chemical properties, *functional characteristics when coupled with a known or disclosed correlation between function and structure*, or some combination of such characteristics." *Enzo Biochem, Inc. v. Gen-Probe Inc.*, 323 F.3d 956, 964, (Fed. Cir. 2002).

In this case, the Specification has a single working example of an antibody, AMY-33 (*see* FF 18). In addition, the Specification does not describe any of the specific structural features, physical or chemical properties, or other information which would permit identification of antibodies which would bind beta-amyloid and which would have the

functional capacity to inhibit aggregation as well as the AMY-33 antibody. The claim is entirely functional.

Indeed, while the post-filing date evidence demonstrates that the 10D5 antibody, a known prior art antibody (FF 6), shares the functional requirements of the claim (FF 11), the Specification provides no description which would have permitted the ordinary artisan to recognize that the 10D5 antibody satisfied the functional requirements of claim 5.

The present case is therefore analogous to *Rochester* and *Centocor*. In *Rochester*, the patent "describes in detail how to make cells that express either COX-1 or COX-2, but not both ..., as well as 'assays for screening compounds, including peptides, polynucleotides, and small organic molecules to identify those that inhibit the expression or activity of the PGHS-2 gene product.'" *Rochester*, 358 F.3d at 927.

Rochester held that even if a DNA sequence might support a claim to hybridizing nucleic acids, the "same is not necessarily true in the chemical arts more generally. Even with the three-dimensional structures of enzymes such as COX-1 and COX-2 in hand, it may even now not be within the ordinary skill in the art to predict what compounds might bind to and inhibit them." *Rochester*, 358 F.3d at 925.

Thus, in *Rochester*, the court found unpersuasive the instant argument by Appellant that "the generation of antibodies, but also the selection of those antibodies with the enumerated specific properties by appropriate assays is well known in the existing art" (App. Br. 69). The court found that the ordinary artisan could not predict which compounds might satisfy the

functional requirements of the claim, the same situation which applies in the instant fact pattern.

Appellant also relies upon the USPTO written description guidelines, and in particular, Example 13, drawn to support for a "claim drawn to 'an isolated antibody capable of binding to antigen X'" (*see* App. Br. 70). Appellant also relies upon a Board decision (*see* Reply Br. 35-37). We find that the decision of the Federal Circuit in *Centocor* is more closely related to this issue.

In *Centocor*, the Court addressed Example 13 of the USPTO written description guidelines. The Court found that the "PTO guidelines conclude that characterization of the protein alone may be sufficient under circumstances where 'one of skill in the art would have recognized that the disclosure of the adequately described [protein] X put the applicant in possession of antibodies which bind to [protein] X.'" *Centocor Ortho Biotech, Inc. v. Abbott Laboratories*, 636 F.3d 1341, 1351 (Fed. Cir. 2011).

Centocor distinguished the USPTO written description guideline situation, where the claim simply required binding an antigen, from the more complicated situation where specific properties beyond simply binding of antigen by the antibody are required. *Id.* at 1352. *Centocor* found that "[c]laiming antibodies with specific properties, e.g., an antibody that binds to human TNF-α with A2 specificity, can result in a claim that does not meet written description even if the human TNF-α protein is disclosed because antibodies with those properties have not been adequately described." *Id.* at 1352.

The instant situation is virtually identical to the *Centocor* situation, since in both cases the antigen of interest was known, but there was no description in the Specification or prior art of the specific properties required. In the instant case, there was no description of any "genetically-engineered" antibodies which were "effective to bind β-amyloid and to inhibit aggregation of the soluble β-amyloid in said subject to an extent at least as great as that obtainable with antibody AMY-33.".

As *Ariad* points out, the "written description requirement also ensures that when a patent claims a genus by its function or result, the specification recites sufficient materials to accomplish that function—a problem that is particularly acute in the biological arts." *Ariad*, 598 F.3d at 1352-3. That is the central issue here, where at best a single species of antibody which satisfies the functional requirements of claim 5 is disclosed. We conclude that Appellant's claims "merely recite a description of the problem to be solved while claiming all solutions to it and ... cover any compound later actually invented and determined to fall within the claim's functional boundaries— leaving it to the pharmaceutical industry to complete an unfinished invention." *Ariad*, 598 F.3d at 1353.

Conclusion of Law

The evidence of record supports the Examiner's conclusion that the disclosure of the Specification failed to demonstrate possession and descriptive support for Claim 5.

SUMMARY

In summary, we affirm the rejection of claims 5, 7-9, 17, 19-21, and 31-38 under 35 U.S.C. § 251 for lack of defect or error in the original patent and as not being an error correctable by reissue.

We reverse the rejection of claims 5, 7-9, 17, 19-21 and 31-38 under 35 U.S.C. § 103(a) as obvious over Walker, Hanan, Bacskai, Friden, and Becker.

We reverse the rejection of claims 17, 19-21 and 35-38 under 35 U.S.C. § 103(a) as obvious over Becker, Cordell, Spillantini, and Kirschner.

We reverse the rejection of claims 5, 7-9, and 31-34 under 35 U.S.C. § 112, first paragraph, new matter, as failing to satisfy the written description requirement.

We affirm the rejection of claims 5, 7-9, and 31-34 under 35 U.S.C. § 112, first paragraph, as failing to satisfy the written description requirement.

No time period for taking any subsequent action in connection with this appeal may be extended under 37 C.F.R. § 1.136(a).

AFFIRMED

cdc

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

Ex parte MING CHANG WU, PEI-YU CHIOU, and AARON T. OHTA

Appeal 2016-000348
Application 13/926,101
Technology Center 2800

Before JAMES C. HOUSEL, JENNIFER R. GUPTA, and LILAN REN, *Administrative Patent Judges.*

GUPTA, *Administrative Patent Judge.*

DECISION ON APPEAL[1]

Appellants[2] appeal under 35 U.S.C. § 134(a) from the Examiner's decision finally rejecting claims 5, 9–27, 29–52, 54–61, and 63–72. We have jurisdiction under 35 U.S.C. § 6(b).

We AFFIRM.

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[1] In this Decision, we refer to the Non-Final Office Action mailed October 2, 2014 ("Non-Final Act."), the Appeal Brief filed March 17, 2015 ("App. Br."), the Examiner's Answer mailed August 4, 2015 ("Ans."), and the Reply Brief filed October 5, 2015 ("Reply Br.").

[2] Appellants identify the real party in interest as Regents of the University of California and Berkeley Lights, Inc. App. Br. 1.

Claim 5, reproduced below, is illustrative of the claims on appeal.

> 5. A process of manipulating cells or particles by light induced dielectrophoresis in an apparatus comprising a first layer, a second layer, a light source, and a microvision-based pattern recognition subsystem, said process comprising:
>
> applying a biasing voltage between said first layer and said second layer at least one of which comprises a photoconductive material configured for conversion of received light to a local electric field in a vicinity of said received light, wherein said cells or particles are disposed in a volume between said first layer and said second layer; and
>
> controlling with said microvision-based pattern recognition subsystem, in response to registering positions of said particles or cells as determined from microscopic imaging, a pattern of light projected from said light source onto said photoconductive material to create a corresponding pattern of virtual electrodes at said surface of said photoconductive material, each said virtual electrode attracting or repelling ones of said cells or particles in said volume by dielectrophoresis.

DISCUSSION

The application on appeal is a division of U.S. Patent Application Serial No. 13/287,093, which was filed on November 1, 2011, and issued as U.S. Patent No. RE 44,711 on January 21, 2014, which is a reissue of U.S. Patent Application Serial No. 11/105,304 (hereinafter "the '304 application"), which was filed April 12, 2005, and issued as U.S. Patent No. 7,612,355 B2 (hereinafter "the '355 patent") on November 3, 2009.

During prosecution of the '304 application, the Examiner issued Restriction Requirement. The restriction set forth two groups of claims as follows:

I: Claims 7–37, drawn to apparatus for manipulating cells or particles by light induced dielectrophoresis, classified in class 204, subclass 643; and

II: Claims 40–43 and 48–52, drawn to a method of manipulating particle or cellular objects retained within a liquid, classified in class 435, subclass 173.1.

The Restriction Requirement determined Inventions I and II (hereafter referred to as "Groups I and II") to be related, but patentably distinct.

In response to the Restriction Requirement, Applicants elected to prosecute Group I, apparatus claims 7–37. Applicants' response did not dispute the propriety of the Examiner's restriction requirement, and canceled the non-elected claims.

On November 3, 2009, the '304 application issued as the '355 patent, containing four apparatus claims. A divisional application presenting the non-elected claims was never filed during the pendency of the '304 application.

On November 1, 2011, Applicants filed Reissue Application Serial No. 13/287,093. The Examiner issued a two-way restriction between claims directed to an apparatus (Group I, claims 1–25, 67–82, and 88–94), and claims directed to a process (Group II, claims 26–66, 83–87, 95–104, and 105–111).

In response to the restriction, Applicants elected the apparatus claims (Group I), and canceled the non-elected claims.

On June 25, 2013, Applicants filed the instant reissue application, which is a division of Reissue Application Serial No. 13/287,093. All original patent claims were canceled and reissue claims 5, 9–27, 29 61, and 63–72, directed to a process are pending.

Claims 5, 9–27, 29–52, 54–61, and 63–72 stand rejected as being based upon a defective reissue declaration under 35 U.S.C. § 251.

Appellants argue the claims as a group. *See generally* App. Br. 3–9. We select claim 5 as representative of the rejected claims, and the remaining claims on appeal will stand or fall with claim 5.

The requirements for reissuance of a patent are set forth in 35 U.S.C. § 251. The first paragraph of Section 251 reads as follows:

> Whenever any patent is, through error, deemed wholly or partly inoperative or invalid, by reason of a defective specification or drawing, *or by reason of the patentee claiming more or less than he had a right to claim in the patent*, the Director shall, on the surrender of such patent and the payment of the fee required by law, reissue the patent for the invention disclosed in the original patent, and in accordance with a new and amended application, for the unexpired part of the term of the original patent. No new matter shall be introduced into the application for reissue. [Emphasis added].

The Federal Circuit has explained that section 251 "is remedial in nature, based on fundamental principles of equity and fairness, and should be construed liberally." *In re Weiler*, 790 F.2d 1576, 1579 (Fed. Cir. 1986). Nevertheless, the court has also made it clear that "not every event or circumstance that might be labeled 'error' is correctable by reissue." *Id.*

Appellants argue that the Examiner erred in determining that the "process claims, regardless of their subject matter, are precluded from reissue due to the Restriction and subsequent Election in the Original Application." App. Br. 6. Specifically, Appellants contend that our reviewing court's decision in *In re Doyle*, 293 F.3d 1355 (Fed. Cir. 2002), not *In re Orita*, 550 F.2d 1277 (CCPA 1977), is instructive in deciding whether Appellants' failure to present the process claims on appeal is an error correctable by reissue. *Id.* at 7; *see also* Reply Br. 2–3.

Doyle involved a matter of first impression: "whether failure to present a so-called linking claim, a claim broad enough to read on—or link—two or more groups of claims subject to a restriction requirement, is an error correctable by resissue." *Doyle*, 293 F.3d at 1358. The Federal Circuit answered this question in the affirmative. *Id.* at 1360. In reaching its conclusion, the court found that the "*Orita* doctrine"[3] did not apply to the facts involved in *Doyle* for two reasons: (1) the new claims were neither identical nor substantially similar to the nonelected claims, and (2) the new claims could have prosecuted with the elected group without running afoul of the restriction requirement. *Id.*

We agree with the Examiner that the facts and circumstances in the present reissue application are distinguishable from those of *Doyle*, which carved out a narrow exception to the *Orita* doctrine. As the Examiner points out, the claims on appeal in the present case are distinguishable from the claims at issue in *Doyle* because they are not directed to genus claims that are broad enough to read on or link both the elected and non-elected claims. The fact that the claims presented in *Doyle* were linking claims was at the heart of the matter decided in *Doyle*. *Doyle*, 293 F.3d at 1358; *see also Landmark Screens LLC v. Morgan, Lewis, & Bockius, LLP*, 676 F.3d 1354, 1364 (Fed. Cir. 2012) (explaining that *Doyle* is properly invoked "[w]hen the reissue claims are *broader* than the issued claims" (emphasis added)). Thus, despite Appellants' contentions, we do not consider this difference to be "immaterial." *See* Reply Br. 2.

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[3] The "*Orita* doctrine" precludes applicants from obtaining substantially identical claims to those of non-elected groups identified in an examiner's restriction requirement when such claims could not have been prosecuted in the application from which they were restricted. *Doyle*, 293 F.3d at 1359.

As discussed above, in the present case Appellants acquiesced to the restriction requirement in the '304 application, electing apparatus claims (Group I), and cancelling the non-elected claims (Group II, method claims), and then, in this reissue, introduced new process claims 5, 9–27, 29–52, 54–61, and 63–72. Unlike the new claims in *Doyle*, the process claims on appeal are not so broad that they would read on or link the claims of Groups I and II in the restriction requirement made in the '304 application. Rather, Appellants' new process claims are narrower than the original non-elected group of method claims, which are not limited to being performed in an apparatus comprising "a microvision-based pattern recognition subsystem" configured to control light projected from a light source "in response to registering positions of said particles or cells as determined from microscopic imaging."

Even if the claims are not viewed as narrower than the original non-elected group of method claims, as the Examiner point outs, the process claims on appeal are patentably distinct from the apparatus claims elected during prosecution of the '304 application. Non-Final Act. 4; Ans. 6–7. As a result, if presented to the Examiner during the prosecution of the '304 application, the new process claims would have been restricted into either original Group II or a group other than the elected Group I. Consequently, Appellants could not have prosecuted the process claims on appeal with the issued claims.

Moreover, as well-stated by the Examiner in the Answer (Ans. 8), Appellants lost their ability for rejoinder by voluntarily cancelling the withdrawn claims before rejoinder occurred. *Id.* (citing *Manuel of Patenting Examining Procedure* § 821.04(b) ("MPEP")).

The facts in the instant appeal track more closely to *Orita* than to *Doyle*. *Orita*, 550 F.2d at 1278–1279. Although the appealed reissue claims are not identical to the canceled, non-elected claims of the original patent application, they are narrower than, and encompass the subject matter of, each of the canceled, non-elected claims. Appellants, here, acquiesced to the restriction requirement in the '304 application by cancelling the non-elected claims (method claims), and are now trying to obtain claims of the same type as the canceled claims via reissue. *Orita*, 550 F.2d at 1280; *see also In re Smyser*, 135 F.2d 747, 749–50 (CCPA 1943), and *In re Cornell*, 150 F.2d 702, 704 (CCPA 1945). Under the rationale set forth in *Smyser and Cornell* (cases upon which *Orita* rests), however, Appellants are not entitled to obtain method claims *per se*, regardless of scope, because they conceded by their acquiescence in the restriction requirement that they were not entitled to claim method claims in their original application. *Smyser*, 135 F.2d at 751; *see also Cornell*, 150 F.2d at 704.

Finally, as pointed out by the court in *Orita*:

> . . . granting by reissue claims substantially identical to those non-elected in application I would be ignoring the proper restriction requirement set forth in that application in which appellants acquiesced. Indeed, appellants' misapplication of section 251 would, if permitted, circumvent the copendency requirement of section 120, incorporated by reference in section 121 (see note 2 supra). Should appellants prevail, the copendency requirement would be meaningless, for should an applicant fail to file a divisional application while maintaining copendency as required by section 120, he could simply revert to section 251 in order to cure his mistake. Section 251 is not a pancea designed to cure every mistake which might be committed by an applicant or his attorney . . . [Footnote omitted].

Orita, 550 F.2d at 1280–1281. The same concerns of *Orita* are equally applicable here.

In sum, a preponderance of the evidence supports the Examiner's conclusion that Appellants' asserted error is not an error correctable by reissue. Non-Final Act. 5; *see also* Reply Br. 4 (where Appellants state that their mistake was "failure to add — in the Response of February 26, 2009 — method claims having each and every limitation of apparatus claims 12 and 24."). Accordingly, we sustain the Examiner's rejection of claims 5, 9–27, 29–52, 54–61, and 63–72.

DECISION

For the above reasons, the Examiner's rejection of claims 5, 9–27, 29–52, 54–61, and 63–72 is affirmed.

No time period for taking any subsequent action in connection with this appeal may be extended under 37 C.F.R. § 1.136(a).

AFFIRMED

EXHIBIT H

2014-1050
(Serial No. 12/827,650)

UNITED STATES COURT OF APPEALS FOR THE FEDERAL CIRCUIT

IN RE EDWARD BEVERLY MORRISON, PAVEL TISHIN, PETR VALENTINOVICH KUZMIN

Appeal from the United States Patent and Trademark Office,
Patent Trial and Appeal Board.

BRIEF FOR APPELLEE—DIRECTOR OF THE UNITED STATES PATENT AND TRADEMARK OFFICE

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February 18, 2014

REPRESENTATIVE CLAIM

28. A method for providing a tow assembly for an electromagnetic airborne survey system at an assembly location, comprising:

providing a plurality of hollow transmitter support frame sections;

providing a transmitter coil for generating a primary magnetic field;

providing connector cables and a tow cable; and

assembling the transmitter support frame sections and the transmitter coil and the cables to provide a tow assembly having a flexible hollow transmitter support frame that is circular or approximates a circle having the transmitter coil disposed within the hollow transmitter support frame and formed from serially connecting the transmitter support frame sections, with the connector cables each having a first end connected to a respective location on a circumference of the transmitter support frame and a second end connected to a first end of the tow cable.

A3; A200.

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STATEMENT OF RELATED CASES

The Director is not aware of any other appeal in connection with this case that has previously been before this Court, or that is currently pending in any other court. The Director is also unaware of any related cases pending in this or any other court that will directly affect, or be directly affected by, this Court's decision in the pending appeal.

I. STATEMENT OF THE ISSUE

Edward Beverly Morrison, Pavel Tishin, and Petr Valentinovich Kuzmin ("Morrison") are the inventors of U.S. Patent No. 7,157,914 ("the '914 patent"). Morrison originally claimed multiple, distinct inventions in the application leading to the '914 patent. Accordingly, the Examiner restricted the claims into three groups. Morrison elected the first group and prosecuted those claims to allowance, but did not file divisional application(s) to the nonelected claims or to any other subject matter prior to issuance of the '914 patent. Morrison now attempts to prosecute subject matter distinct from his elected invention by filing a reissue application. The issue before this Court is whether the Board properly determined that Morrison's failure to timely file a divisional application is not correctable by reissue.

II. STATEMENT OF THE CASE

The '914 patent, entitled "Airborne Electromagnetic Time Domain System, Computer Product and Method," issued on January 2, 2007 with twenty-seven claims. A18, A20-35. On June 30, 2010, Morrison filed a reissue application[1] adding new claims. A17, A36-37, A134. The Examiner finally rejected the new claims under 35 U.S.C. § 251 for lack of defect or error in the

[1] The present reissue application is a divisional of reissue application no. 12/343,321, filed November 16, 1999, which issued on March 15, 2011 as U.S. RE42,217.

original patent correctable by reissue. A242-57 (Examiner's Answer). Morrison appealed to the Board of Patent Appeals and Interferences ("Board"), which affirmed. A1-12. This appeal followed.

A. Morrison Discloses a Method and Apparatus for Geological Mapping Using Time Domain Electromagnetic Surveying

Time domain electromagnetic ("TDEM") surveying allows for the geological exploration of the earth. In simplistic terms, TDEM surveying involves electromagnetic pulses that penetrate the earth's surface. The feedback information produced from the electromagnetic pulses allows geophysicists to accurately map underground features such as water sources, mineral deposits, soil features, *etc.*, from above the earth. *See generally* United States Environmental Protection Agency, "Time-Domain Electromagnetic Methods," http://www.epa.gov/esd/cmb/GeophysicsWebsite/pages/reference/methods/Surface_Geophysical_Methods/Electromagnetic_Methods/Time-Domain_Electromagnetic_Methods.htm (last visited Jan. 8, 2014); *see also* A20 (col. 1, ll. 14-35).

The airborne TDEM system disclosed in the '914 patent consists of a transmitter **16** and a receiver **18** assembly towed from, *e.g.*, a helicopter. A21 (col. 4, ll. 12-19); A23 (Fig. 1). The transmitter includes a wire coil **28** attached to a support frame **20**. A21 (col. 4, ll. 26-32); A22 (col. 5, ll. 13-20); A25 (Fig. 3). The transmitter coil **28** carries a time-varying (on-off) current that in turn

generates periodic electromagnetic field pulses. A20 (col. 1, ll. 26-29); A33 (col. 7, ll. 11-16). The receiver, equipped with a sensor coil **50**, measures the earth's response to the pulses. A20 (col. 1, ll. 26-35); A22 (col. 6, l. 9). A computer program analyzes the data the receiver obtains to produce useable survey data. A22 (col. 6, ll. 64-66).

While airborne TDEM surveying was known in the art, the '914 patent states that its system provides improved sensor resolution over known systems through the use of a dual-mode gain amplifier linked to the sensor. A21 (col. 3, ll. 25-26); *see also* A33 (col. 7, ll. 10-25). The dual-mode gain amplifier limits signal amplification when the transmitter is pulsed "on," but allows for high signal amplification when the transmitter is pulsed "off." A33 (col. 7, ll. 10-25). The resulting increased signal-to-noise ratio and reduced electrical interference improves overall sensor resolution. *Id.*

B. Prosecution of the Original Application

The '914 patent originated as U.S. patent application no. 10/716,813, which was filed on November 20, 2003. During prosecution of the application, the Examiner issued a restriction requirement,[2] separating the pending claims

[2] Section 121 of the Patent Act grants the Director the authority to limit a patent to one invention. 35 U.S.C. § 121. In a so-called "restriction requirement," the examiner typically organizes the claims into groups (*i.e.*, independent or distinct inventions) and requires the applicant to elect one group for prosecution. 37 C.F.R. § 1.142(a). Claims to the invention(s) not elected are

into three groups for purposes of examination:

- Group I, claims 1, 3-10, 12-13, 15-28, and 32-33, drawn to *a method and apparatus using a tow assembly*, the assembly comprising a flexible frame, the receiver flexibly connected to a receiver support frame and a dual mode gain amplifier, classified in class 324, subclass 330;

- Group II, claim 29, drawn to *a computer program* comprising a computer readable medium and instructions to operate a general electromagnetic antenna assembly, classified in class 702, subclass 5; and

- Group III, claims 30-31 and 34-35, drawn to *trailing airborne surveying systems* having specific antenna structures, classified in class 343, subclass 707.

A205-10 (emphases added).

The Examiner explained that the independent claims of each group had non-overlapping features and thus represented distinct subcombinations. A207-08; *see also* Manual of Patent Examining Procedure ("MPEP") § 806.05(d) (discussing restriction of claims to distinct subcombinations). The claims of Group III were separately grouped from the claims of Groups I and II because the Group III-claims required specific antenna (*i.e.*, transmitter and receiver) structure not found in the claims of Groups I and II. A207-08.

Specifically, independent claims 30 and 34 of Group III required a receiver support frame and a transmitter support frame connected to one another

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withdrawn from consideration, and if not later subject to a rejoinder process, are canceled prior to issuance. 37 C.F.R. § 1.142(b).

by way of a plurality of connecting cables (shown, for example, as tension rope **40** in Figure 4) evenly distributed along the circumference of the support frames. A316-17, 319-20 (nonelected claims 30 and 34); *see also* A22 (col. 5, ll. 40-47); A26 (Fig. 4) (reproduced below with red annotation).

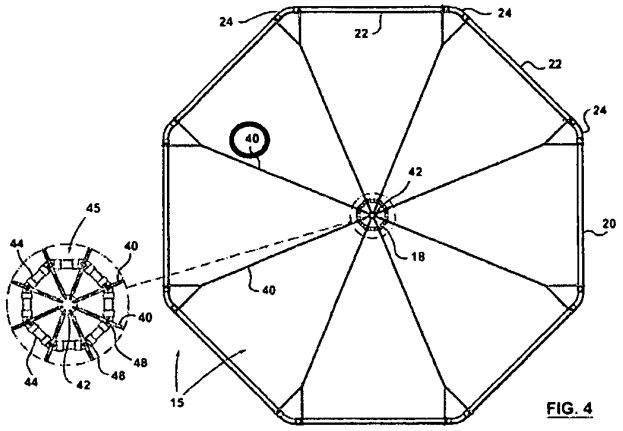

FIG. 4

Independent claim 31 of Group III specified that the receiver support frame consist of a plurality of interconnected frame members that are easily assembled and disassembled. A317-18 (nonelected claim 31).

In contrast, the independent claims of Groups I and II recited none of these features. A208. The claims of Group I focused on other features—specifically, the dual-mode gain amplifier and a sensor means flexibly connected to the receiver support frame. *Id.* And the claim of Group II recited a computer readable medium. *Id.* The Examiner also noted that the art recognized the claims of each group as having distinct subject matter and

separate classification, further supporting restriction of the claims into the three groups. *Id.*

In response to the restriction requirement, Morrison elected without traverse the claims of Group I, and withdrew claims 29-31 and 34-35 "without prejudice to the right to file one or more divisional applications comprising the subject matter of species (II) or (III)." A322; A6 (FF12). Immediately thereafter the Examiner allowed claims 1, 3-10, 12-13, 15-28, and 32-33 of Group I (renumbered as claims 1-27), and with Morrison's permission, canceled the nonelected claims of Groups II and III. *See* A324-27 (Notice of Allowability and Examiner's Amendment).

The '914 patent issued on January 2, 2007. A18. Independent claims 1 and 26 recite various embodiments of "[a]n airborne time domain electromagnetic surveying system." A33 (col. 8, ll. 15-42); A34 (col. 10, l. 51-col. 11, l. 13). Remaining independent claims 20 and 27 recite methods for "producing survey data." A34 (col. 9, l. 64-col. 10, l. 31); A35 (col. 11, l. 13-col. 12, l. 23). Morrison did not file any divisional application(s) to the nonelected subject matter or to any other non-claimed subject matter prior to issuance. *See, e.g.*, A6 (FF12).

C. Prosecution of the Reissue Applications

As described in his opening Brief, Br. at 5-6, Morrison filed two reissue applications from the '914 patent. The first, U.S. Application No. 12/343,321, contained the complete set of patented claims 1-27 from the '914 patent, as well as new claims 28-33 directed to a method for providing a tow assembly. *See* A328-29 (new claims 28-33). Consistent with the original prosecution, the Examiner restricted the new claims from the patented claims. A220-24. The Examiner explained that because the new claims do not require the dual-mode gain amplifier and the flexible connection between the sensor and receiver support frame characteristic of the patented claims, they are properly restricted from the patented claims. A221.

Morrison again elected to prosecute claims 1-27 to allowance, but this time filed a divisional application—the second reissue application and the application underlying this appeal—containing the nonelected reissue claims 28-33. A36-37. In his reissue declaration, A58-61, Morrison attested that the '914 patent was "wholly or partly inoperative by reason of an error in claiming less than I had a right to claim in the '914 patent," A59. Specifically, Morrison declared that he had claimed his invention too narrowly by overlooking embodiments that pertain to a method for providing a tow assembly at an assembly location. *Id.*

The Examiner rejected claims 28-33 and later-added claims 34-36 under 35 U.S.C. § 251 for lack of defect or error in the original patent correctable by reissue. *See* A242-57. Comparing the proposed reissue claims with the nonelected claims of Group III of Morrison's original application, the Examiner found that the reissue claims relate to subject matter not distinct from the nonelected claims, as both sets of claims are directed to the specific features of the transmitter and receiver structures of the towed assembly. A245. The Examiner explained that, had the reissue claims been presented in the original prosecution, those claims could not have been prosecuted in that application because they would have been grouped with the nonelected claims of Group III, or alternatively, in a separate group of claims. A245-46. Relying on MPEP § 1412.01, the Examiner concluded that Morrison's failure to file a divisional application to the nonelected subject matter prior to issuance of the '914 patent is not an error correctable by reissue. A246.

D. The Board's Decision

The Board affirmed the Examiner's rejection of all of the pending claims under 35 U.S.C. § 251 for lack of defect or error in the '914 patent correctable by reissue. A12. First, the Board found independent claim 28, as follows, representative:

> 28. A method for providing a tow assembly for an electromagnetic airborne survey system at an assembly location, comprising:
>
> providing a plurality of hollow transmitter support frame sections;
>
> providing a transmitter coil for generating a primary magnetic field;
>
> providing connector cables and a tow cable; and
>
> assembling the transmitter support frame sections and the transmitter coil and the cables to provide a tow assembly having a flexible hollow transmitter support frame that is circular or approximates a circle having the transmitter coil disposed within the hollow transmitter support frame and formed from serially connecting the transmitter support frame sections, with the connector cables each having a first end connected to a respective location on a circumference of the transmitter support frame and a second end connected to a first end of the tow cable.

A3; *see also* A200.

The Board then addressed the rejection under § 251, and found that the evidence of record supported the Examiner's conclusion that the '914 patent does not contain an error correctable by reissue. A4-12. The Board agreed with the Examiner that Morrison's reissue claims are substantially similar to the nonelected claims of Morrison's original application. A9. Specifically, the Board compared the subject matter of representative reissue claim 28 to that of original, nonelected claim 34 of Group III and found no substantial difference between the scope and structural features recited in the claims. A9-11. The

Board concluded that the reissue claims would have been grouped with nonelected Group III. A11. And because Morrison chose not to file a divisional application to the nonelected subject matter, the Board explained, he is precluded from pursuing those claims under the *Orita* doctrine. *Id.* (citing *In re Orita*, 550 F.2d 1277 (CCPA 1977)).

The Board also found unpersuasive Morrison's argument that his reissue application is proper because the reissue claims contain subject matter not presented in any of the original claims. A11. Even assuming that the reissue claims were never expressly "non-elected," they would have been grouped into another nonelected group had they been presented and lost through Morrison's failure to file a divisional application to those claims. *Id.* The Board explained that under this Court's decision in *In re Weiler*, 790 F.2d 1576, 1579 (Fed. Cir. 1986), Morrison's failure to file divisional applications to subject matter not previously claimed does not constitute error correctable under § 251. A11.

III. SUMMARY OF THE ARGUMENT

The *Orita-Weiler-Doyle* line of cases prohibits a patentee from using a reissue proceeding to obtain claims that the patentee could not have prosecuted in his or her original application. These cases are based on the rationale that, in acquiescing to an examiner's restriction requirement, the applicant has chosen to pursue claims only to the elected invention. In other words, the applicant has

agreed to independently prosecute any invention distinct from that elected in divisional application(s). This Court accordingly does not treat a patentee's failure to file those divisional application(s) as an "error" correctable under the reissue statute. Otherwise, the patentee who failed to file a divisional application could use a reissue proceeding to circumvent the co-pendency requirement of 35 U.S.C. § 120.

Morrison attempts to do exactly what the *Orita-Weiler-Doyle* line of cases rejects. In response to the Examiner's restriction requirement, Morrison elected without traverse the claims of Group I and prosecuted them to allowance. During pendency of his original application, Morrison did not file divisional application(s) to the nonelected claims or to any other distinct subject matter he disclosed. Morrison now seeks to prosecute an invention that he chose not to pursue in a divisional application. But that invention, as Morrison admits, is distinct from his patented claims and accordingly could not have been prosecuted in his original application. Put differently, because Morrison claimed exactly what he had a right to claim in his original application—*i.e.*, the elected method and apparatus claims—the Board correctly determined that the '914 patent lacks an error correctable under the reissue statute. For this reason, the Board's rejection should be affirmed.

IV. ARGUMENT

A. Standard of Review

Morrison has the burden to show that the Board committed reversible error. *In re Watts*, 354 F.3d 1362, 1369 (Fed. Cir. 2004). Whether the claims of a reissue application are unpatentable under 35 U.S.C. § 251 is a question of law based on underlying findings of fact. *In re Serenkin*, 479 F.3d 1359, 1361 (Fed. Cir. 2007). This Court reviews the Board's legal conclusions *de novo* but affirms the Board's factual findings that are supported by substantial evidence. *In re Jolley*, 308 F.3d 1317, 1320 (Fed. Cir. 2002); *see also* 5 U.S.C. § 706(2)(E). Substantial evidence is "such relevant evidence as a reasonable mind might accept as adequate to support" the conclusion reached. *Consol. Edison Co. v. NLRB*, 305 U.S. 197, 229 (1938). It is "something less than the weight of the evidence but more than a mere scintilla of evidence." *In re Kotzab*, 217 F.3d 1365, 1369 (Fed. Cir. 2000). "[W]here two different, inconsistent conclusions may reasonably be drawn from the evidence in record, an agency's decision to favor one conclusion over the other is the epitome of a decision that must be sustained upon review for substantial evidence." *Jolley*, 308 F.3d at 1329.

B. The Board Correctly Concluded That Morrison's Patent Lacks an Error Correctable by Reissue

Section 251 of the 1952 Patent Act provides for the reissue of a patent that "through error without any deceptive intention, [is] deemed wholly or partly

inoperative or invalid . . . by reason of the patentee claiming more or less than he had a right to claim in the patent." 35 U.S.C. § 251 (2006), *amended by* 35 U.S.C. § 251(a) (2011) (adding paragraph letters and deleting "without any deceptive intention"). Fundamental principles of equity and fairness guide the application of the reissue statute, as Morrison notes. *See* Br. at 19. But "[b]y its terms section 251 restricts reissue to situations in which an error occurred." *In re Doyle*, 291 F.3d 1355, 1358 (Fed. Cir. 2002). And not just any "error" qualifies under the statute. *E.g.*, *Weiler*, 790 F.2d at 1579 ("[N]ot every event or circumstance that might be labeled 'error' is correctable by reissue."). This Court and its predecessor court have generally refused to call "error" the deliberate choices a patent applicant makes (albeit later regretted) to obtain allowance. *See e.g.*, *In re Yamazaki*, 702 F.3d 1327, 1333 (Fed. Cir. 2012) (holding that patentee's failure to prevent a patent from issuing with terminal disclaimer not an error correctable by reissue); *In re Mostafazadeh*, 643 F.3d 1353, 1358 (Fed. Cir. 2011) (reiterating that intentional surrender of subject matter during original prosecution to overcome prior art not an error); *Serenkin*, 479 F.3d at 1363 (holding that a "conscious decision" to accept a later filing date for an application not a correctable error); *In re Mead*, 581 F.2d 251, 257 (CCPA 1978) (holding that patentee's "conscious choice" not to file a

continuing application not correctable error). The *Orita*, *Weiler*, and *Doyle* decisions, relevant to this appeal and discussed in detail below, stand in accord.

1. The *Orita-Weiler-Doyle* line of cases prohibits a patentee from using a reissue proceeding to obtain claims that the patentee could not have prosecuted in his or her original application

Orita, *Weiler*, and *Doyle* hold that when an applicant makes a deliberate choice in response to a restriction requirement to prosecute a single invention and fails to file divisional application(s) to the remaining, nonelected inventions—whether originally claimed or not—an error correctable by § 251 does not exist. These cases are based on the rationale that once an applicant acquiesces to a restriction requirement, any invention distinct from the one elected and prosecuted to allowance can only be pursued in a timely-filed divisional application.

In *Orita*, the CCPA addressed the issue of whether an applicant's failure to file a divisional application to nonelected claims constituted an "error" under § 251. 550 F.2d at 1280. During the prosecution of Orita's original application, the examiner issued a restriction requirement separating the claims into two groups. *Id.* at 1278. The applicants elected the first group and canceled the nonelected claims of the second. *Id.* After a patent issued, applicants filed a reissue application to claims "substantially identical to the originally non-elected claims," and stated in a declaration that they had claimed less than they had a

right to claim because "they had forgotten to timely file a divisional application covering the originally non-elected subject matter." *Id.* at 1278-79.

The court upheld the Board's denial of the reissue, holding that applicants' acquiescence to the examiner's restriction requirement did not constitute an "error" under § 251. The court explained that while "appellants undoubtedly erred by failing to file a timely divisional application," the resulting patent was error-free: "Patentees claimed exactly what they had a right to claim in the patent, no more nor less, and appellants' failure to timely re-file does not change this fact." *Id.* at 1280.

The court also noted that allowing reissue claims substantially identical to those not elected would render the co-pendency requirement of § 120 "meaningless." The court continued:

> for should an applicant fail to file a divisional application while maintaining copendency as required by section 120, he could simply revert to section 251 in order to cure his mistake. Section 251 is not a panacea designed to cure every mistake which might be committed by an applicant or his attorney, and the case at bar exemplifies a mistake which this section cannot cure.

Id. at 1281.

The CCPA's decision gave rise to what is now commonly referred to as "the *Orita* doctrine." It finds its roots in two pre-1952 Patent Act cases: *In re Cornell*, 150 F.2d 702 (CCPA 1945) and *In re Smyser*, 135 F.2d 747 (CCPA 1943). In both of those cases, the CCPA held that the reissue statute did not

authorize claiming of subject matter in a reissue that could not have been included in the original application as a result of applicants' acquiescence in the examiner's requirement for "division." *Cornell*, 150 F.2d at 704-05; *Smyser*, 135 F.2d at 751.

Following *Orita*, which dealt with *claimed* subject matter, this Court held in *Weiler* that error correctable by § 251 does not lie in failing to file divisional applications to *non-claimed* subject matter distinct from the subject matter that the applicant elected. *Weiler*, 790 F.2d at 1580. The examiner in *Weiler* restricted the original claims into three groups. Weiler chose the first group without traverse and prosecuted those claims to allowance, but did not file divisional applications directed to the nonelected subject matter or to any other disclosed subject matter. *Id.* at 1578. After issuance, Weiler filed a reissue application with claims to subject matter disclosed but never claimed in the original application. *Id.* at 1578-79. This Court upheld the Board's rejection of the reissue application for lack of error. In doing so, this Court noted that the subject matter of the claims on appeal was "clearly independent of and distinct" from that of both the elected and nonelected claims, and accordingly, "Weiler would thus have had no right to insert and present [the reissue claims] in the original application after the examiner's requirement for restriction." *Id.* at 1581. Following the logic of *Orita*, this Court held that by acquiescing in an

examiner's restriction requirement, "it cannot on the present record have been error to forego divisional applications on subject matter to which claims had never been made." *Id.* at 1582.

While *Orita* and *Weiler* addressed specific claims to distinct inventions, *Doyle* addressed a "linking" claim, *i.e.*, "a claim broad enough to read on—or link—two or more groups of claims subject to a restriction requirement." 293 F.3d at 1358. This Court characterized the issue in *Doyle* as "whether failure to present a so-called linking claim . . . is an error correctable by reissue." *Id.* Doyle's original application included both compound and method claims. *Id.* at 1356. In response to the examiner's nine-way restriction requirement, Doyle elected the claimed invention of using a genus of catalysts to insert carbenes into certain chemical bonds, and canceled the remaining claims. *Id.* Doyle did not file any divisional applications to the nonelected claims. But following issuance of the patent, Doyle filed a reissue application with claims directed to a method of using a genus of catalysts to insert any prochiral molecule (*i.e.*, not just carbene) into the chemical bonds. *Id.* at 1357. Doyle conceded that the new claims, while broader in scope than the elected invention, read on some of the nonclcctcd claims. *Id.*

The Board upheld the rejection of the claims based on lack of error correctable by reissue. *Id.* at 1358. This Court reversed. In doing so, the Court

first noted that "[t]he so-called *Orita* doctrine . . . precludes a reissue applicant from obtaining substantially identical claims to those of nonelected groups identified in an examiner's restriction requirement when such claims could not have been prosecuted in the application from which they were restricted." *Id.* at 1359. The Court then explained that *Weiler* prevents a reissue applicant from pursuing claims to "a completely separate invention from the issued claims or any of the nonelected claims." *Id.* at 1362. Such claims, the *Doyle* Court explained, if presented during the original prosecution, "would have been subject to the original restriction requirement and the applicant would not have been allowed to prosecute them with the original patent—he would have been required to assert them in a divisional." *Id.*

But in *Doyle*, the Court held that neither *Orita* nor *Weiler* applied because Doyle's reissue claims were "not to an invention distinct from that of the issued claims." *Id.* at 1363. Unlike Orita and Weiler, Doyle could have—and indeed *should have*—prosecuted the broader linking claims (linking the elected and nonelected claims) in the original application. *Id.* And importantly, because Doyle never acquiesced to prosecuting the broader linking claims in a divisional, the estoppel rationale underlying *Orita* and *Weiler* did not apply. *Id.*

The *Orita* and *Weiler* cases stand for the "established principle that applicants are 'estopped from obtaining by reissue claims which, because of a

requirement for restriction in which they had acquiesced, they could not claim in their patent.'" *In re Watkinson*, 900 F.2d 230, 232 (Fed. Cir. 1990) (quoting *Orita*, 550 F.2d at 1280). *Doyle* comports with that principle, as Doyle could have prosecuted his reissue claims in his original application. That he failed to prosecute his reissue claims in his original application constituted a correctable error of claiming less than Doyle had a right to claim under § 251. In contrast, the applicants in *Orita* and *Weiler* could not have pursued their reissue claims in their original applications because those claims were distinct from the elected invention. Again, by acquiescence to a restriction requirement, those applicants agreed to limit their original application to the elected invention. Therefore they were estopped from submitting other, distinct subject matter in reissue that they failed to timely submit in a divisional application during the pendency of their original applications.

2. The '914 patent lacks an error because the now-claimed subject matter could not have been prosecuted in Morrison's original application

In this case, the Board correctly found that Morrison is estopped from prosecuting via reissue claims directed to an invention distinct from his elected—and ultimately patented—invention. A11-12; *see also* A252 (stating that "appellants' new claims [are] directed to an invention that is completely different/patentably distinct from the elected invention of the original patent").

The Examiner restricted the claims of the original application into three independent groups (inventions).[3] A207. Morrison elected Group I without traverse, thereby acquiescing to the Examiner's determination that the *only* invention that the '914 patent could claim was the assembly and method of Group I. A6 (FF12). Put differently, Morrison agreed to prosecute all subject matter distinct from the claims of Group I in timely-filed divisional applications.

Instead of filing divisional applications prior to issuance of the '914 patent, however, Morrison now attempts to claim that nonelected subject matter through reissue. But this Court's precedent of almost 70 years forecloses him from doing so. Specifically, Morrison's reissue claims represent an invention substantially similar to previously-claimed subject matter that he deliberately abandoned after the restriction requirement, and therefore this case squarely fits into the facts of *Orita*, or alternatively, Morrison's reissue claims represent a distinct but "overlooked" invention that he failed to originally claim at all, and this case squarely fits into the facts of *Weiler*. *See* A9-11; *infra* at 24-36.

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[3] Morrison does not challenge the propriety of the original restriction requirement. Nor could he, as "neither this court nor the board has jurisdiction in this proceeding to review the merits of a requirement for restriction under section 121, as a restriction requirement is a matter within the discretion of the examiner and not tantamount to a rejection of claims." *Watkinson*, 900 F.2d at 233.

But whichever case applies directly to the facts at hand makes no difference in the end. It is dispositive here that the reissue claims are distinct from Morrison's elected invention, *i.e.*, the claims of Group I, as Morrison has consistently acknowledged throughout prosecution. *See* A155 (Response to Non-Final Office Action) (stating that the reissue claims are "independent and distinct from the restricted group of claims" and "not properly grouped within the prior restriction"); A187-88 (Appeal Brief) (stating that the reissue claims "do[] not fall within the Group I invention that was elected in the . . . application that matured into the original '913 [*sic*] patent").[4] Morrison also admits to this Court that his reissue claims are distinct from the claims of his elected invention. *See* Br. at 14 ("these inventions are separate and distinct, even though related as subcombinations"). Morrison accordingly could not have prosecuted the distinct reissue claims in his original application because of his acquiescence to the restriction requirement, and his resulting patent lacks an error correctable by reissue. *Doyle*, 293 F.3d at 1361; *Watkinson*, 900 F.2d at 232; *Weiler*, 790 F.2d

[4] Morrison's position was also made clear in oral argument before the Board. *See* A300 (Oral Argument Transcript) (JUDGE SAADAT: So I just want to make the record clear, had these claims been part of the original application do you think they would have been restricted into a different group, or they would be part of group I? MR. MARCUS: In my opinion, they would have been restricted into a different group . . . . JUDGE SAADAT: So, if you accept that as a plausible situation, these claims would have been restricted into a different group than group I. MR. MARCUS: In my view, yes.").

at 1582, *Orita*, 550 F.2d at 1280; *Cornell*, 150 F.2d at 704; *Smyser*, 135 F.2d at 751.

Specifically, § 251 requires error in the issued patent. *See, e.g.*, *Doyle*, 293 F.3d at 1361. The thrust of the *Orita-Weiler-Doyle* line of cases is that when a patentee has claimed exactly that to which he is entitled in a patent because he has agreed to claim only the elected invention—no error exists in that patent. *Orita*, 550 F.2d at 1280. And here, Morrison claimed exactly what he had a right to claim—the elected claims of Group I that issued as claims 1-27 of the '914 patent. Thus, Morrison's repeated assertion that his "error" lies not in failing to file a divisional application with his current reissue claim, but in failing to present those claims during prosecution of the original application, *see, e.g.*, Br. at 25, 26, 36, lacks merit. *Even if* Morrison had presented the reissue claims during the original prosecution of the '914 patent *and* the Examiner had entered those claims, the result is still a dead end. After having been presented, those new claims would have been restricted and withdrawn as directed to nonelected subject matter, *see* MPEP § 821.03, and consequently would have been lost through Morrison's failure to file a timely divisional application, *Doyle*, 293 F.3d at 1362.

Put differently, no matter how Morrison characterizes the "error" he made during the original prosecution, his issued patent lacks an error correctable by

reissue "because the matter sought on reissue could not have been prosecuted originally." *Doyle*, 293 F.3d at 1361. In this case, the Board merely followed this Court's well-established precedent that whenever an examiner issues a restriction requirement, she is by definition requiring the applicant to claim every other distinct invention disclosed in the specification—whether originally claimed or not—in a timely-filed divisional application. By acquiescing to the restriction requirement, the applicant has agreed to do so. As this Court has explained, there is nothing unfair about applying the reissue statute in such a way that it holds the patentee to his word, *Doyle*, 293 F.3d at 1360, and prevents circumvention of the co-pendency requirement of 35 U.S.C. § 120, *Orita*, 550 F.2d at 1280-81.

In sum, reissue does not exist to correct Morrison's "error." As this Court has repeatedly explained, reissue simply is not "a panacea" to correct every prosecution mistake. *See MBO Labs., Inc. v. Becton, Dickinson & Co.*, 602 F.3d 1306, 1313 (Fed. Cir. 2010); *Serenkin*, 479 F.3d at 1362; *Doyle*, 293 F.3d at 1358; *Nupla Corp. v. IXL Mfg. Co.*, 114 F.3d 191, 195 (Fed. Cir. 1997); *Weiler*, 790 F.2d at 1582.

3. *Weiler* dictates that Morrison cannot claim in reissue distinct subject matter that was disclosed but not originally claimed in the '914 patent

Morrison asserts that his reissue claims represent a disclosed but "overlooked embodiment" of his invention, and that the claims are proper subject matter for a reissue application. Br. at 21. Not so. Even if he never presented the reissue claims in his original application, Morrison's failure to timely file a divisional application to the subject matter of those claims during the prosecution of the '914 patent does not constitute an error correctable by reissue. *Weiler*, 790 F.2d at 1579. As explained above, this conclusion is based on the rationale that failing to file a divisional application to distinct subject matter—whether originally claimed or not—that could not have been prosecuted in the original application due to an examiner's restriction requirement is not an "error" within the meaning of § 251.

Assuming that the reissue claims are distinct from the nonelected claims of Group III, the facts of *Weiler* are identical to those here. The examiner in *Weiler*, like the Examiner during prosecution of the '914 patent, issued a restriction requirement. 790 F.2d at 1578. The applicants in *Weiler*, like Morrison, deliberately chose to claim one invention without traverse, but failed to file any divisional applications to the non-claimed subject matter. *Id.* Finally, the applicants in *Weiler*, again like Morrison, filed a reissue application with

claims to subject matter not claimed at all in the original application. *Id.* at 1578-79. But this Court held that "an inventor's failure to claim particular subject matter was not the result of the 'error' required by § 251." *Id.* at 1583. Thus, even if Morrison is correct that the reissue claims are distinct from the original, nonelected claims of Group III (and would not have been grouped with those claims), this Court's holding in *Weiler* would still preclude him from obtaining the claims in reissue. *Id.* at 1582 ("If it were not error to forego divisional applications on subject matter to which claims had been made in the original application, it cannot on the present record have been error to forego divisional applications on subject matter to which claims had never been made.").

Morrison attempts to cast doubt over *Weiler* by mischaracterizing its holding, and by relying on subsequent cases (which could not have overruled *Weiler*) and commentary in Chisum. Br. at 21-24, 34. But these arguments lack merit.

First, contrary to Morrison's arguments otherwise, the "intent to claim" issue addressed in *Weiler* does not alter its holding that failure to file a divisional application does not constitute correctable error under § 251. The *Weiler* Court addressed "intent to claim" because lack of an "intent to claim" formed the basis of the Board's rejection of Weiler's claims. Specifically, the Board found no

error in Weiler's original patent because, in its view, Weiler had no "intent to claim" the subject matter of reissue claims 13 and 19. 790 F.2d at 1580. The Board based its holding on the fact that Weiler never presented those claims during prosecution of his original application, and the Solicitor's brief in the appeal pointed out that Weiler's specification provided only minimal disclosure for those claims. *Id.* at 1579-80. But in addressing the "intent to claim" issue, this Court characterized the Board's focus on Weiler's "intent to claim" "infelicitous," and "reflect[ing] non-statutory language." *Id.* at 1580, 1583. The *Weiler* Court explained that the "central question" before it was "not whether there is disclosure, but whether Weiler ha[d] established 'error' which can be remedied by reissue." *Id.* at 1582. The Court concluded that Weiler's alleged "error"—the failure to file divisional applications to the previously unclaimed subject matter—is not one the reissue statute serves to remedy. *Id.* The same is true for Morrison's "error" here.

Morrison attempts to color the clear holding of *Weiler* as dicta. Br. at 33. But this Court's extensive analysis of *Weiler* in its subsequent *Doyle* opinion refutes Morrison's position. *See Doyle*, 293 F.3d at 1362-63. As the *Doyle* Court explained, "*Weiler* applies only to an attempt to assert new claims in reissue that read on a separate invention and not on the subject matter of the issued claims." *Id.* at 1363. *Doyle* further explained that because Weiler's

reissue claims 13 and 19 were directed to a distinct invention, "they too would have been subject to the original restriction requirement and the applicant would not have been allowed to prosecute them with the original patent—he would have been required to assert them in a divisional." *Id.* at 1362. *Doyle* concludes:

> Under *In re Orita*, of course, there is no correctable error in failing to prosecute divisional applications on inventions of the nonelected groups identified by the examiner in the original restriction requirement. In *In re Weiler*, we held that *the result should be the same* with respect to claims 13 and 19, which also asserted *wholly distinct inventions from that covered by the issued patent.*

Id. at 1362 (emphases added).

All parties agree that Morrison's reissue claims, like the reissue claims in *Weiler*, are distinct from his issued claims. As *Doyle* explains, whether under *Orita* or under *Weiler*, no error lies in failing to file divisional applications to claims "wholly distinct from that covered by the issued patent." *Id.* Otherwise, "the copendency requirement would become meaningless, for should an applicant fail to file a divisional application while maintaining copendency as required by section 120, he could simply revert to section 251 in order to cure his mistake." *Orita*, 550 F.2d at 1281.

Second, Morrison's invocation of *In re Amos*, 953 F.2d 613 (Fed. Cir. 1991) lacks merit. Morrison relies on *Amos* for the proposition that "overlooked embodiments" may be claimed through reissue. Br. at 22-25. But the only issue

this Court addressed in *Amos* was whether an applicant's "intent to claim" may serve as an independent basis for denying a reissue application under § 251. *Amos*, 953 F.2d at 616. And this Court held, as it did in *Weiler*, that "intent to claim" is not dispositive of any inquiry under § 251, and so reversed the Board's decision. *Id.* at 619. In contrast to the facts of *Amos*, the Board in this case did not refuse Morrison's reissue application for an alleged lack of "intent to claim." The Board upheld the Examiner's rejection of the reissue claims for lack of an error under § 251—a rejection that the *Amos* Court notes is proper under the statute. *Id.* at 617. Moreover, instead of criticizing *Weiler* as Morrison appears to assert, Br. at 35, *Amos* positively cites to *Weiler*. 953 F.2d at 617 (citing 790 F.2d at 1581). Thus, *Amos* does not stand for the proposition that "overlooked embodiments" are always proper subject matter for reissuance simply because they are disclosed in an original application, and its holding is inapposite here.

Third, Morrison's speculation that "there is no essential or substantive difference between reissue claims that broaden the scope of what is covered in the original patent, and reissue claims that cover subject matter not claimed at all in the original patent," Br. at 34 (citing 4A Donald S. Chisum, *Chisum on Patents* § 15.03[2][b][iv] (2004)), ignores the important distinction underlying the *Orita-Weiler-Doyle* line of cases. In each case denying reissue, the applicants *acquiesced* to an examiner's restriction requirement. They *agreed* to

prosecute all subject matter distinct from the elected invention—whether classified in a nonelected group of claims or never claimed at all—in timely-filed divisional applications. In other words, the applicants chose *what* to prosecute and thus *what not* to prosecute. As a result, they were "estopped from obtaining by reissue claims which, because of a requirement for restriction in which they had acquiesced, they could not claim in their patent." *Orita*, 550 F.2d at 1280.

This scenario—in which an applicant chooses the invention to pursue—stands in stark contrast with that in which an examiner makes no restriction requirement during the original prosecution. In the latter scenario, a patentee can claim through reissue any invention otherwise meeting all other statutory requirements under the Patent Act, because that patentee has made *no agreement* respecting the subject matter that might have been claimed in the original application. *See In re Staats*, 671 F.3d 1350, 1355 (Fed. Cir. 2012) (holding that patentee could obtain claims in a continuation reissue application to embodiment not claimed within two-year time period); 37 C.F.R. § 1.176(b) (discussing restriction between subject matter of original patent claims and previously unclaimed subject matter in reissue application). And, as Morrison must admit, none of the cases on which he appears to rely for his "overlooked embodiments" argument involved restriction requirements. *See* Br. at 25 (acknowledging that

the facts of *Amos* do not include a restriction requirement); *see also In re Swartzel*, 60 F.3d 843, 1995 WL 389111, *2 (Fed. Cir. 1995) (unpublished) (declining to extend the *Orita* "divisional doctrine" where no restriction requirement issued and applicant misunderstood scope of claims).

Moreover, although it would not change the outcome here, Morrison has made no allegation that he miscommunicated with his prosecuting attorney or agent or that he or the prosecuting attorney or agent lacked a full appreciation of the scope of disclosed subject matter, which as Chisum acknowledges, typically supports reissue applications to broader subject matter. *Chisum on Patents* § 15.03[2][b][iv]; *see also In re Wilder*, 736 F.2d 1516, 1519 (Fed. Cir. 1984) (holding that attorney's failure to appreciate the full scope of the invention resulted in error in the issued patent correctable by reissue).

4. *Orita* prevents Morrison from obtaining in reissue subject matter not elected in the original prosecution of the '914 patent

This Court need not decide Morrison's "overlooked embodiments" argument, because as the Board found, the facts of this case are on all fours with those of *Orita*. As Morrison acknowledges, Br. at 25-26, a patentee's failure to timely file a divisional application to nonelected subject matter in response to a restriction requirement is not an error correctable by reissue. *Orita*, 550 F.2d at 1280-81. Morrison nonetheless asserts that the *Orita* doctrine does not apply

here because the reissue claims "were never the subject of a restriction requirement." Br. at 25. Morrison is mistaken.

As an initial matter, Morrison's attempt to impose an *ipsis verbis* test for applying the *Orita* doctrine should be rejected. *See* Br. at 25-26 (arguing that the *Orita* doctrine only applies to claims that were actually nonelected in the original application). Nothing in *Orita* requires that the claims sought in reissue recite identical words as the nonelected claims of the original prosecution in order to run afoul of its holding. *Orita* instead involves a determination of whether the reissue claims would have been grouped with the nonelected claims because they are directed to the same subject matter. In other words, "substantially identical" as used in *Orita* refers to those "reissue claims which, because of a requirement for restriction in which [applicants] had acquiesced, they could not claim in their patent." *Orita*, 550 F.2d at 1280.

And here, the Board found that Morrison could not have prosecuted his reissue claims in his original application because those claims would have been withdrawn for belonging to a nonelected group of claims—*i.e.*, Group III. The Board compared reissue claim 28 and original, nonelected claim 34 of Group III and found that, although they are directed to different statutory categories, the claims have substantially the same scope and structural features and therefore

would have been classified in the same inventive group. A9-11. The Board's finding is supported by the words of the claims themselves.

Specifically, both reissue claim 28 and original, nonelected claim 34 recite specific structural features of a particular tow assembly for airborne electromagnetic surveying. A10. Claim 28 recites "providing a plurality of hollow transmitter support frame sections." *Id.* Claim 34 correspondingly recites "a transmitter section . . . consisting of a plurality of interconnectable transmitter section frame members." A320. Although the specification does not use the term "hollow" as recited in claim 28, the specification describes the transmitter section **16** as consisting of a support frame **20** consisting of a plurality of "substantially tube sections **22**." A21 (col. 4, ll. 26-30). The specification further explains the various tube sections are interconnected. *Id.* (col. 4, ll. 30-32). Thus, claim 28 recites a plurality of hollow transmitter frame sections substantially identical to the interconnectable transmitter section frame members recited in claim 34.

Reissue claim 28 also recites "providing a transmitter coil for generating a primary magnetic field . . . having the transmitter coil disposed within the hollow transmitter support frame." A3. Morrison contends the Board omitted the "transmitter coil disposed within the hollow transmitter support frame" in its analysis. Br. 27-28. But nonelected claim 34 similarly recites "the transmitter

section houses an elastically suspended transmitter coil." A320. The specification discloses the transmitter coil **28** is strung along the bottom of support frame **20** by attaching the transmitter coil from multiple points along the support frame **20** by a suitable form of attachment. A22 (col. 5, ll. 15-19). The specification further points out that the transmitter coil **28** can be disposed inside the support frame. *Id.* (col. 5, ll. 19-20). Therefore, claim 28 recites a transmitter coil disposed within the hollow transmitter support frame substantially identical to the elastically suspended transmitter coil housed within the transmitter section of claim 34.

Reissue claim 28 then recites "providing connector cables and a tow cable . . . with the connector cables each having a first end connected to a respective location on a circumference of the transmitter support frame and a second end connected to the first end of the tow cable." A3; A200. Similarly, claim 34 states "[w]herein the tow assembly apparatus is connected to the aircraft by means of at least one cable means connected to the transmitter section at a plurality of points." A320. The specification discloses the rope **26** is used to suspend the support frame **20** at points along the circumference thereof and attached to a central tow cable. A22 (col. 5, ll. 5-13). Thus, reissue claim 28 and original, nonelected claim 34 both recite cables connecting the transmitter to a tow cable at various points.

Further, claim 28 recites "assembling the transmitter support frame sections and the transmitter coil and the cables to provide a tow assembly having a . . . frame that is circular or approximates a circle." A3. Similarly, claim 34 incorporates "a transmitter section having a central axis . . . and the transmitter section frame members define a polygonal profile." A320. The specification contemplates that the support frame **15** of the tow assembly has a polygonal shape approximating a circular shape. A21 (col. 4, ll. 51-55). Therefore, reissue claim 28 and original, nonelected claim 34 both embody a circularly-shaped transmitter.

Claim 28 additionally incorporates "serially connecting the transmitter support frame sections." Morrison again argues the Board intentionally omitted this feature in its analysis. Br. 27-28. But the specification describes the support frame **20** consisting of tube sections **22** whereby adding tube sections **22** together provides a support frame. *See* A21 (col. 4, ll. 42-47). Claim 34 analogously recites a "transmitter section . . . consisting of a plurality of interconnectable transmitter section frame members, such that the transmitter section can be assembled." A320.

Morrison points out that claim 34 recites structural limitations related to the receiver section of the tow assembly. Br. at 29. But the absence of these features from claim 28 does not mean that claim 28 would have been classified

in a different inventive group than claim 34. Two claims can be indistinct and grouped together even though they do not recite identical limitations. This makes sense, as no two claims recite the exact same limitations or are of the exact same scope in a single application. *See* 37 C.F.R. § 1.75(b) ("More than one claim may be presented provided they differ substantially from each other and are not unduly multiplied.").

Morrison also argues that reissue claim 28 would not have been grouped with original claim 34 because claim 34 is an apparatus claim while claim 28 is a method claim having "manipulative steps." Br. at 27-29. Not so. As a general matter, no rule requires an automatic restriction between apparatus and method claims. Indeed, the Examiner in Morrison's original application grouped both method and apparatus claims into Group I. A205. Similarly here, the Board found no substantial difference between claim 28 and claim 34 "even though they are directed to different statutory categories." A11. Moreover, claims related as a process of making and a product made are grouped together when the process cannot make a product materially different from the product claimed, or the product cannot be made by any other process. *See* MPEP § 806.05(f). Here, claim 28 recites a method of making a tow assembly having specific structural features that are recited in claim 34. Thus, reissue claim 28 and claim 34 would have been joined together in nonelected Group III. A9.

Morrison's additional argument that the Board's analysis was "speculative" lacks merit. Br. at 29. Many questions of patent law require backward-looking inquiries (*e.g.*, obviousness and damages), and the analysis is no different with respect to the *Orita* doctrine. The only way the USPTO can give effect to *Orita* (and *Weiler*) is to necessarily look back in time and answer the question of whether the reissue applicant *could have* prosecuted his claims in his original application. *Orita*, 550 F.2d at 1280. That inquiry is not based on pure speculation, but on consideration of the original restriction requirement and the distinctions and similarities (if any) between the claims. Here, the Board compared the subject matter of the representative reissue claim to a nonelected claim, and found no patentable distinction between the two. That is not "speculation," but fact-finding based on the words and scope of the claims themselves.

5. Morrison's "law of the case" argument does not help him

Finally, Morrison asserts that the Board ignored the "law of the case," which Morrison refers to as "the Examiner's findings justifying the original restriction requirement between the elected claims of Group I and the non-elected Groups II and III." Br. at 30-31. Morrison's assertion misses the point. The issue in this case is whether the '914 patent contains an error correctable in the reissue application on appeal. Again, the *Orita-Weiler-Doyle* case law

requires a determination of whether the reissue claims would have been grouped with the original, elected claims of Group I. That the original claims of each of Groups I, II, and III were distinct from one another does not necessarily answer the question of whether the presently-appealed reissue claims are distinct from the claims of Group I. And in answering that question, the Board did not ignore what Morrison calls the "law of the case," and neither does the Director. Indeed, the fact that two different examiners in two applications restricted the reissue claims from the patented claims only further confirms the Director's point—that the reissue claims are distinct from the claims of Group I and therefore could not have been prosecuted in Morrison's original application. And whether the facts of this case align more closely to *Weiler* or to *Doyle* makes no difference to the outcome. That Morrison could not have prosecuted his reissue claims in his original application is dispositive, because he is now estopped from obtaining those claims in reissue. *Watkinson*, 900 F.2d at 232.

V. CONCLUSION

For the foregoing reasons, the Board's conclusion that the '914 patent lacks an error correctable by reissue is correct. This Court should affirm the Board's decision.

Respectfully submitted,

February 18, 2014

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CERTIFICATE OF SERVICE

I hereby certify that on February 18, 2014, I electronically filed the foregoing BRIEF FOR APPELLEE—DIRECTOR OF THE UNITED STATES PATENT AND TRADEMARK OFFICE using the Court's CM/ECF filing system. Counsel for appellant was electronically served by and through the Court's CM/ECF filing system per Fed. R. App. P. 25 and Fed. Cir. R. 25(a) and 25(b).

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RULE 32(a)(7)(C) CERTIFICATE OF COMPLIANCE

I certify pursuant to Fed. R. App. Proc. 32(a)(7) that the foregoing BRIEF FOR APPELLEE—DIRECTOR OF THE UNITED STATES PATENT AND TRADEMARK OFFICE complies with the type-volume limitation required by the Court's rule. The total number of words in the foregoing brief, excluding the table of contents and the table of authorities, is 8471 words as calculated using the Word® software program.

/s/ Kristi L. R. Sawert
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2012-1689
(Serial No. 11/358,951)

UNITED STATES COURT OF APPEALS FOR THE FEDERAL CIRCUIT

IN RE BEKA SOLOMON

Appeal from the United States Patent and Trademark Office,
Patent Trial and Appeal Board.

BRIEF FOR APPELLEE—ACTING DIRECTOR OF THE UNITED STATES PATENT AND TRADEMARK OFFICE

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March 15, 2013

REPRESENTATIVE CLAIM

5. A method of inhibiting aggregation of soluble β-amyloid in a human subject, comprising

causing an effective amount of an anti-β-amyloid antibody, or an antigen-binding fragment thereof, to come into contact with the soluble β-amyloid,

said antibody or fragment being effective to bind β-amyloid and to inhibit aggregation of the soluble β-amyloid in said subject to an extent at least as great as that obtainable with antibody AMY-33, thereby inhibiting aggregation of the soluble β-amyloid,

wherein said anti-β-amyloid antibody is one that is obtainable using an immunogen consisting of a peptide consisting of residues 1-28 of β-amyloid, and said antigen binding fragment is an antigen binding fragment of said antibody,

and wherein said antibody or fragment is not conjugated with a detectable moiety.

A00003 (formatting and emphasis added).

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STATEMENT OF RELATED CASES

The Acting Director is not aware of any other appeal in connection with this case that has previously been before this Court, or that is currently pending in any other court. The Acting Director is also unaware of any related cases pending in this or any other court that will directly affect, or be directly affected by, this Court's decision in the pending appeal.

I. STATEMENT OF THE ISSUES

Beka Solomon is the inventor of U.S. Patent No. 5,688,651 ("the '651 patent"). Solomon originally claimed multiple inventions in the application leading to the '651 patent. Accordingly, the Examiner restricted the claims into three groups. Solomon elected the first group and prosecuted those claims to allowance, but failed to file divisional application(s) to the nonelected claims or to any other subject matter prior to issuance of the '651 patent. Solomon now attempts to prosecute subject matter distinct from her elected invention by filing a reissue application. The first issue before this Court is whether the Board properly determined that Solomon's failure to timely file a divisional application cannot be corrected by reissue. If the Court affirms the Board on this first issue, it need not consider the next issue.

Solomon claims a method of inhibiting aggregation of a protein—β-amyloid—in a human by causing an antibody specific for β-amyloid or fragment of that antibody to bind β-amyloid with an inhibition strength at least as great as "AMY-33," an antibody already known in the art. The specification fails to disclose any antibody or fragment thereof that meets that description. Nor does it describe sufficient structural features that are common to the genus of antibodies required to practice the claimed method. The second issue, should

the Court need to reach it, is whether substantial evidence supports the Board's finding that the claimed method is not adequately supported by the specification.

II. STATEMENT OF THE CASE

The '651 patent, entitled "Prevention of Protein Aggregation," issued on November 18, 1997 with four claims. A00041-54. On February 22, 2006, Solomon filed a reissue application[1] canceling the original patent claims and adding new claims. A00039-40; A00075-77. The Examiner finally rejected all of the pending claims 5, 7-9, 17, 19-21, and 31-38 under 35 U.S.C. § 251 for lack of defect or error in the original patent correctable by reissue, and under 35 U.S.C. § 103(a) as obvious over certain prior art references. A00915-91 (Examiner's Answer). The Examiner also rejected claims 5, 7-9, and 31-34 under 35 U.S.C. § 112, first paragraph, for improper introduction of new matter and for lack of written description support in the specification. *Id.* Solomon appealed to the Board of Patent Appeals and Interferences ("Board"),[2] which affirmed the Examiner's reissue and written description rejections, but reversed the prior art and new matter rejections. A00002-30. This appeal followed.

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[1] The present application is the child of reissue application no. 09/441,140, filed November 16, 1999, which is currently pending in the Office.

[2] Effective September 16, 2012, Congress renamed the "Board of Patent Appeals and Interferences" as the "Patent Trial and Appeal Board." 35 U.S.C. § 6(a) (2011).

III. STATEMENT OF THE FACTS

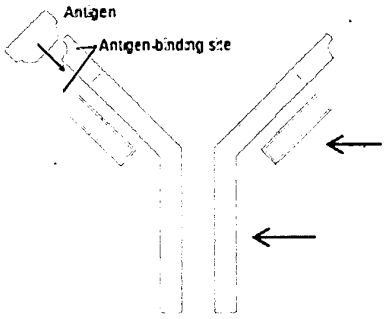
This appeal involves disputes centering on both the technology and the prosecution history. The Acting Director first describes the disclosure of the '651 patent, and then describes the prosecution before the Office that led up to the filing of the presently-appealed reissue application.

A. Solomon's '651 Patent: The Selection and Use of Antibodies or Antibody Fragments to Inhibit Protein Aggregation

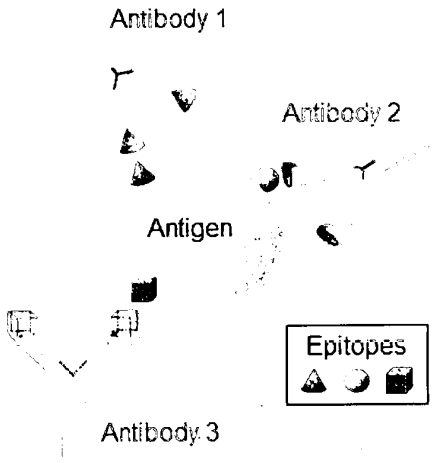
Following synthesis, proteins fold into a particular three-dimensional shape to fulfill their different functions. Occasionally, however, proteins fail to properly fold and subsequently accumulate and clump together. *See* A00041 (col. 1, ll. 12-22). This abnormal association of misfolded proteins is known in the art as protein aggregation, and is implicated in several disease states, including Down's syndrome and Alzheimer's disease. *Id.* (col. 1, ll. 42-46). The '651 patent proposes the use of "anti-aggregation molecules"—particularly antibodies and antibody fragments—to prevent protein aggregation and disassociate protein aggregates *in vivo*. *Id.* (col. 1, ll. 4-8).

Antibodies are Y-shaped proteins that play a significant role in the immune response. Antibodies work by binding an antigen, *e.g.*, a protein that evokes an immune response. As shown in the following diagram, the Y-shaped structure consists of two identical "light" polypeptide chains and two identical

"heavy" polypeptide chains. Each light chain connects with a heavy chain to create the "arms" of the Y-shaped molecule.



The upper arms of the antibody form a region called the "antigen-binding site." The antigen-binding site regions are highly variable, consisting of an amino acid sequence unique to each antibody. The specific region of the antigen to which the antigen-binding site binds is called an "epitope." A single antigen (shown in the diagram below) may have several different epitopes (illustrated as varying shapes), which consist of short amino acid sequences generally 5 to 8 residues in length. In nature, many different antibodies (shown in different colors) will bind to a single antigen, but those antibodies typically do not bind to the same epitope on that antigen.



For example, the antibody 1 of the diagram only binds to the "green" epitope,

whereas antibody 2 only binds to the "blue" epitope. In the laboratory, antibodies can be "raised against" (or created to bind to) particular antigens or even specific epitopes. Antibodies genetically engineered from the same clone—*i.e.*, monoclonal antibodies—are identical and therefore bind to the same epitope.

The '651 patent discloses a method of selecting antibodies or antibody fragments *in vitro* (in a test tube), and a method of using those antibodies or antibody fragments to inhibit protein aggregation and disassociate protein aggregates *in vivo*. A00042 (col. 3, ll. 40-55). The specification proposes several aggregating proteins as antibody targets, of which β-amyloid—a protein present in the brain "plaques" of Alzheimer's patients—is preferred. A00043 (col. 5, ll. 50-66).

The '651 patent shows that a commercially-available monoclonal antibody known as AMY-33 binds to and inhibits *in vitro* aggregation of β-amyloid. A00048 (col. 15, ll. 43-46); A00042 (col. 3, ll. 8-11). The '651 patent postulates that AMY-33 recognizes an epitope located somewhere within the first 28 amino acids (*i.e.*, amino acids 1-28) of β-amyloid, A00048 (col. 15, ll. 43-46), and "probably" residues 25-28, *id.* (col. 16, ll. 5-9). On the other hand, the '651 patent shows that another commercially-available monoclonal antibody known as 6F/3D has only partial or no appreciable effect on β-amyloid

aggregation *in vitro*. A00048 (col. 15, ll. 49-53). The '651 patent postulates that 6F/3D recognizes an epitope located somewhere within amino acids 8-17 of β-amyloid. *Id.*

B. Prosecution of the '651 Patent

The '651 patent originated as U.S. patent application no. 08/358,786, which was filed on December 16, 1994 with 24 claims. The Examiner issued a restriction requirement,[3] separating the claims into three groups for purposes of examination:

- Group I, claims 1-6, drawn to a method of selection for anti-aggregation molecules;
- Group II, claims 7-23, drawn to a method of treating a protein aggregation disease by administering an expression vector encoding the anti-aggregation molecule, a pharmaceutical composition, and expression vectors; and
- Group III, claim 24, drawn to a method of treating a protein aggregation disease by administering a monoclonal antibody.

A01352-54.

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[3] Section 121 of the Patent Act grants the Director the authority to limit a patent to one invention. 35 U.S.C. § 121. In a so-called "restriction requirement," the examiner typically organizes the claims into groups (*i.e.*, independent or distinct inventions) and requires the applicant to elect one group for prosecution. 37 C.F.R. § 1.142(a). Claims to the invention(s) not elected are withdrawn from consideration, and if not later subject to a rejoinder process, are canceled prior to issuance. 37 C.F.R. § 1.142(b).

The Examiner explained that the different methods distinguish the invention of Group I from the inventions of Groups II and III. A01353. Specifically, the invention recited in the claims of Group I is directed to a method for using an *in vitro* assay to identify molecules that prevent protein aggregation, whereas the inventions recited in the claims of Groups II and III are directed to methods of treating a disease associated with protein aggregation by administering an aggregation-inhibiting nucleic acid molecule or antibody, respectively. *Id.* The Examiner also noted that the art recognizes each invention as having divergent subject matter and separate classification. *Id.*

In response to the restriction requirement, Solomon elected without traverse the claims of Group I, and "withdr[e]w claims 7-24 without prejudice." A01359; *see also* A01363 (noting the election without traverse of Group I). Eventually, the Examiner allowed claims 1, 3, 4, and 6 of Group I (renumbered 1, 2, 3, and 4, respectively), and with Solomon's permission, canceled the nonelected claims of Groups II and III. *See* A01374-77 (Notice of Allowability and Examiner's Amendment).

The '651 patent issued on November 18, 1997. A00053. Claims 1 and 3, the only independent claims, both recite a "method of selecting an anti-aggregation molecule having the chaperone-like activity of anti-aggregation." A00049 (col. 18, ll. 14-15 & 39-40). Solomon failed to file any divisional or

continuation application(s) to the nonelected subject matter or to any other non-claimed subject matter prior to issuance. *See, e.g.*, A00133-34 ("It is noted that there are no divisionals or continuations filed from the parent 08/358,786 application.").

C. Prosecution of the Reissue Application

On February 22, 2006, Solomon filed the reissue application underlying this appeal. A00039-40. In her first reissue declaration ("the 2006 declaration"), A00055-58, Solomon attested that the '651 patent was "wholly or partly inoperative or invalid by reason of the patentee claiming more or less than it had the right to claim in the patent," A00056. Specifically, Solomon declared that all parties involved in the prosecution of the original application focused their attentions on patenting the method of selecting an anti-aggregation molecule as embodied in issued claims 1-4 to the exclusion of other inventions disclosed in the application. A00056. Solomon further explained:

> It was only recently that the Applicant/Patentee and the Assignee realized that the concepts fully disclosed in the specification directed to pharmaceutical compositions for preventing or reducing aggregation of an aggregating protein or for disaggregating preaggregated aggregates of the aggregating protein and methods of disaggregating an aggregate of or preventing the aggregation of an aggregating protein by causing an anti-aggregation molecule to come into contact with the aggregate or with the soluble aggregating protein were not claimed *per se*, and further they could be and should be now so claimed.

A000056-57.

In a preliminary amendment accompanying the reissue application, Solomon canceled issued claims 1-4 of the '651 patent and submitted new claims 5-28. A00075-77. Independent claim 5 recited a method for preventing or reducing the aggregation of an aggregating protein by administering an anti-aggregation molecule, whereas the only other independent claim 17 recited a method for disassociating (*i.e.*, "disaggregating") a protein aggregate by administering an anti-aggregation molecule. A00075-76. Several dependent claims limited the aggregating protein to β-amyloid, carboxypeptidase A, and others. *See* A00075-76 (claims 10-16); A00077 (claims 22-28).

The Examiner issued a restriction requirement requiring Solomon to select one particular aggregating protein for examination. A00117-22. The Examiner explained that the aggregating proteins are unique in that they possess different amino acid sequences, are implicated in different diseases, and are inhibited by different anti-aggregating agents. A00120-21. In response, Solomon elected to prosecute the claimed methods as relating to β-amyloid. A00123.

In the first Office Action on the merits, the Examiner rejected the claims under 35 U.S.C. § 251 for lack of defect or error in the original patent correctable by reissue. A00133-35. Comparing the proposed reissue claims with nonelected claim 24 of Solomon's original application, the Examiner

concluded that the reissue claims relate to subject matter not patentably distinct from the nonelected claim, as both are directed to the use of an antibody to treat a human suffering from a protein-aggregation disease. A00135. Relying on the Manual of Patent Examining Procedure ("MPEP") § 1412.01 and cases cited therein, the Examiner explained that Solomon's failure to timely file a divisional application to the nonelected subject matter prior to issuance of the '651 patent is not an error correctable by reissue. A00133-34.

Solomon eventually submitted a substitute declaration ("the 2008 declaration"). A00427-31. In the 2008 declaration, Solomon attested that she submitted a claim to a "generic method of treating a protein aggregation disease" during the original prosecution of the '651 patent, but chose not to prosecute that claim in a divisional application. A00428-29. Later, however, she and the assignee realized that no claims had ever been submitted "specifically directed to methods of disaggregating an aggregate of or inhibiting the aggregation of soluble β-amyloid" with anti-β-amyloid antibodies or fragments thereof. A00429. Thus, Solomon continued, "[i]t was realized that the failure to claim these concepts was an error without deceptive intent and that they could and should be now so claimed." *Id.*

The Examiner, however, maintained the rejection under § 251. The Examiner found no patentable distinction between a method for "reversing

aggregation" as claimed in original claim 24 and a method for "inhibiting aggregation" or "disaggregating an aggregate" as claimed in reissue claims 5 and 17. A00555. Indeed, the Examiner explained, the subject matter of the reissue claims falls within the scope of original claim 24. A00558-59. Finally, the Examiner noted that, had the reissue claims been presented in the original prosecution, those claims could not have been prosecuted in that application because they encompass subject matter distinct from the invention Solomon elected. A00556-58.

D. The Board's Decision

The Board affirmed the Examiner's rejection of all of the pending claims under 35 U.S.C. § 251 for lack of defect or error in the original patent correctable by reissue, and the rejection of claims 5, 7-9, and 31-34 under 35 U.S.C. § 112, first paragraph for failing to satisfy the written description requirement. A00030.

First, the Board found independent claim 5, as follows, representative:

> 5. A method of inhibiting aggregation of soluble β-amyloid in a human subject, comprising
>
> causing an effective amount of an anti-β-amyloid, or an antigen-binding fragment thereof, to come into contact with the soluble β-amyloid,
>
> *said antibody or fragment being effective to bind β-amyloid and to inhibit aggregation of the soluble β-amyloid in said subject to an extent at least as great as that obtainable with*

> *antibody AMY-33*, thereby inhibiting aggregation of the soluble β-amyloid,
>
> wherein said anti-β-amyloid antibody is one that is obtainable using an immunogen consisting of a peptide consisting of residues 1-28 of β-amyloid, and said antigen binding fragment is an antigen binding fragment of said antibody,
>
> and wherein said antibody or fragment is not conjugated with a detectable moiety.

A00003 (formatting and emphasis added).

The Board then addressed the rejection under § 251, and found that the evidence of record supported the Examiner's conclusion that the original patent does not contain an error. A00005-12. The Board agreed with the Examiner that Solomon's reissue claims are drawn to methods of treating protein aggregation diseases and therefore are substantially similar to the nonelected claims of Solomon's original application. A00008. Because Solomon failed to file a divisional application to the nonelected subject matter, the Board concluded that she is precluded from pursuing those claims under the *Orita* doctrine. A00009 (citing *In re Orita*, 550 F.2d 1277 (CCPA 1977)). The Board explained that allowing Solomon to pursue claims to a nonelected invention in reissue would render the co-pendency requirement of 35 U.S.C. § 120[4]

[4] Section 120 provides that any continuation or divisional application of an originally filed application must be filed "before the patenting or abandonment of or termination of proceedings on the first application or on an

"'meaningless,'" a result the *Orita* Court found undesirable. A00011-12 (quoting *Orita*, 550 F.2d at 1281).

The Board also found unpersuasive Solomon's reliance on this Court's decision in *In re Doyle*, 293 F.3d 1355 (Fed. Cir. 2002), noting that the reissue claim presented in that case was broader than all of the original claims and therefore encompassed the elected invention. A00010. But here, the Board explained, the reissue claims would have either fallen within nonelected Group III or another nonelected group had the claims been presented in the original application. A00010-11. And in any event, the newly introduced claims do not encompass the elected invention. A00011 (stating that the reissue claims "d[o] not encompass nor relate to the elected Group I which was prosecuted to issuance").

With respect to the rejection under section 112, first paragraph, written description, the Board agreed with the Examiner that the specification failed to adequately describe a genus of antibodies or fragments of those antibodies that "bind[] β-amyloid and inhibit[] aggregation of soluble β-amyloid to an extent at least as great as that obtainable with antibody AMY-33." A00026. The Board found no examples of any antibodies (or fragments thereof) in the specification

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application similarly entitled to the benefit of the filing date of the first application" in order to receive the benefit of the original application's filing date. 35 U.S.C. § 120 (2011).

that meet that description. A00029. Accordingly, the Board concluded that Solomon's claims "'merely recite a description of the problem to be solved while claiming all solutions to it and . . . cover any compound later actually invented and determined to fall within the claim's functional boundaries—leaving it to the pharmaceutical industry to complete an unfinished invention.'" *Id.* (quoting *Ariad Pharms., Inc. v. Eli Lilly and Co.*, 598 F.3d 1336, 1353 (Fed. Cir. 2010) (*en banc*)).

IV. SUMMARY OF THE ARGUMENT

The *Orita-Weiler-Doyle* line of cases prohibits a patentee from using a reissue proceeding to obtain claims that the patentee could not have prosecuted in his or her original application. These cases are based on the rationale that, in acquiescing to an examiner's restriction requirement, the applicant has chosen to pursue claims only to the elected invention. In other words, the applicant has agreed to independently prosecute any invention distinct from that elected in divisional application(s). This Court accordingly does not treat a patentee's failure to file those divisional application(s) as an "error" correctable under the reissue statute. Otherwise, the co-pendency requirement of 35 U.S.C. § 120 would be eviscerated.

Solomon attempts to do exactly what the *Orita-Weiler-Doyle* line of cases rejects. In response to the Examiner's restriction requirement, Solomon elected

without traverse claims to a method of selecting an anti-aggregation molecule *in vitro* and prosecuted them to allowance. Those claims were distinct from the nonelected claims directed to a method of treating protein aggregation in a human by administering an "anti-aggregation" antibody. Solomon failed, however, during the pendency of her application to file divisional applications to the nonelected claims or to any other distinct subject matter she disclosed.

Solomon's attempt to blunt the impact of *Orita-Weiler-Doyle* fails. Her assertion that her "error" lies not in failing to file divisional application(s), but in failing to present the reissue claims during prosecution of the '651 patent reflects an attempt to get an improper do-over: to prosecute an invention that is distinct from one she expressly chose to pursue in her original application. Indeed, because Solomon claimed exactly what she had a right to claim in her original application—*i.e.*, the elected *in vitro* method claims—the Board correctly determined that the '651 patent lacks an error correctable under the reissue statute.

Should this Court need to reach the issue, the Board also correctly determined that some of Solomon's claims fail to comply with the written description requirement. The rejected claims encompass a broad genus of antibodies (or fragments thereof) that bind β-amyloid with an inhibition strength "at least as great as" a prior art antibody known as AMY-33. But the

specification fails to describe any antibody or fragment thereof falling within the claimed genus, or sufficient structural features common to that genus. For these reasons, the Board's rejection should be affirmed.

V. ARGUMENT

A. Standard of Review

Solomon has the burden to show that the Board committed reversible error. *In re Watts*, 354 F.3d 1362, 1369 (Fed. Cir. 2004). Whether the claims of a reissue application are unpatentable under 35 U.S.C. § 251 is a question of law based on underlying findings of fact. *In re Serenkin*, 479 F.3d 1359, 1361 (Fed. Cir. 2007). Whether a claim comports with the written description standard of 35 U.S.C. § 112(a)[5] is a question of fact. *Ariad*, 598 F.3d at 1351.

This Court reviews the Board's legal conclusions *de novo* but affirms the Board's factual findings that are supported by substantial evidence. *In re Jolley*, 308 F.3d 1317, 1320 (Fed. Cir. 2002); *see also* 5 U.S.C. § 706(2)(E). Substantial evidence is "such relevant evidence as a reasonable mind might accept as adequate to support" the conclusion reached. *Consol. Edison Co. v. NLRB*, 305 U.S. 197, 229 (1938). It is "something less than the weight of the evidence but more than a mere scintilla of evidence." *In re Kotzab*, 217 F.3d

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[5] In the Leahy-Smith America Invents Act ("AIA"), Congress added a paragraph letter to the first paragraph of § 112, without otherwise substantively changing its content. 35 U.S.C. § 112(a) (2011).

1365, 1369 (Fed. Cir. 2000). "[W]here two different, inconsistent conclusions may reasonably be drawn from the evidence in record, an agency's decision to favor one conclusion over the other is the epitome of a decision that must be sustained upon review for substantial evidence." *Jolley*, 308 F.3d at 1329.

B. The Board Correctly Concluded That Solomon's Patent Lacks an Error Correctable by Reissue

Section 251 of the 1952 Patent Act provides for the reissue of a patent that "through error without any deceptive intention, [is] deemed wholly or partly inoperative or invalid . . . by reason of the patentee claiming more or less than he had a right to claim in the patent." 35 U.S.C. § 251 (2006), *amended by* 35 U.S.C. § 251(a) (2011) (adding paragraph letters and deleting "without any deceptive intention"). Fundamental principles of equity and fairness guide the application of the reissue statute. But "[b]y its terms section 251 restricts reissue to situations in which an error occurred." *Doyle*, 291 F.3d at 1358. And not just any "error" qualifies under the statute. *E.g.*, *In re Weiler*, 790 F.2d 1576, 1579 (Fed. Cir. 1986) ("[N]ot every event or circumstance that might be labeled 'error' is correctable by reissue.").

This Court and its predecessor court have generally refused to call "error" the deliberate choices a patent applicant makes (albeit later regretted) to obtain allowance. *See e.g.*, *In re Yamazaki*, 702 F.3d 1327, 1333 (Fed. Cir. 2012) (holding that patentee's failure to prevent a patent from issuing with terminal

disclaimer not an error correctable by reissue); *In re Mostafazadeh*, 643 F.3d 1353, 1358 (Fed. Cir. 2011) (reiterating that intentional surrender of subject matter during original prosecution to overcome prior art not an error); *Serenkin*, 479 F.3d at 1363 (holding that a "conscious decision" to accept a later filing date for application not a correctable error); *In re Mead*, 581 F.2d 251, 257 (CCPA 1978) (holding that patentee's "conscious choice" not to file a continuing application not correctable error). The *Orita*, *Weiler*, and *Doyle* decisions, relevant to this appeal and discussed in detail below, stand in accord.

1. The *Orita-Weiler-Doyle* line of cases prohibits a patentee from using a reissue proceeding to obtain claims that the patentee could not have prosecuted in his or her original application

Orita, *Weiler*, and *Doyle* hold that when an applicant makes a deliberate choice in response to a restriction requirement to prosecute a single invention and fails to file divisional application(s) to the remaining, nonelected inventions—whether originally claimed or not—error under § 251 does not exist. These cases are based on the rationale that once an applicant acquiesces to a restriction requirement, any invention distinct from the one elected and prosecuted to allowance can only be pursued in a timely-filed divisional application.

In *Orita*, the CCPA addressed the issue of whether an applicant's failure to file a divisional application to nonelected claims constituted an "error" under

§ 251. 550 F.2d at 1280. During the prosecution of Orita's original application, the examiner issued a restriction requirement separating the claims into two groups. *Id.* at 1278. The applicants elected the first group and canceled the nonelected claims of the second. *Id.* After issuance, applicants filed a reissue application to claims "substantially identical to the originally non-elected claims," and stated in a declaration that they had claimed less than they had a right to claim because "they had forgotten to timely file a divisional application covering the originally non-elected subject matter." *Id.* at 1279.

The court upheld the Board's denial of the reissue, holding that applicants' acquiescence to the examiner's restriction requirement did not constitute an "error" under § 251. The court explained that while "appellants undoubtedly erred by failing to file a timely divisional application," the resulting patent was error-free: "Patentees claimed exactly what they had a right to claim in the patent, no more nor less, and appellants' failure to timely re-file does not change this fact." *Id.* at 1280.

The court also noted that allowing reissue claims substantially identical to those not elected would render the co-pendency requirement of § 120 "meaningless." The court continued:

> for should an applicant fail to file a divisional application while maintaining copendency as required by section 120, he could simply revert to section 251 in order to cure his mistake. Section 251 is not a panacea designed to cure every mistake which might be

> committed by an applicant or his attorney, and the case at bar exemplifies a mistake which this section cannot cure.

Id. at 1281.

The CCPA's decision gave rise to what is now commonly referred to as "the *Orita* doctrine." It finds its roots in two pre-1952 Patent Act cases: *In re Cornell*, 150 F.2d 702 (CCPA 1945) and *In re Smyser*, 135 F.2d 747 (CCPA 1943). In both of these cases, the CCPA held that the reissue statute did not authorize claiming of subject matter in a reissue that could not have been included in the original application as a result of applicants' acquiescence in the examiner's requirement for "division." *Cornell*, 150 F.2d at 704; *Smyser*, 135 F.2d at 751.

Following *Orita*, which dealt with *claimed* subject matter, this Court held in *Weiler* that error under § 251 does not lie in failing to file divisional applications to *non-claimed* subject matter distinct from the subject matter that the applicant elected. *Weiler*, 790 F.2d at 1580. The examiner in *Weiler* restricted the original claims into three groups. Weiler chose the first group without traverse and prosecuted those claims to allowance, but failed to file divisional applications directed to the nonelected subject matter or to any other disclosed subject matter. *Id.* at 1578. After issuance, Weiler filed a reissue application with claims to subject matter disclosed but never claimed in the original application. *Id.* at 1578-79. This Court upheld the Board's rejection of

the reissue application for lack of error. In doing so, this Court noted that the subject matter of the claims on appeal was "clearly independent of and distinct" from that of both the elected and nonelected claims, and accordingly, "Weiler would thus have had no right to insert and present [the reissue claims] in the original application after the examiner's requirement for restriction." *Id.* at 1581. Following the logic of *Orita*, this Court held that by acquiescing in an examiner's restriction requirement, "it cannot on the present record have been error to forego divisional applications on subject matter to which claims had never been made." *Id.* at 1582.

While *Orita* and *Weiler* addressed specific claims to distinct inventions, *Doyle* addressed a "linking" claim, "a claim broad enough to read on—or link—two or more groups of claims subject to a restriction requirement." 293 F.3d at 1358. This Court characterized the issue in *Doyle* as "whether failure to present a so-called linking claim . . . is an error correctable by reissue." *Id.* Doyle's original application included both compound and method claims. *Id.* at 1356. In response to the examiner's nine-way restriction requirement, Doyle elected the claimed invention of using a genus of catalysts to insert carbenes into certain chemical bonds, and canceled the remaining claims. *Id.* Doyle did not file any divisional applications to the nonelected claims. But following issuance of the patent, Doyle filed a reissue application with claims directed to a method of

using a genus of catalysts to insert any prochiral molecule (*i.e.*, not just carbene) into the chemical bonds. *Id.* at 1357. Doyle conceded that the new claims, while broader in scope than the elected invention, read on some of the nonelected claims. *Id.*

The Board upheld the rejection of the claims based on lack of error correctable by reissue. *Id.* at 1358. This Court reversed. In doing so, the Court first noted that "[t]he so-called *Orita* doctrine . . . precludes a reissue applicant from obtaining substantially identical claims to those of nonelected groups identified in an examiner's restriction requirement when such claims could not have been prosecuted in the application from which they were restricted." *Id.* at 1359. The Court then explained that *Weiler* prevents a reissue applicant from pursuing claims to "a completely separate invention from the issued claims or any of the nonelected claims." *Id.* at 1362. Such claims, the *Doyle* Court explained, if presented during the original prosecution, "would have been subject to the original restriction requirement and the applicant would not have been allowed to prosecute them with the original patent—he would have been required to assert them in a divisional." *Id.*

The *Doyle* Court held, however, that neither *Orita* nor *Weiler* applied to Doyle because his new claims "are genus claims, whereas the nonelected claims are species that fall within the new genus claims. In other words, the reissue

claims are substantially broader than the claims of the nonelected groups." *Id.* at 1360. Unlike Orita and Weiler, Doyle could have—and indeed *should have*—prosecuted the broader linking claims (linking the elected and nonelected claims) in the original application. *Id.* And importantly, because Doyle did not agree to prosecute the broader linking claims in a divisional, the estoppel rationale underlying *Orita* and *Weiler* did not apply. *Id.*

The *Orita* and *Weiler* cases stand for the "established principle that applicants are 'estopped from obtaining by reissue claims which, because of a requirement for restriction in which they had acquiesced, they could not claim in their patent.'" *In re Watkinson*, 900 F.2d 230, 232 (Fed. Cir. 1990) (quoting *Orita*, 550 F.2d at 1280). *Doyle* comports with that principle, as Doyle could have prosecuted his reissue claims in his original application. That he failed to do so constituted the error of claiming less than Doyle had a right to claim under § 251. In contrast, the applicants in *Orita* and *Weiler* could not have pursued their reissue claims in their original applications because those claims were distinct from the elected invention. Again, those applicants agreed to limit their original application to the elected invention, and therefore were estopped from submitting other, distinct subject matter in reissue that they failed to timely submit in a divisional application during the pendency of their original applications.

2. The '651 patent lacks an error because the now-claimed subject matter could not have been prosecuted in Solomon's original application

In this case, the Board correctly found that Solomon is estopped from prosecuting via reissue claims directed to an invention distinct from her elected—and ultimately patented—claimed invention. A00008-09; *see also* A00943 (stating that "the present claims are directed to a patentably distinct invention from the elected group of the patent"). The Examiner restricted the claims of the original application into three independent groups (inventions). A01352-54. The invention recited in the claims of Group I was directed to a method for using an *in vitro* assay to select molecules that prevent protein aggregation, whereas the inventions recited in the claims of Groups II and III were directed to methods of treating a disease associated with protein aggregation by administering an aggregation-inhibiting nucleic acid molecule or antibody, respectively. A01353; *see also* A00008 (citing FF1-2). Solomon elected Group I without traverse, thereby acquiescing to the Examiner's determination that the *only* invention that the '651 patent could claim was an *in vitro* method for selecting anti-protein aggregation molecules. A00006 (FF2). Put differently, Solomon agreed to prosecute all subject matter distinct from the claimed molecule selection method in timely-filed divisional applications.

Instead of filing divisional applications prior to issuance of the '651 patent, however, Solomon now attempts to claim that subject matter through reissue. But this Court's precedent of almost 70 years forecloses her from doing so. And whether Solomon deliberately abandoned the now-claimed subject matter following the restriction requirement, or simply failed to originally claim it at all (presented as alternative arguments in sections 3 and 4, below) makes no difference in the end. The reissue claims are distinct from Solomon's elected invention, and therefore could not have been prosecuted in her original application. *Doyle*, 293 F.3d at 1361; *Watkinson*, 900 F.2d at 232; *Weiler*, 790 F.2d at 1582, *Orita*, 550 F.2d at 1280; *Cornell*, 150 F.2d at 704; *Smyser*, 135 F.2d at 751.

Specifically, issued claim 1 of the '651 patent recites a method of selecting an anti-aggregation molecule having specific properties, through five specific *in vitro* reaction steps (*i.e.*, denaturing, mixing, incubating, selecting, and testing). A00049. Reissue claim 5, on the other hand, is directed to a method of inhibiting aggregation of β-amyloid *in vivo* by administering certain antibodies. A00003. In other words, claim 5 treats a disease in a human by administering a specific therapeutic, whereas issued claim 1 tests and identifies certain molecules in a laboratory setting. Thus, the subject matter Solomon now attempts to claim does not share the same purpose, method steps, or outcome as

the invention she elected during the original prosecution of the '651 patent. *See* A00011 (stating that "newly introduced claims 5-28 . . . did not encompass nor relate to methods of selecting anti-aggregation molecules of the elected Group I which was prosecuted to issuance"); *see also* MPEP § 802.01(I) (stating that two inventions are distinct for purposes of restriction when they are unconnected in design, operation, or effect).

In sum, reissue does not exist to correct Solomon's "error." As this Court has repeatedly explained, reissue simply is not "a panacea" to correct every prosecution mistake. *See MBO Labs., Inc. v. Becton, Dickinson & Co.*, 602 F.3d 1306, 1313 (Fed. Cir. 2010); *Serenkin*, 479 F.3d at 1362; *Doyle*, 293 F.3d at 1358; *Nupla Corp. v. IXL Mfg. Co.*, 114 F.3d 191, 195 (Fed. Cir. 1997); *Weiler*, 790 F.2d at 1582.

3. The *Orita* doctrine prevents Solomon from obtaining in reissue subject matter not elected in the original prosecution of the '651 patent

As Solomon acknowledges, Br. at 19-20, a patentee's failure to timely file a divisional application to nonelected subject matter in response to a restriction requirement is not an error correctable by reissue. *Orita*, 550 F.2d at 1280-81. Solomon nonetheless asserts that the *Orita* doctrine does not apply to the instant facts because "[t]he present claims are not the same as, or substantially identical

to, the claim in the original patent application that was cancelled subsequent to a restriction requirement." Br. at 27. Solomon is mistaken.

As an initial matter, Solomon's attempt to impose an *ipsis verbis* test for applying the *Orita* doctrine, Br. at 27-29, should be rejected. Nothing in *Orita* requires that the claims sought in reissue recite identical words as the nonelected claims of the original prosecution in order to run afoul of its holding. *Orita* instead requires a determination of whether the reissue claims would have been grouped with the nonelected claims because they are directed to the same subject matter.

And here, although worded differently, both reissue claim 5 and original, nonelected claim 24 are directed to the use of an antibody to treat a human suffering from a protein-aggregation disease. In other words, both claim 5 and original claim 24 concern the same host (a human), the same method step (administering an antibody to the human), and the same biological mechanism (causing an antibody to come into contact with an aggregating protein). Thus, as the Board correctly found, reissue claim 5 would have been categorized in the same inventive group as original claim 24. A00008.

Solomon's argument that claim 5 is not directed to a method of treating a protein aggregation disease rings hollow; as the Examiner aptly observed, but for the disease, no reason exists to administer the antibodies to the human in the

first place. A00944. Indeed, the specification makes clear that once an appropriate antibody is selected (through the *in vitro* method of issued claim 1), the "invention provides for its use therapeutically to prevent or reduce protein aggregation in vivo." A00043 (col. 6, ll. 1-5). The skilled artisan would therefore recognize that inhibiting aggregation of soluble β-amyloid as recited in reissue claim 5 constitutes the same therapeutic use originally recited in nonelected claim 24. A00944.

Solomon also argues that claim 5 is "quite different" from original, nonelected claim 24 because claim 24 states that the antibody "reverses" aggregation, whereas claim 5 states that the antibody "inhibits" aggregation. Br. at 28. But the original Examiner distinguished claim 24 from the only other human treatment claim presented during the original prosecution (*i.e.*, original claim 23) on the basis of the therapeutic administered (monoclonal antibody versus nucleic acid), and not on the biological effect of the antibody itself (*i.e.*, whether it "reverses" or "inhibits" aggregation). Moreover, Solomon fails to show that such a distinction even exists. A01353. As the current Examiner explained, the specification contemplates that a monoclonal antibody that binds to an aggregated protein and causes disaggregation would also necessarily bind to a soluble protein to prevent aggregation. A00953-54 (citing A00043 (col. 4, ll. 62-66 & col. 5, ll. 23-29)). Solomon presents no evidence rebutting the

Examiner's finding that an antibody that "inhibits" aggregation also "reverses" aggregation. *In re Geisler*, 116 F.3d 1465, 1470 (Fed. Cir. 1997) (attorney argument no substitute for evidence).

Solomon's argument that the *Orita* doctrine does not apply because claim 5 is narrower than original claim 24 (and therefore not of "substantially similar scope" as allegedly required by *Doyle*), Br. at 28-29, misses the mark. This Court noted that the reissue claims asserted in *Doyle* were "significantly broader" than both the elected and nonelected claims, and therefore could have been prosecuted in the original patent because they read on the *elected* group of claims. 293 F.3d at 1361. Here, however, not only is reissue claim 5 *narrower* than nonelected claim 24, it also bears no relationship to the elected group of claims. In other words, reissue claim 5—unlike the reissue claim presented in *Doyle*—is not a linking claim that could have been prosecuted in the application that matured into the '651 patent. A00010. Moreover, if the *Orita* doctrine forecloses obtaining a broader genus claim (like original, nonelected claim 24) as Solomon admits, then permitting a narrower species claim (like representative claim 5) via reissue makes little sense. Thus *Doyle*, contrary to Solomon's arguments, does not support the notion that narrowing the subject matter of original claim 24 to a specific species of protein provides an escape route from the *Orita* doctrine.

Finally, Solomon's reference to the restriction requirement issued in the present reissue application bears no relevance to the issue of whether the '651 patent contains an error correctable by reissue. As the Examiner pointed out, that each of the proteins listed in the specification are *distinct from one another* does not mean that a therapeutic method targeting one of those proteins is distinct from a broader claim to the therapeutic method not limited to a particular protein, like that recited in original claim 24. A00944-45.

4. Alternatively, this Court's decision in *Weiler* dictates that Solomon cannot claim in reissue distinct subject matter that was disclosed but not originally claimed in the '651 patent

Even if the reissue claims are directed to a different invention from original, nonelected claim 24 as Solomon asserts, the result is the same. After affirmatively electing an invention to prosecute, Solomon's failure to timely file a divisional application to distinct subject matter disclosed but never claimed during the prosecution of the '651 patent does not constitute an error correctable by reissue. *Weiler*, 790 F.2d at 1579. As explained above, this holding is based on the rationale that failing to file a divisional application to any subject matter—whether claimed or not—that could not have been prosecuted in the original application due to an examiner's restriction requirement is not an "error" within the meaning of § 251.

The facts of *Weiler* are identical to those here. The examiner in *Weiler*, like the Examiner during prosecution of the '651 patent, issued a restriction requirement. 790 F.2d at 1578. The applicants in *Weiler*, like Solomon, deliberately chose to claim one invention without traverse, but failed to file any divisional applications to the non-claimed subject matter. *Id.* Finally, the applicants in *Weiler*, again like Solomon, filed a reissue application with claims to subject matter not claimed at all in the original application, and asserted that the reissue claims "should have been made in the original application." *Id.* at 1578-79. But this Court held that "an inventor's failure to claim particular subject matter was not the result of the 'error' required by § 251." *Id.* at 1583. Thus, even if Solomon is correct that the reissue claims are distinct from original, nonelected claim 24 (and would not have been grouped with that claim), Br. at 24, this Court's holding in *Weiler* would still preclude her from obtaining the claims in reissue.

Solomon attempts to cast doubt over *Weiler* by relying on subsequent cases, which could not have overruled *Weiler*, and commentary in Chisum. Br. at 21-24. But these arguments lack merit. *First*, contrary to Solomon's arguments otherwise, the holdings of *Doyle* and *Landmark Screens, LLC v. Morgan, Lewis, & Bockius, LLP*, 676 F.3d 1354 (Fed. Cir. 2012), are consistent with that of *Weiler*. In both *Doyle* and *Landmark*, the patentee presented a claim

in reissue that *could* have been prosecuted in the original patent because it encompassed within its scope the elected claims. Thus, neither patentee in those cases—unlike the patentees in *Orita* and *Weiler*—was estopped from presenting broader claims in reissue.[6]

Solomon's invocation of *In re Amos*, 953 F.2d 613 (Fed. Cir. 1991), Br. at 23-24, fares no better. In *Amos*, this Court reiterated that an applicant's "objective intent to claim" does not serve as an independent basis for denying a reissue application under § 251, and so reversed the Board's decision. *Id.* at 618-19. In this case, the Board did not refuse Solomon's reissue application for an alleged lack of an "objective intent to claim" as Solomon appears to imply. Instead, the Board upheld the Examiner's rejection of the reissue claims for lack of an error under § 251—a rejection that the *Amos* Court notes is proper under the statute. *Id.* at 617. Moreover, instead of "distinguishing" *Weiler* as Solomon asserts, *Amos* positively cites to *Weiler*. 953 F.2d at 617. The *Weiler* Court also observed that an "objective intent to claim" rejection lacks any

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[6] Solomon's second block quote from *Landmark*, Br. at 22, is misleading in that it implies—via omitted text—that this Court found that Landmark's reissue application met the requirements of § 251 because the claims of the reissue application were broader than the *nonelected* claims of Landmark's original application. In fact, the district court made this finding. 676 F.3d at 1365. This Court explained that "[w]hen the reissue claims are broader than the *issued* claims," § 251 may be satisfied by the patentee asserting that he claimed less than he had a right to claim in the patent. *Id.* at 1364 (emphasis added).

statutory basis in § 251. 790 F.2d at 1581. And given that the issue in *Amos* was whether the "original patent" language of § 251 is conclusively determined through a written description analysis, and not whether the patentee had demonstrated "error" under § 251, this Court called the *Amos* parties' attention to *Weiler* "inexplicable." 953 F.2d at 618 n.3. *Amos* is similarly inapposite here.

Second, Chisum's speculation that "there is no essential or substantive difference between reissue claims that broaden the scope of what is covered in the original patent claims and reissue claims that cover subject matter not claimed at all in the original patent," *see* Br. at 23, ignores the important distinction underlying the *Orita-Weiler-Doyle* line of cases. In each case denying reissue, the applicants had *acquiesced* to an examiner's restriction requirement. They *agreed* to prosecute all subject matter distinct from the elected invention—whether classified in a nonelected group of claims or never claimed at all—in timely-filed separate applications. In other words, the applicants chose *what* to prosecute and thus *what not* to prosecute. As a result, they were "estopped from obtaining by reissue claims which, because of a requirement for restriction in which they had acquiesced, they could not claim in their patent." *Orita*, 550 F.2d at 1280.

This scenario—in which an applicant chooses the invention to pursue—stands in stark contrast with that in which an examiner makes no restriction requirement during the original prosecution. In the latter scenario, a patentee can claim through reissue any invention otherwise meeting all other statutory requirements under the Patent Act, because that patentee has made *no agreement* respecting the subject matter that might have been claimed in the original application. *See In re Staats*, 671 F.3d 1350, 1355 (Fed. Cir. 2012) (holding that patentee could obtain claims in reissue application to embodiment not claimed within two-year time period). Moreover, although it would not change the outcome here, Solomon has made no allegation that she miscommunicated with her prosecuting attorney or agent or that she or the prosecuting attorney or agent lacked a full appreciation of the scope of disclosed subject matter, which as Chisum acknowledges, typically supports reissue applications to broader subject matter. 4A Donald S. Chisum, *Chisum on Patents* § 15.03[2][b][iv] (2004); *see also, e.g.*, *Hester Indus., Inc. v. Stein, Inc.*, 142 F.3d 1472, 1479-80 (Fed. Cir. 1998).

Solomon also asserts that this Court wrongly decided *Weiler* because, in coming to its decision, the Court ignored an agency procedural rule,

37 C.F.R. § 1.145. Br. at 24-25.[7] Solomon relies on this rule for the notion that she could have "inserted and presented" her reissue claims in her original application, and accordingly her "error" lies not in failing to file a divisional application to those claims, but in failing to present those claims during prosecution of the original application. Br. at 27. Solomon misses the point.

Section 251 requires error *in the issued patent*. *See, e.g.*, *Doyle*, 293 F.3d at 1361. The thrust of the *Orita-Weiler-Doyle* line of cases is that when a patentee has claimed exactly that to which they are entitled in a patent because they have agreed to claim only the elected invention—no error exists *in that patent*. *Orita*, 550 F.2d at 1280. And here, Solomon claimed exactly what she had a right to claim—the elected method claims of selecting an anti-aggregation molecule *in vitro*. Thus, *even if* Solomon had presented the reissue claims during the original prosecution of the '651 patent *and* the Examiner had entered that amendment under Rule 145, the result is still a dead end. After having been presented, those new claims would have been withdrawn as directed to nonelected subject matter, *see* MPEP § 821.03, and consequently would have been lost through Solomon's failure to file a timely divisional application, *Doyle*, 293 F.3d at 1362.

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[7] Because *Weiler* is binding precedent, this argument is better suited to the *en banc* Court. *Newell Cos. v. Kenney Mfg. Co.*, 864 F.2d 757, 765 (Fed. Cir. 1988).

Moreover, Solomon's argument that the Examiner might not have issued a second restriction requirement per MPEP § 803, Br. at 25, cannot be squared with the original prosecution of the '651 patent. At the very least, the reissue claims and the original, nonelected claims share the common feature of being directed to the administration of a therapeutic in a human (*i.e.*, an *in vivo* use), whereas the elected invention recites a method of selecting antibodies in a laboratory (*i.e.*, an *in vitro* use). Thus, the original Examiner would have properly restricted the reissue claims from the elected *in vitro* method claims of Group I for the same reasons that she separated the *in vivo* human treatment claims of Groups II and III from the elected *in vitro* method claims in the first place. *See* A01353.

Solomon's mention of the prohibition against double patenting rejections, Br. at 25-26, is a non sequitur. Once the USPTO restricts the claims of an application and therefore forces an applicant to file a second, divisional application to the nonelected claims, 35 U.S.C. § 121 prevents the Office from rejecting the claims of that divisional application over the original, restricted application. That makes sense, as the Office cannot issue a double patenting rejection when the Office itself forced the applicant into filing the second application. But here, no divisional applications were filed; thus, whether or not § 251 would have barred a double patenting rejection simply has no

applicability. "In other words, this tortured hypothetical does not correspond to the record in this case," and accordingly fails to change the outcome. *Perricone v. Medicis Pharm. Corp.*, 432 F.3d 1368, 1375 (Fed. Cir. 2005).

In sum, no matter how Solomon spins the "error" she made during the original prosecution, her *issued patent* lacks an error correctable by reissue "because the matter sought on reissue could not have been prosecuted originally." *Doyle*, 293 F.3d at 1361. This Court should therefore uphold the Board's denial of Solomon's reissue application.

5. Solomon's remaining arguments lack merit

Solomon argues that the Board's reference to specific language in her 2006 declaration without reference to the specific language in her 2008 declaration is reversible error. Br. at 15. It is not. As an initial matter, the Board committed no error because Solomon essentially made the same statements in both the 2006 declaration and the 2008 declaration. Specifically, Solomon stated in the 2006 declaration that the reissue claims cover concepts "not claimed *per se*," A00057, whereas in the 2008 declaration she stated that "no claims had ever been submitted" to those concepts, A00429. Thus, each declaration characterizes her "error" as not claiming the subject matter of the reissue claims during the original prosecution, which is consistent with Solomon's argument before this Court.

But even if, *arguendo*, the declarations make materially different statements as to the origin of Solomon's "error," the Board's consideration of the 2006 declaration does not compel reversal of its decision. Under the "harmless error" rule, "the appellant must not only show the existence of error, but also show that the error was in fact harmful because it affected the decision below." *Watts*, 354 F.3d at 1369. And here, no matter the specific words the Board quoted, Solomon's issued patent simply contains no "error" correctable under the reissue statute. *Supra* at 17-37; *see also* A00011.

Finally, the Board did not "establish" a *per se* rule as Solomon contends. Br. at 20-21. The Board merely followed this Court's well-established precedent that whenever an examiner issues a restriction requirement, she is by definition requiring the applicant to claim every other distinct invention disclosed in the specification—whether originally claimed or not—in a timely-filed divisional application. And by acquiescing to the restriction requirement, the applicant has agreed to do so. As this Court has explained, there is nothing unfair about applying the reissue statute in such a way that it holds the patentee to his word, *Doyle*, 293 F.3d at 1360, and prevents circumvention of the co-pendency requirement of 35 U.S.C. § 120, *Orita*, 550 F.2d at 1280-81.

C. Substantial Evidence Supports the Board's Finding That Claims 5, 7-9, and 31-34 Lack Adequate Written Description in the Specification

Section 112(a) requires that the specification contain a written description of the invention. 35 U.S.C. 112(a) (2011). "[T]he hallmark of written description is disclosure." *Ariad*, 598 F.3d at 1351. In other words, the specification must "convey[] to those skilled in the art that the inventor had possession of" and "actually invented" the claimed subject matter. *Id.* "A 'mere wish or plan' for obtaining the claimed invention is not adequate written description." *Centocor Ortho Biotech, Inc. v. Abbott Labs.*, 636 F.3d 1341, 1348 (Fed. Cir. 2011) (quoting *Regents of the Univ. of Cal. v. Eli Lilly & Co.*, 119 F.3d 1559, 1566 (Fed. Cir. 1997)).

1. Solomon's disclosure fails to meet any of the written description tests for a genus of antibodies

Representative claim 5 is broad, claiming a genus of anti-β-amyloid antibodies (or fragments thereof) that are capable of both binding to β-amyloid and inhibiting aggregation of β-amyloid "to an extent at least as great as" prior art monoclonal antibody AMY-33. A00034. Typically, for a claim to a genus of antibodies, the written description requirement is satisfied by the disclosure of "a representative number of species falling within the scope of the genus," or an

established "correlation between structure and function." *Ariad*, 598 F.3d at 1350.[8] Solomon's disclosure satisfies neither of these tests.

First, the specification fails to disclose *any* antibody or antibody fragment that binds to β-amyloid and inhibits β-amyloid aggregation at least as well as AMY-33. A00029. The specification only discloses two relevant antibodies, AMY-33 and 6F/3D, neither of which falls within the scope of the claim of representative claim 5. A00048 (Example 2). Claim 5 does not encompass 6F/3D because that antibody does not function as well as AMY-33. *Id.* (col. 15, ll. 49-53) (disclosing that 6F/3D had partial or no appreciable effect on β-amyloid aggregation *in vitro* compared to AMY-33). Claim 5 also does not encompass AMY-33 because that antibody *is* the comparative molecule recited in the claim, *i.e.*, the antibody to which all antibodies or antibody fragments must be compared. Thus, contrary to Solomon's assertions otherwise, Br. at 32, 33, & 39, AMY-33 does not serve as an example of a species falling within the scope of the genus of antibodies encompassed by the claim.

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[8] The third test commonly used to show possession of chemical molecules, *i.e.*, the disclosure of "structural features common to the members of the genus so that one of skill in the art can visualize or recognize the members of the genus," *Ariad*, 598 F.3d at 1350 (quotation omitted), is not commonly used in the antibody art. Persons of skill in this art generally do not consider disclosure of the complete amino acid sequence of an antibody necessary to show possession. *See Written Description Training Materials Rev. 1, March 25, 2008* at 46 (available at http://www.uspto.gov/web/menu/written. pdf) (hereinafter "*PTO Guidelines*").

While this Court has declined to set forth a bright-line rule regarding the number of species sufficient to describe a genus, *Ariad*, 598 F.3d at 1351, the "representative number of species" test is not met where, as here, the number is zero. *See, e.g.*, *Centocor*, 636 F.3d at 1350-51; *Ariad*, 598 F.3d at 1356-58 (finding lack of written description for claimed method of reducing NF-κB activity where specification failed to show any molecule that actually reduced NF-κB activity); *Univ. of Rochester v. G.D. Searle & Co.*, 358 F.3d 916, 927-928 (Fed. Cir. 2004) (finding lack of written description for claimed method of selectively inhibiting COX-2 enzyme where specification failed to describe any specific compound that could selectively inhibit COX-2).

Second, the specification fails to disclose any correlation between structure and function sufficient to show that Solomon actually invented the claimed genus of antibodies or antibody fragments. The function claimed is binding to β-amyloid and inhibiting β-amyloid aggregation at least as well as AMY-33. The specification fails to provide any structure of an antibody or antibody fragment capable of performing these functions at least as well as AMY-33. Moreover, while the specification provides some structure of the antigen by expressing preference for an antibody that binds to an epitope located somewhere within the first 28 amino acids of β-amyloid, *see, e.g.*, A00043 (col. 6, ll. 23-26), the specification does not definitively disclose *which* epitope(s)

within those residues the antibody or antibody fragment must bind in order to have the function of inhibiting β-amyloid aggregation at least as well as AMY-33. Such a disclosure is critical here, as the specification warns that an appropriate antibody must be "directed to the *appropriate epitope* on the β-amyloid molecule" in order to inhibit aggregation while still allowing normal functioning of the protein. A00042 (col. 3, ll. 24-27) (emphasis added); *see also Ariad*, 598 F.3d at 1351 (sufficiency of disclosure must be determined on a case-by-case basis).

The absence of disclosure is also particularly problematic here because the specification clearly conveys to the skilled artisan that not every antibody or antibody fragment raised against amino acid residues 1-28 will actually have the claimed function of inhibiting β-amyloid aggregation. As shown in the specification, monoclonal antibody 6F/3D had only partial or no appreciable effect on β-amyloid aggregation *in vitro*, even though it ostensibly binds to an epitope found within amino acids 8-17 of β-amyloid. A00048 (col. 15, ll. 49-53). Moreover, during prosecution, Solomon called "the bold statement that all antibodies that recognize an epitope between 1-28 of Aβ will prevent aggregation of β-amyloid or will cause aggregated β-amyloid to disaggregate, [] unsupportable and untrue." A00512.

Accordingly, Solomon's specification fails to show adequate possession of the claimed genus of antibodies through either the disclosure of a representative number of species or the disclosure of a function-structure correlation. Solomon's arguments to the contrary fail.

2. Solomon's rebuttal arguments fail to show that she had possession of the large genus of antibodies and antibody fragments encompassed by the scope of representative claim 5

Solomon asserts that recitation of the claimed antibodies' "properties" sufficiently describes a genus of antibodies and antibody fragments having the claimed functions. Br. at 32-33. Not so. The "properties" to which Solomon refers are the wished-for functions of binding to and inhibiting β-amyloid aggregation as well as AMY-33. *Halliburton Energy Servs., Inc. v. M-I LLC*, 514 F.3d 1244, 1255 (Fed. Cir. 2008) (explaining that functional claim language defines an element by "what it does rather than what it is") (quotation omitted); *see also* A00980. And "functional characteristics" satisfy the written description requirement only where they are "coupled with a known or disclosed correlation between function and structure." *Enzo Biochem, Inc. v. Gen-Probe Inc.*, 323 F.3d 956, 964 (Fed. Cir. 2002) (quotation omitted).

As explained immediately above, the specification provides no correlation between the claimed function of binding to and inhibiting β-amyloid aggregation and the structure of an antibody or antibody fragment capable of performing

these functions at least as well as AMY-33. And in addition to failing to provide even one example of an antibody or antibody fragment that possesses the claimed functions, the specification also "teaches nothing about the structure, epitope characterization, binding affinity, specificity, or pharmacological properties common" to the antibodies of the claimed genus. *In re Alonso*, 545 F.3d 1015, 1021-22 (Fed. Cir. 2008). Accordingly, Solomon's reliance on the functional abilities of the claimed antibodies fails.

Solomon also argues that her specification requires little detail because finding members of the claimed genus of antibodies would be a "simple matter," because skill in the antibody art is high and the field is mature. Br. at 33 (citing *Capon v. Eshhar*, 418 F.3d 1349, 1357-58 (Fed. Cir. 2005)). Solomon relies on Example 13 of the agency's *PTO Guidelines* as evidence for that notion. Br. at 33-37. But Example 13 is inapposite to Solomon's claim.

As explained in the *PTO Guidelines*, production of antibodies that bind to a well-characterized antigen, such as β-amyloid here, is conventional. *PTO Guidelines* at 46. But Solomon's claim is not limited to an antibody that merely binds β-amyloid. A00028-29. The antibody or antibody fragment must *also* inhibit β-amyloid aggregation, and do so *at least as well* as AMY-33. As this Court explained in *Centocor*, claiming antibodies with specific properties beyond mere binding ability—*i.e.*, a subgenus of binding antibodies—requires

more disclosure than just the antigen. 636 F.3d at 1352 ("Claiming antibodies with specific properties, e.g., an antibody that binds to human TNF-α with A2 specificity, can result in a claim that does not meet written description even if the human TNF-α protein is disclosed . . . ."); *see also* A00028.

Solomon's related argument that *Centocor* is inapplicable to this case, Br. at 36-38, also lacks merit. Solomon correctly notes that Centocor's claims lacked sufficient written description because, at the time of Centocor's application, it was not technologically possible to make the fully-humanized antibodies that Centocor claimed. *Centocor*, 636 F.3d at 1352. But this Court's explanation of why Centocor's arguments failed applies directly to this case. Specifically, Centocor—like Solomon here—claimed a large genus of antibodies but failed to disclose any single antibody falling within the scope of its claims. *Id.* at 1348. And Centocor—like Solomon here—indirectly relied on the *PTO Guidelines* to argue that no examples are necessary to meet the written description requirement for a genus of antibodies claimed by their binding affinity for a certain protein and ability to inhibit certain biological reactions. *Id.* at 1349 (describing Centocor's argument that the claimed antibodies were adequately described by their binding affinity to TNF-α and ability to competitively inhibit binding of the A2 mouse antibody to TNF-α).

This Court disagreed, explaining that Example 13 of the *PTO Guidelines* only applies where "the applicant is disclosing a novel protein and then claiming both the protein and an antibody that binds to it." *Id.* at 1351. This Court then held that both Example 13 as well as its own precedent stand for the proposition that an applicant may claim an antibody to a particular antigen without disclosing an example of that antibody only where (1) the applicant discovers and fully characterizes a novel antigen to which the antibody binds, and (2) the creation of those antibodies is routine. *Id.* at 1352; *see also Noelle v. Lederman*, 355 F.3d 1343, 1349 (Fed. Cir. 2004) (finding that disclosure of novel, fully-characterized mouse antigen showed possession of a genus of mouse antibodies, but did not similarly show possession of a genus of human antibodies because the human antigen was unknown).

Here, as in *Centocor*, there is no dispute that the antigen—*i.e.*, β-amyloid—was known in the art prior to Solomon's filing date. *See, e.g.*, A00041 (col. 1, ll. 47-50). Further, while creation of some antibodies simply binding to β-amyloid *in vitro* was conventional, selection of the subgenus of antibodies that both bind to β-amyloid and inhibit β-amyloid aggregation in a human was not equally conventional, as the specification itself demonstrates, *see* A00048 (col. 15, ll. 49-53) (showing that 6F/3D had only partial or no appreciable effect on β-amyloid aggregation *in vitro*, even though it ostensibly

binds to an epitope found within amino acids 8-17 of β-amyloid), and as Solomon has admitted, A00512. *See also* A00982 (Examiner's reasoning that the predictability of producing antibodies having the desired ability to inhibit β-amyloid aggregation is low based on the fact that only half of the antibodies tested in the specification exhibited the desired functions). Thus, as in *Centocor*, Example 13 of the *PTO Guidelines* does not allow Solomon to claim a genus of antibodies by merely disclosing an antigen.

Finally, Solomon implies that she should receive a patent because she was the first to discover that antibodies can be used to inhibit soluble β-amyloid aggregation. Br. at 38. Solomon's contribution to the sciences is not the dispute. As this Court explained in *Ariad*, "[p]atents are not awarded for academic theories, no matter how groundbreaking or necessary to the later patentable inventions of others." 598 F.3d at 1353. Here, Solomon's specification does not identify "the practical implications of [her] research," *i.e.*, the antibodies and antibody fragments that can be administered to a human to bind to β-amyloid and inhibit β-amyloid aggregation at least as well as AMY-33. *Id.* Rather, as the Board explained, Solomon's disclosure provides a plan for obtaining the claimed antibodies, "'leaving it to the pharmaceutical industry to complete an unfinished invention.'" A00029 (quoting *Ariad*, 598 F.3d at 1353).

Solomon's specification fails to show that she invented, or had possession of, a genus of antibodies or antibody fragments falling within the broad scope of representative claim 5. Accordingly, the Board's written description rejection is supported by substantial evidence and should be affirmed should the Court need to reach this issue.

VI. CONCLUSION

For the foregoing reasons, the Board's conclusion that the '651 patent lacks an error correctable by reissue is correct, and its finding that claims 5, 7-9, and 31-34 lack sufficient written description under § 112, first paragraph, is supported by substantial evidence. This Court should affirm the Board's decision.

Respectfully submitted,

March 15, 2013

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CERTIFICATE OF SERVICE

I hereby certify that on March 15, 2013, I electronically filed the foregoing BRIEF FOR APPELLEE—ACTING DIRECTOR OF THE UNITED STATES PATENT AND TRADEMARK OFFICE using the Court's CM/ECF filing system. Counsel for appellant was electronically served by and through the Court's CM/ECF filing system per Fed. R. App. P. 25 and Fed. Cir. R. 25(a) and 25(b).

/s/ Kristi L. R. Sawert
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RULE 32(a)(7)(C) CERTIFICATE OF COMPLIANCE

I certify pursuant to Fed. R. App. Proc. 32(a)(7) that the foregoing BRIEF FOR APPELLEE—ACTING DIRECTOR OF THE UNITED STATES PATENT AND TRADEMARK OFFICE complies with the type-volume limitation required by the Court's rule. The total number of words in the foregoing brief, excluding the table of contents and the table of authorities, is 10,812 words as calculated using the Word® software program.

/s/ Kristi L. R. Sawert
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EXHIBIT I

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Polyethylene glycol (PEG)-conjugated therapeutic peptides/proteins have been shown to exhibit clinical properties superior to those of their corresponding unmodified parent molecules. However, the desirable pharmacological features gained by protein PEGylation become irrelevant if conjugates are inactivated or cannot reach their target tissues. Here we describe the design and synthesis of MAL-FMS-OSU. This bifunctional agent enables PEG chains to be linked to peptides and proteins through a slowly hydrolysable chemical bond. PEG-FMS-peptide/protein conjugates thus formed undergo spontaneous hydrolysis at a slow rate upon incubation at pH 8.5, 37 °C with a $t_{1/2}$ value of 8–14 ± 2 h, generating the unmodified parent molecule. The validity of this approach was studied with exendin-4 and human growth hormone. A single subcutaneous administration of $PEG_{40,000}$-FMS-exendin-4 facilitated a prolonged and stable reduction in glucose levels in mice ($t_{1/2}$ = 30 ± 2 h) and exceeded the effect obtained by the same dose of the native hormone by 7–8 times.

Recently, several polyethylene glycol (PEG)[1]-conjugated therapeutic proteins have been shown to exhibit clinical properties superior to those of their corresponding unmodified parent proteins (1–3). Apparently, covalent binding of PEG to proteins produces steric interference, protecting conjugates from proteolysis and decreasing their rate of clearance from the circulation system via intracellular uptake and kidney filtration (2, 6, 7).

To achieve these desired pharmacological features, several low molecular weight PEG chains or a single high molecular weight PEG must be linked to a protein (8). However, this often drastically decreases the biological or pharmacological potency of the protein (4, 5). Therefore, to maximize protection while minimizing reductions in bioactivity, PEGylation is usually applied to higher molecular weight proteins that are less likely to be inactivated by the procedure. However even such modification may lead to marked reduction in activity. For example, a currently used 40-kDa PEG-interferon-α2a exhibits only 7% of the activity of the native cytokine, thus necessitating the administration of higher doses (9). Furthermore, in contrast to its parent protein this 40-kDa PEG-interferon-α2a does not readily penetrate all target tissues, as it is restricted to the blood and interstitial fluid (10).

Several attempts to overcome these obstacles through reversible protein/peptide PEG-assisted delivery (*e.g.* by employing detachable PEG chains) have been reported (11–15). They suffer, however, from major disadvantages including their dependence on enzymatic hydrolysis as the rate-determining step of PEG-parent molecule dissociation (11–13), potentially making the behavior of the molecules unpredictable and their utilization impractical because of steric considerations and inappropriate enzyme availability. An additional disadvantage is the nonspecific PEGylation of undesired protein/peptide functional moieties (*e.g.* hydroxyls, imidazole, thiols (14)), potentially causing post-PEGylation inter- and intramolecular reactions (14, 15) that could generate complex mixtures and immune-related risks.

In view of these issues, we tested a novel approach for designing PEG-peptide/protein conjugates from which PEG is released by spontaneous chemical hydrolysis. Reversible PEG-protein conjugation permits time-dependent reactivation of inactive PEGylated molecules. An appropriate conjugate would have to undergo slow and spontaneous hydrolysis under physiological conditions to yield the intact parent molecule (16–19). Our contributions are brought here in detail, using the antidiabetic peptide exendin-4 and human growth hormone (hGH) as *in vivo* and *in vitro* models, respectively.

EXPERIMENTAL PROCEDURES

Materials—Exendin-4 (HGEGTFTSDLSKQMEEEAVRLFIEWLKNGGPSSGAPPPS-NH_2) and a non-lysine-containing synthetic peptide of 27 amino acids (AEISGQLSYVRDVNSWQHIWTNVSIEN) were synthesized by the solid phase method using a multiple peptide synthesizer, AMS 422 (Abimed Analyser Technik, GmbH). An Fmoc (*N*-(9-fluorenyl)methoxycarbonyl) strategy was employed throughout the peptide chain assembly. α-Lactalbumin, 5,5′-dithiobis(2-nitrobenzoic acid), reduced glutathione, and trinitrobenzene sulfonic acid were purchased from Sigma. $PEG_{40,000}$-OSu (mPEG2-*N*-hydroxysuccinimide ester) was purchased from Shearwater (Huntsville, AL). All other materials used in this study were of analytical grade. A Centricon-50 ultrafiltration device for aqueous solutions was purchased from Millipore S.A.

Ultraviolet spectra were obtained by using a Beckman DU 7500 spectrophotometer in 1-cm path length UV cuvettes. Mass spectra were determined using MALDI-TOF and ESMS techniques (Bruker-Reflex-Reflectron model and VG-platform-II electrospray single quadropole mass spectrometer, Micro Mass, respectively).

HPLC analyses were performed using a Spectra-Physics SP8800 liquid chromatography system equipped with an Applied Biosystems 757 variable wavelength absorbance detector and a Spectra-SYSTEM

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[1] The abbreviations used are: PEG, polyethylene glycol; hGH, human growth hormone; MALDI-TOF, matrix-assisted laser desorption ionization time-of-flight; ESMS, electrospray single quadropole mass spectrometer; MAL, maleimide; FMS, 9-hydroxymethyl-7-sulfofluorene-*N*-hydroxysuccinimide; OSu, hydroxysuccinimide ester; HPLC, high pressure liquid chromatography; TLC, thin layer chromatography; PBS, phosphate-buffered saline.

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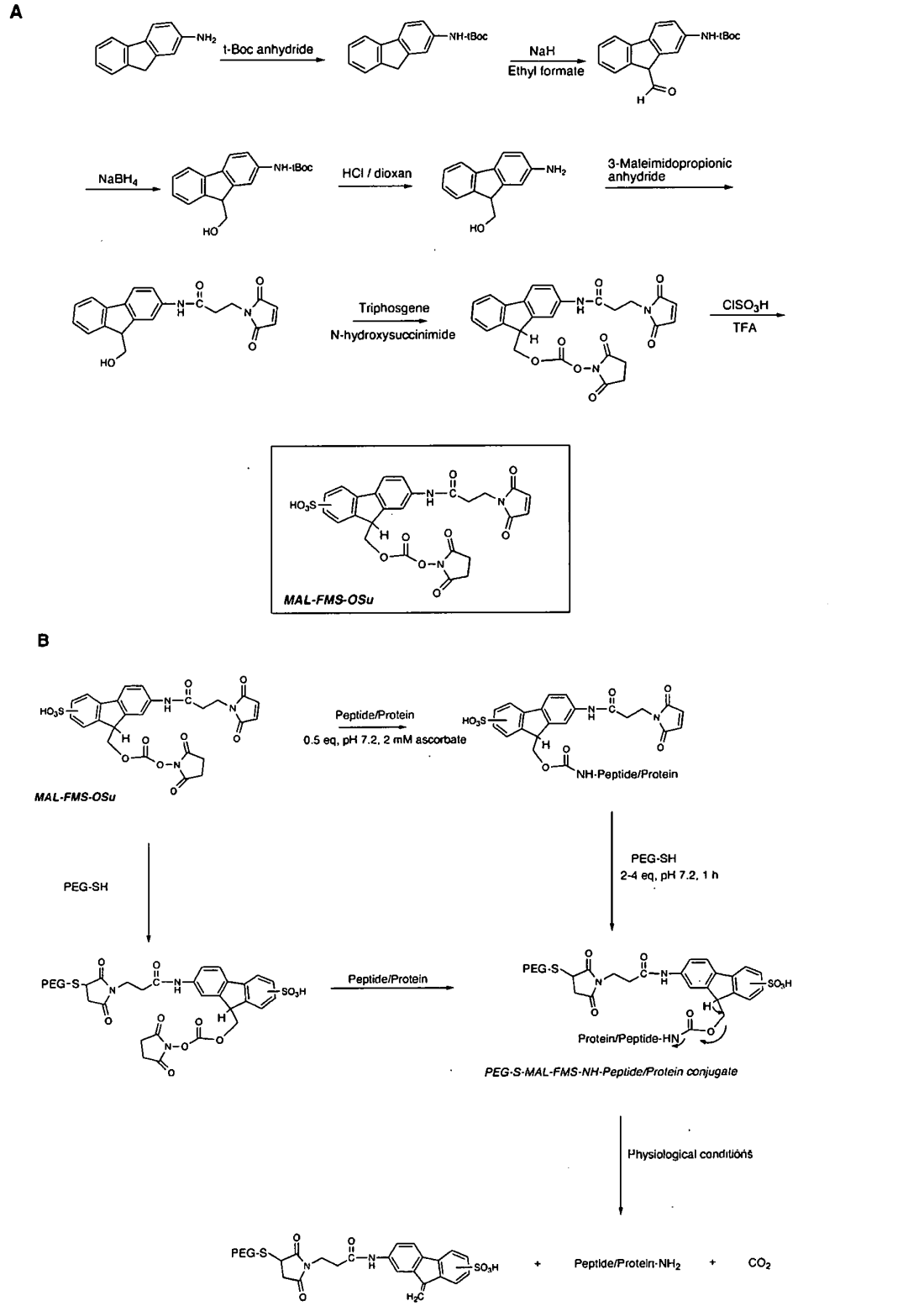

FIG. 1. **Schematic illustration of the synthesis and the structure of MAL-FMS-OSu (*A*) and of the two possible experimental procedures for preparing PEG-FMS-peptide/protein conjugates (*B*).**

P2000 liquid chromatography system equipped with a Spectra-SYSTEM AS100 autosampler and a Spectra-SYSTEM UV1000, all controlled by a ThermoQuest chromatography data system (ThermoQuest Inc., San Jose, CA). The column effluents were monitored by UV absorbance at 220 nm. Analytical reverse phase-HPLC was performed using a pre-packed Chromolith™ Performance RP-18e column (4.6 ×

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100 mm, Merck, Darmstadt, Germany). The column was eluted with a binary gradient of 10–100% solution B over 10 min with a flow rate of 3 ml/min (solution A was 0.1% trifluoroacetic acid in H_2O and solution B was 0.1% trifluoroacetic acid in acetonitrile:H_2O; 3:1, v/v). PEGylated compounds were analyzed using an RP-4 column (250 × 4 mm, 5-μm bead size, Vydac, Hesperia, CA) with a binary gradient of 10–100% solution B for 50 min at a flow rate of 1 ml/min.

Preparative separations were performed with pre-packed Vydac RP-18 or RP-4 columns (250 × 22 mm). The column was eluted with 10–100% solution B over 60 min (12 ml/min).

MAL-FMS-OSu—9-Hydroxymethyl-2-amino fluorene was synthesized with an 84% yield as described elsewhere (20). Conditions were as follows: TLC, chloroform:methanol, 9:1 (v/v); R_F = 0.38; calculated ESMS = 211.26 Da, and found ESMS for $[M+H]^+$ = 212.10 Da.

3-Maleimidopropionic Anhydride—3-Maleimidopropionic acid (1 g, 5.9 mmol) and *N,N'*-dicyclohexylcarbodiimide (0.69 g, 2.95 mmol) were dissolved in 10 ml of *N,N*-dimethylformamide and incubated for 4 h. Dicyclohexylurea was filtered out, and the anhydride formed was kept at 4 °C.

9-Hydroxymethyl-2-amino fluorene (0.4 g, 1.9 mmol) and $NaHCO_3$ (0.74 g, 8.85 mmol) were dissolved in water and 3-maleimidopropionic anhydride in *N,N*-dimethylformamide (12 ml, 2.95 mmol) was added. The reaction mixture was stirred for 40 min, and product formation was monitored by analytical HPLC on a Chromolith column (R_t = 4.46 min, 10–100% solution B in 10 min, 3 ml/min). The crude product was purified using preparative HPLC (RP-18 column, 10–100% acetonitril: water (75:25; v/v), 60 min, 12 ml/min). The yield was 57%, 1.08 mmol, 0.39 g; calculated ESMS = 362.38 Da, and found ESMS = 362.42 Da.

Pyridine (0.167 ml, 2 mmol) was added dropwise to a stirred solution of 9-hydroxymethyl-2-(amino-3-maleimidopropionate)fluorene (0.37g, 1.02 mmol) and triphosgene (0.425 g, 1.43 mmol) in dry tetrahydrofuran (10 ml). After 20 min the precipitated pyridine hydrochloride salt was filtered out, and the tetrahydrofuran was removed by vacuum. The oil obtained was dissolved in 10 ml of dry tetrahydrofuran with *N*-hydroxysuccinimide (0.61g, 5.3 mmol). Pyridine (0.26 ml, 3.2 mmol) was then added, and the solution was stirred for 20 min. Some additional precipitated pyridine hydrochloride salt was filtered out, and the tetrahydrofuran was removed by vacuum. The oil obtained was dissolved in chloroform (100 ml) and washed with an aqueous $NaHCO_3$ solution (0.5 N, 3 × 50 ml), HCl (0.1 N, 3 × 50 ml), water (2 × 50 ml), and brine. The chloroform was removed by vacuum, and the product was desiccated. Conditions were as follows: yield was 89%, 0.9 mmol, 0.45 g; HPLC (Chromolith column) R_t = 5.7 min (10–100% solution B in 10 min, 3 ml/min). Calculated ESMS = 503 Da, found ESMS for $[M+Na]^+$ = 526.38 Da, and found ESMS for $[M+K]^+$ = 542.30 Da.

9-Hydroxymethyl-2-(amino-3-maleimidopropionate)-7-sulfofluorene N-Hydroxysuccinimide—To a solution of 9-hydroxymethyl-2-(amino-3-maleimidopropionate)-fluorene *N*-hydroxysuccinimide (0.2 mmol, 0.1 g) in trifluoroacetic acid (10 ml), chlorosulfonic acid (0.5 ml) was added. After 15 min, cold ether (90 ml) was added, and the precipitated product was washed three times with ether and desiccated. Conditions were as follows: yield: 95%, 0.11 g, 0.19 mmol; HPLC (Chromolith column) R_t = 2.65 min (10–100% solution B in 10 min, 3 ml/min). The calculated ESMS = 583 Da; found ESMS for $[M-H]^+$ = 582.24 Da, for $[M+H]^+$ = 584.52 Da, and for $[M+Na]^+$ = 606.47 Da.

$PEG_{40,000}$-SH—$PEG_{40,000}$-OSu was dissolved at a concentration of 40 mg/ml in an aqueous solution of cystamine-di-HCl (1 M) and brought to pH 8.5 with $NaHCO_3$. Reaction was carried out for 2 h at 25 °C. The product thus obtained was dialyzed overnight against 0.1 M $NaHCO_3$, treated with 30 mM dithiothreitol (25 °C, 1 h), and redialyzed against 0.01 M HCl. $PEG_{40,000}$-SH was obtained with a 93% yield. It contained 1 mol sulfhydryl moiety per mol of $PEG_{40,000}$ as determined with 5,5'-dithiobis(2-nitrobenzoic acid) in the presence of ascorbate.

Preparation of PEG_{5000}-2-Sulfo-9-fluorenylmethoxycarbonyl N-Hydroxysuccinimide—MAL-FMS-OSu (1.8 mg, 3 μmol) was added to a solution of PEG_{5000}-SH (15 mg, ~3 μmol) in phosphate-buffered saline (PBS) (0.5 ml, pH 7.4), and the reaction solution was stirred for 30 min. The product PEG_{5000}-FMS-OSu was purified by HPLC on an RP-4 column and lyophilized.

PEG_{5000}-FMS-Exendin-4—PEG_{5000}-FMS-OSu (2.3 mg, ~0.42 μmol) was added to a solution of exendin-4 (1.6 mg, 0.4 μmol) in PBS (0.5 ml, pH 8), and the reaction solution was stirred for 2 h. The product PEG_{5000}-FMS-exendin-4 was purified by HPLC on an RP-4 column and lyophilized.

Preparation of $PEG_{40,000}$-FMS-Exendin-4—To a stirred solution of exendin-4 (1 mg in 1.0 ml of 0.1 M phosphate buffer, 2 mM ascorbate, pH 7.2, 0.239 mM) was added 280 μg of MAL-FMS-OSu (28 μl from a fresh solution of MAL-FMS-OSu in *N,N*-dimethylformamide, 10 mg/ml, 2.0 molar excess over the peptide). After 7 min, solid $PEG_{40,000}$-SH was added to a final concentration of 0.5 mM (2.1 molar excess over the peptide). The reaction was carried out for 1 h, dialyzed overnight against H_2O, and then further filtered through Centricon with a molecular mass cutoff of 50 kDa to remove any residual exendin-4 or MAL-FMS-exendin-4 that had not linked to $PEG_{40,000}$-SH. The concentration of the conjugate was determined by amino acid analysis according to the content of aspartic acid (two residues), alanine (two residues) and valine (one residue), or calculated from the absorbance of the conjugate at 280 nm. The calculated extinction coefficient at 280 nm was 26,940 ± 100, a value that is the additive absorbance of exendin-4 (ϵ_{280} = 5740) and $PEG_{40,000}$-FMS (ϵ_{280} = 21,200). A solution of 20 μM $PEG_{40,000}$-FMS-exendin had A = 0.51. Thus the conjugate had a 1:1 $PEG_{40,000}$-FMS/exendin stoichiometry.

Preparation of $PEG_{40,000}$-Exendin-4—Exendin-4 (0.3 mg, 75 nmol) was dissolved in PBS, pH 7.5, and reacted with $PEG_{40,000}$-OSu (20 mg, 470 nmol) for 3 h, filtered (7 times) through Centricon (cut off, 50 kDa), and characterized by MALDI-TOF mass spectrometry (found: 48,074 Da).

$PEG_{40,000}$-FMS-peptide 27 was prepared as described for $PEG_{40,000}$-

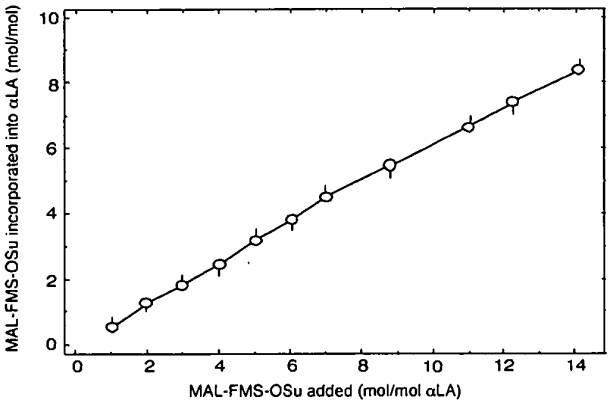

FIG. 2. **Degree of incorporation of MAL-FMS-OSu at pH 7.2 into α-lactalbumin as a function of the amount of added reagent.** To samples of α-lactalbumin (1.0 ml of 1 mg/ml in 0.1 M phosphate buffer, pH 7.2) MAL-FMS-OSu was added at concentrations ranging from 1 eq up to 14 molar eq of MAL-FMS-OSu. For each treatment the amount incorporated into the protein was determined by the absorbance at 280 nm after dialysis and by quantitating the amount of unmodified amino side chain moieties with trinitrobenzene sulfonic acid.

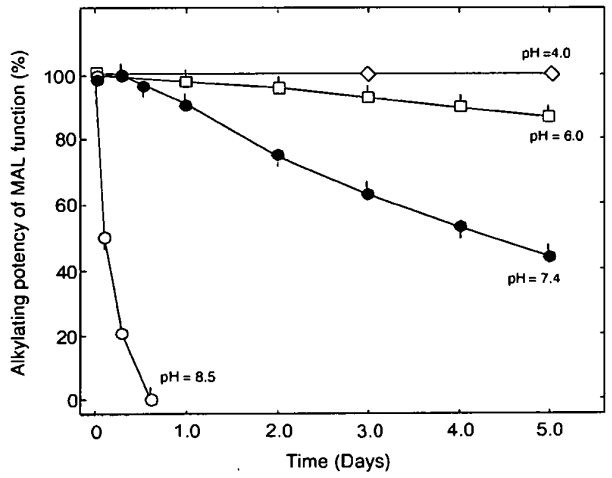

FIG. 3. **Stability of the maleimide functional moiety in MAL-FMS-OSu in aqueous solutions having different pH values.** MAL-FMS-OSu (1 mM) was incubated at room temperature in H_2O (pH 6.0), in 0.007 M acetic acid (pH~4.0), in 0.1 M phosphate buffer (pH 7.4), and in 0.1 M $NaHCO_3$ (pH 8.5). At the indicated time points, aliquots were allowed to react with a slight excess of reduced glutathione (15 min at pH 7.2), and the concentration of unreacted reduced glutathione was determined with 5,5'-dithiobis(2-nitrobenzoic acid).

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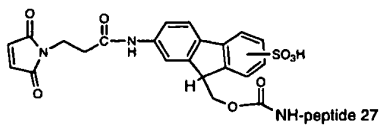
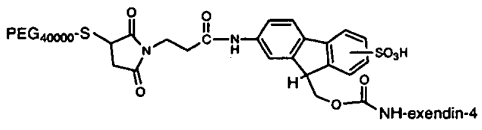
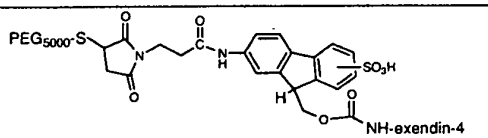
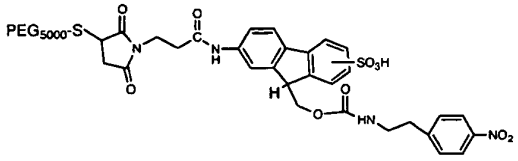
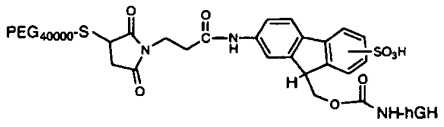
TABLE I
Structures and half-lives of PEG-FMS conjugates

	Structure	k (h^{-1})[b]	$t_{1/2}$ (h)[c]
Mal-FMS-Peptide 27[a]		0.082	8.4
PEG_{40000}-FMS-exendin-4		0.058	11.9
PEG_{5000}-FMS-exendin-4		0.050	13.8
PEG_{5000}-FMS-4-nitro-phenethylamine		0.074	9.4
PEG_{40000}-FMS-human growth hormone (hGH)		0.059	11.8

[a] Rate of hydrolysis was followed by trinitrobenzene sulfonic acid assay.
[b] k is the slope constant derived from the plot of ln(PEG-FMS conjugate) against time (h).
[c] $t_{1/2}$ was determined from the formula $t_{1/2} = \ln 2/k$.

FMS-exendin-4. MAL-FMS-OSu (280 μg) was added to a solution of hGH (4.5 mg, 0.225 μmol) in 0.5 ml of PBS, pH 7.4. After 10 min $PEG_{40,000}$-SH (10 mg) was added, and the reaction mixture was stirred for 1 h and dialyzed overnight against water. An hGH displacement assay was performed as described elsewhere (21).

Glucose-lowering Assay of $PEG_{40,000}$-FMS-Exendin-4—Three groups of CD1 mice (n = 6 per group) subcutaneously received saline, native exendin-4 (4 μg/mouse), or $PEG_{40,000}$-FMS-exendin-4 (4 μg of peptide/mouse). Circulating glucose levels were measured with a glucose analyzer (Beckman Instruments) by the glucose oxidase method. Blood samples for the blood glucose analyses were taken from the tail veins. The level of glucose in normal healthy CD1-mice was 140 ± 7 mg/dl (7.77 mM).

Glucose-lowering Assay of Released Exendin-4—$PEG_{40,000}$-FMS-exendin-4 (0.36 mg containing 30 μg of exendin-4) was incubated for 24 h at pH 8.5, 37 °C. The released peptide was separated from the $PEG_{40,000}$-sulfodibezofulvene using HPLC on a Chromolith column (10–100% solution B in 10 min, 3 ml/min), and the peptide solution was lyophilized. The peptide concentration was determined by its absorbance at 220 nm. Native exendin-4 and the released exendin-4 were dissolved in saline and administered subcutaneously at identical doses to five groups of CD1 mice (n = 3 per group). Circulating glucose levels were then determined as described above.

RESULTS

We have designed, synthesized, and characterized MAL-FMS-OSu, Fig. 1*A*). This is a water-soluble heterobifunctional reagent consisting of a sulfonated fluorenyloxycarbonyl *N*-hydroxysuccinimide ester that reacts with peptide and protein amino groups. A maleimide group was attached to the fluorenyl backbone to enable coupling to sulfhydryl-containing PEG-SH (Fig. 1*B*). At 320 nm, where peptides or proteins absorb negli-

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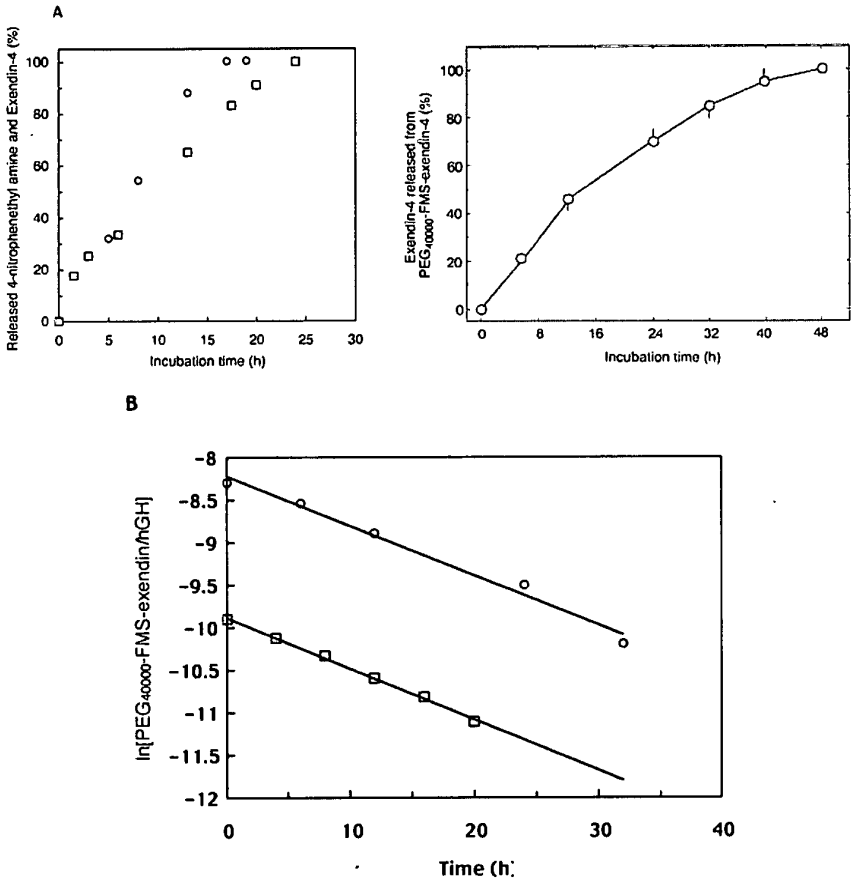

FIG. 4. The rate of hydrolysis of PEG-FMS conjugates upon incubation at pH 8.5, 37 °C is shown in *A*. Solutions of $PEG_{40,000}$-FMS-exendin-4 (*right*, O), PEG_{5000}-FMS-exendin-4 (*left*, O), and PEG_{5000}-FMS-4-nitro-phenethyl amine (*left*, □) were incubated in PBS at pH 8.5, 37 °C. At the indicated time points, aliquots (50 μl) were analyzed using HPLC on an RP-4 column. Results are expressed as percent of the maximal peak area of released exendin-4 and 4-nitrophenethylamine as a function of time. *B*, the concentration of $PEG_{40,000}$-FMS-exendin-4 (O) and $PEG_{40,000}$-FMS-hGH (□) was determined for each time point by HPLC. The linear plot obtained indicates that the rate of hydrolysis is of first order reaction. The half-life time of the conjugates was calculated from $t_{1/2} = \ln 2/k$, when k is the slope of the linear plot (h^{-1}).

gibly, MAL-FMS-OSu (either free or covalently bound to proteins) absorbs with a molar extinction coefficient (ϵ_{320}) of 16,100 ± 150 mol^{-1} cm^{-1}.

Degree of MAL-FMS Incorporation as a Function of the Spacer Added—In Fig. 2, samples of α-lactalbumin (1 mg/ml) were modified with increasing concentrations of MAL-FMS-OSu in 0.1 M PBS, pH 7.2, for 30 min at 25 °C. That is, under experimental conditions where the maleimide-alkylating capacity of the spacer remains nonmodified (see the next paragraph). About 1 mol of MAL-FMS was incorporated into α-lactalbumin per 2.1 mol of reagent added, up to a 14 molar excess over the protein (Fig. 2).

Stability of the MAL Function in Aqueous Solutions—Fig. 3 demonstrates the potency of the reaction of MAL-FMS-OSu with reduced glutathione as a function of time at different pH values. MAL-FMS-OSu was found fully stable at pH 4–6 over a period of a day or two, and at pH 7.2 it is stable over a period of several hours. At pH 8.5 the maleimido moiety is destroyed at an accelerated rate ($t_{1/2}$ = 2.7 ± 0.3 h, Fig. 3).

Two possible pathways for the related PEGylation of target peptides, proteins, and small molecules are suggested (Fig. 1*B*). One involves attaching MAL-FMS-OSu to the amine component of the target and then substituting PEG-SH for the maleimide moiety. The second option involves preparing PEG-FMS-OSu, which then reacts with the amine component of the target (Fig. 1*B*). This pathway is suitable for sulfhydryl- or disulfide-containing peptides and proteins.

Rate of Hydrolysis of PEG-FMS Conjugates—Four PEG-FMS conjugates and one MAL-FMS conjugate were prepared using the described pathways (see Table I and "Experimental Procedures"). The hydrolysis rates and reaction orders of PEG_{5000}-FMS-4-nitro-phenethylamine, PEG_{5000}-FMS-exendin-4, $PEG_{40,000}$-FMS-exendin-4, and $PEG_{40,000}$-FMS-hGH were evaluated at pH 8.5, 37 °C (Table I). The hydrolysis rate at pH 8.5 is equivalent to the hydrolysis rate in serum as shown previously (16–19). The release of 4-nitro-phenethylamine, exendin-4, and hGH was monitored by reverse phase-HPLC and was determined to be a first order reaction (Table I and Figs. 4 and 5). As shown (Fig. 4 and Table I), the peptides and protein were released from the conjugates in a slow homogenous fashion, with $t_{1/2}$ of 9.4, 13.8, 11.9, and 11.8 h. Exendin-4 was fully released from the $PEG_{40,000}$ conjugate after 48 h of incubation.

Demonstrating the Validity of Reversible PEGylation with hGH and Exendin-4—*In vitro*, $PEG_{40,000}$-FMS-hGH exhibits no receptor binding affinity. Upon incubation, recognition of the released hGH by the native hGH receptor was preserved (Fig. 5).

Exendin-4 is an insulinotropic glucagon-like peptide-1 agonist that associates with β-pancreatic cells, elevates endogenous cAMP levels, enhances secretion of insulin, and lowers circulating glucose levels (22–25). A profound pharmacological advantage of this glucagon-like peptide-1 agonist is that (following administration at any dosage) the circulating blood glucose level never falls below a threshold that in non-diabetic healthy CD1 mice is 74 ± 7 mg/dl (26). Fig. 6 shows the glucose-lowering profile of subcutaneously administered native exendin-4 and of $PEG_{40,000}$-FMS-exendin-4 at a dose of 4 μg of peptide/mouse relative to a saline-treated group of mice. With the native peptide, blood glucose level declined 26–28% (from 140 to 104–101 mg/dl) with the largest percent change in blood glucose level occurring 0.5–1 h after administration. Glucose concentrations then returned to their initial levels with a $t_{1/2}$ of

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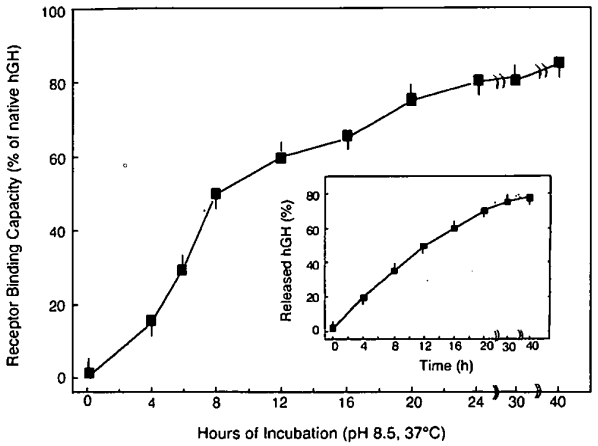

FIG. 5. **Time course of reactivation of $PEG_{40,000}$-FMS-hGH upon incubation at pH 8.5, 37 °C.** $PEG_{40,000}$-FMS-hGH (1 mg/ml) was incubated in 0.1 M phosphate buffer, 0.6% bovine serum albumin, and 2 mM NaN_3 at 37 °C. Aliquots were withdrawn at the indicated time points and analyzed for their potencies in displacing ^{125}I-hGH from enriched hGH receptor preparation extracted from rabbit liver plasma membranes. Native hGH displaces ^{125}I-hGH in this assay half-maximally at a concentration of 0.3 ± 0.03 nM. An hGH derivative exhibiting half-maximal displacement in this assay at a concentration of 3.0 ± 0.3 nM is considered to have 10% of the binding potency of the native receptor. The rate of release of hGH from PEG_{40}-FMS-hGH upon incubation at pH 8.5, 37 °C is shown in the *inset*. $PEG_{40,000}$FMS-hGH (1 mg of protein) was incubated as described above. At the indicated time points, 0.1-ml aliquots were withdrawn and subjected to analytical HPLC analysis.

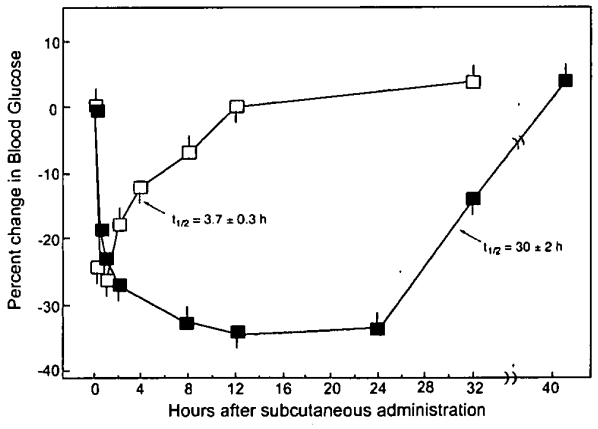

FIG. 6. **Glucose-lowering patterns of native exendin-4 and $PEG_{40,000}$-FMS-exendin-4 following a single subcutaneous administration to CD1-mice.** Three groups of CD1 mice (n = 6 per group) underwent one subcutaneous administration of saline, native exendin-4 (□, 4 μg/mouse), or $PEG_{40,000}$-FMS-exendin-4 (■, 4 μg of peptide/mouse). Circulating glucose levels were then monitored. Results are expressed as percent decrease in plasma glucose concentration in the groups treated with exendin-4 or $PEG_{40,000}$-FMS-exendin-4 relative to that found in the saline-treated group measured at the same time point during the day.

3.7 ± 0.3 h. Following the subcutaneous administration of $PEG_{40,000}$-FMS-exendin-4, the decrease in blood glucose level took place at a more moderate rate. Circulating glucose reached its lowest concentration 8–12 h after administration (92 mg/dl, 33%). Stable, low circulating glucose concentrations were then maintained for a further 12 h. Return to initial glucose levels took place with a $t_{1/2}$ of 30 ± 2 h, which is 7.5 times longer than that obtained by the same dose of the native hormone.

Irreversibly conjugated $PEG_{40,000}$-exendin-4 has only ≤1% of

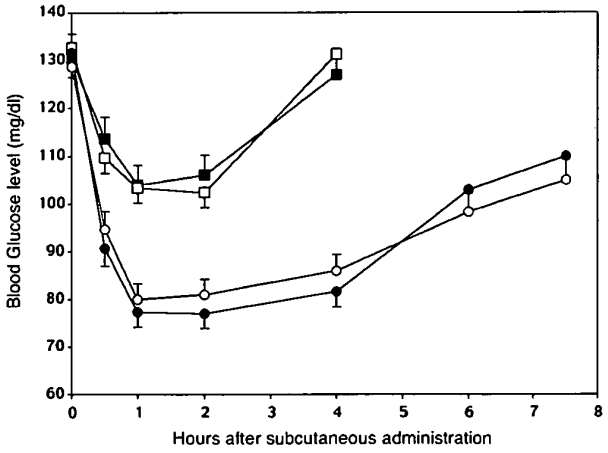

FIG. 7. **Glucose-lowering patterns of exendin-4 released from $PEG_{40,000}$-FMS-exendin-4.** Five groups of CD1 mice (n = 3 per group) underwent one subcutaneous administration of saline, native exendin-4, or released exendin-4 (1 and 0.1 μg of peptide/mouse, respectively). Circulating glucose levels were then determined. Results are expressed as plasma glucose levels (mg/dl) measured following subcutaneous administration. ■, native exendin-4 (0.1 μg); □, released exendin-4 (0.1 μg); ●, native exendin-4 (1 μg); ○, released exendin-4 (1 μg).

the activity of native exendin-4 (IC_{50} = 250 ± 30 pmol/mouse *versus* IC_{50} = 2.5 ± 0.24 pmol/mouse for the native exendin-4, not shown).

Biological Potency of Exendin-4 that Was Released from $PEG_{40,000}$-FMS-Exendin-4 Conjugate—Fig. 7 shows the glucose-lowering pattern of exendin-4 released from the $PEG_{40,000}$-FMS-exendin-4 conjugate by incubation at pH 8.5, 37 °C for 24 h. The glucose-lowering pattern of the released exendin-4 was indistinguishable from that obtained by the native peptide; both reduced blood glucose levels by 30 and 50% 1 h after subcutaneous administration of 0.1 and 1 μg of peptide/mouse, respectively.

DISCUSSION

PEGylation's previously mentioned side effect of drastically decreasing drug bioactivity has meant that only a limited number of such conjugates have found clinical application (5). This happens despite the profound advantages that steric protection confers in elongating the lifetimes of the drugs *in vivo*, protecting them from proteolysis and shielding them from the immune system (4–8). Theoretically, decreased bioactivity can be overcome by linking the PEG chains via a chemical bond sensitive to mild alkaline and/or acid hydrolysis or enzymatically able to be cleaved by serum proteases or esterases. Obviously, an inconsistent rate of hydrolysis would render such an approach impractical. A prerequisite condition is therefore that the hydrolysis of the PEG chains from the conjugate takes place at a slow rate and in a homogenous fashion under the strictly homeostatic pH and temperature conditions of the mammalian circulatory system (27).

Previously, we prepared FMS-OSu (17–19) as a reversible protein modifier. Protein-linked FMS moieties undergo slow and spontaneous hydrolysis under physiological conditions, generating the unmodified parent molecule. Hydrolysis of FMS-protein conjugates at 37 °C in normal human serum, in aqueous buffers of pH 8.5, or in the circulatory system *in vivo* takes place with a $t_{1/2}$ of 5–7 h. Using the FMS moiety as the scaffold for the suggested reversible PEGylation technology enables the hydrolysis rates of various PEG-FMS-protein conjugates to be predicted. The $t_{1/2}$ for the hydrolysis of modified

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small molecules and PEG-conjugated polypeptides and proteins falls within a relatively narrow range of 8–14 h (Table I). The constant hydrolysis rate for PEG-FMS-protein conjugates is caused by the β-elimination reaction, which occurs at position 9 of the fluorenyl moiety (Fig. 1*B*) and is solely dependent on the pH of the surrounding medium. Thus, in contrast to what occurs in studies based on enzymatic hydrolytic bond cleavage, similar PEG-FMS hydrolysis rates are expected from all conjugates regardless of the identity of the protein/peptide moiety conjugated. Using the present approach one can further control the hydrolysis rate by substituting the fluorenyl moiety with electron-withdrawing or electron-inducing groups that increase or decrease, respectively, the hydrolysis rate. The number of PEG-FMS chains attached to a drug should also affect the rate at which the native drug is released.

Exendin-4 contains one His N^{α} amino function and two Lys N^{ϵ} amino groups, enabling modification at these three positions. Indeed, N-terminal amino acid sequencing revealed that, although the $PEG_{40,000}$-FMS-exendin-4 product eluted as a single peak on HPLC, it was actually a mixture containing primarily the N^{α}-modified hormone. Similarly, hGH contains several amino functions that may be conjugated. However, in view of the reversibility of the PEGylation reaction, which regenerate the native peptide and protein hormones, this point deserves only minor consideration.

Calculations based on the hydrolysis rates of FMS-exendin-4 and $PEG_{40,000}$-FMS-exendin-4 at 37 °C in normal sera and PBS, pH 8.5, respectively, revealed that ~4% of exendin-4 is released from the conjugate each hour *in vivo* (19). We further hypothesized that this release rate if combined with prolonged maintenance of the conjugate in the circulatory system prior to exendin-4 hydrolysis should yield a long lasting glucose-lowering signal in mice, as indeed is found following a single subcutaneous administration of $PEG_{40,000}$-FMS-exendin-4 conjugate (Fig. 6).

It should be noted that the covalently linked exendin-4 is fully active following its release from $PEG_{40,000}$-FMS-exendin-4 by incubation (Fig. 7), suggesting that the conjugation *per se* does not lead to irreversible modification and/or denaturation of the peptide.

The sulfonated fulvene moiety is not toxic as previously shown (19). High molecular weight PEG is known to be safe in terms of toxicity and immunogenicity and is widely used in the food, cosmetic, and pharmaceutical industries (28, 29).

In summary a conceptual approach termed "reversible PEGylation" was implemented here with low molecular weight polypeptides and proteins. This novel approach enables the desirable pharmacological features associated with PEGylation to be conferred on low molecular weight peptide and protein drugs that would otherwise have been fully or partially inactivated by this technique. A pharmacologically "silent" conjugate that is "trapped" in the circulatory system releases the covalently linked parent peptide or protein with a desirable pharmacokinetic profile. This new development is expected to extend the lifetime, bioavailability, and efficacy of existing peptide drugs and to extend the same in peptide drug candidates that may yet be discovered by genomics and proteomics.

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Prolonging the Action of Protein and Peptide Drugs by a Novel Approach of Reversible Polyethylene Glycol Modification

Haim Tsubery, Marina Mironchik, Mati Fridkin and Yoram Shechter

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PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 15/646,822

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	280
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	600
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	2160
TOTAL CLAIMS (37 CFR 1.16(i))	16 minus 20=	*			OR	x 80 =	0.00
INDEPENDENT CLAIMS (37 CFR 1.16(h))	6 minus 3 =	* 3				x 420 =	1260
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						1200
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							0.00
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	5500

APPLICATION AS AMENDED - PART II

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT A	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT B	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.

** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".

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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
15/646,822	07/11/2017	Daniel J. Wendt	30610/44556C1-R2

CONFIRMATION NO. 7727

90849
Marshall, Gerstein & Borun LLP (Biomarin)
233 South Wacker Drive
6300 Willis Tower
Chicago, IL 60606

FORMALITIES LETTER

OC000000096490770

Date Mailed: 01/05/2018

NOTICE OF INCOMPLETE REPLY (REISSUE)

Filing Date Granted

The U.S. Patent and Trademark Office has received your reply on 10/04/2017 to the Notice to File Missing Parts (Notice) mailed 08/10/2017 and it has been entered into the reissue application. The reply, however, does not include the following items required in the Notice.

The period of reply remains as set forth in the Notice. You may, however, obtain EXTENSIONS OF TIME under the provisions of 37 CFR 1.136 (a) accompanied by the appropriate fee (37 CFR 1.17(a)).

A complete reply must be timely filed to prevent ABANDONMENT of the above-identified application.

The required items noted below SHOULD be filed along with any items required above. The filing date of this reissue application will be the date of receipt of the items required above.

- The inventor's oath or declaration has not been submitted.

  An inventor's oath or declaration in compliance with 37 CFR 1.63 or 1.64 (and 37 CFR 1.175) executed by or with respect to each inventor is required. For a reissue application, the inventor's oath or declaration must be submitted prior to examination and cannot be postponed. See 37 CFR 1.175(e). An assignee of the entire interest can execute the oath or declaration if: (1) the application does not seek to enlarge the scope of the claims of the original patent; or (2) the application for the original patent was filed under 37 CFR 1.46 by the assignee of the entire interest. A substitute statement under 37 CFR 1.64 in lieu of an oath or declaration may be submitted in the circumstances provided for in 35 U.S.C. 115(d) and 37 CFR 1.64.

Replies must be received in the USPTO within the set time period or must include a proper Certificate of Mailing or Transmission under 37 CFR 1.8 with a mailing or transmission date within the set time period. For more information and a suggested format, see Form PTO/SB/92 and MPEP 512.

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/ddfelix/

Docket No.: 135576.010627/REI/CON
(Prior Docket No.: 30610/44556C1-R2)
(Patent)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Daniel J. Wendt et al.

Application No.: 15/646,822 | Confirmation No.: 7727

Filed: July 11, 2017 | Art Unit: 1629

For: *VARIANTS OF C-TYPE NATRIURETIC PEPTIDE* | Examiner: To be assigned

RESPONSE TO NOTICE OF INCOMPLETE REPLY (REISSUE)
(*Filing Date Granted*)

Submitted via EFS-Web
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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Commissioner:

In response to the Notice of Incomplete Reply (Reissue), Filing Date Granted, dated January 5, 2018, as received in the above-identified continuation reissue patent application, applicant submits herewith the Inventors' Oaths or Declarations (Reissue Application Declarations), in compliance with 37 C.F.R. §1.63 (and 37 C.F.R. §1.175) and signed by inventors Daniel J. Wendt, Shinong Long, Sianna Castillo, Christopher P. Price, Mika Aoyagi-Scharber and Michel Claude Vellard. Also submitted herewith is a Substitute Statement in lieu of an Oath or Declaration for Reissue Patent Application on behalf of inventor Augustus O. Okhamafe, in compliance with 37 C.F.R. §1.64 (and 37 C.F.R. §1.175) and signed by an authorized signatory of BioMarin Pharmaceutical Inc. as assignee, in connection with this application.

The Commissioner is hereby authorized to charge any fees, or additional fees, required for the entry and consideration of this paper and accompanying submissions to Deposit Account No. 50-2678, Order No. 135576.010627/REI/CON. Should an extension of time be required in addition to that requested in a petition for extension of time, the Commissioner is requested to

grant a petition for that extension of time which is required to make this paper and accompanying submissions timely, and is hereby authorized to charge any fee for such an extension of time, or to credit any overpayment, to Deposit Account No. 50-2678, Order No. 135576.010627/REI/CON.

Respectfully submitted,
Greenberg Traurig, LLP

Dated: January 5, 2018

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Electronic Patent Application Fee Transmittal

Application Number:	15646822
Filing Date:	11-Jul-2017
Title of Invention:	Variants of C-Type Natriuretic Peptide
First Named Inventor/Applicant Name:	Daniel J. Wendt
Filer:	Leslie A. Serunian
Attorney Docket Number:	30610/44556C1-R2

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension - 3 months with $0 paid	1253	1	1400	1400
Miscellaneous:				
		Total in USD ($)		**1400**

Electronic Acknowledgement Receipt

EFS ID:	31416823
Application Number:	15646822
International Application Number:	
Confirmation Number:	7727
Title of Invention:	Variants of C-Type Natriuretic Peptide
First Named Inventor/Applicant Name:	Daniel J. Wendt
Customer Number:	90849
Filer:	Leslie A. Serunian
Filer Authorized By:	
Attorney Docket Number:	30610/44556C1-R2
Receipt Date:	05-JAN-2018
Filing Date:	11-JUL-2017
Time Stamp:	13:45:36
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	$1400
RAM confirmation Number	010818INTEFSW00000009502678
Deposit Account	502678
Authorized User	Leslie Serunian
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Warnings:					
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2	Applicant Response to Pre-Exam Formalities Notice	135576_010627REICON_ResptoNoticeofIncompleteReply.pdf	121749 447c32ba53ca3ff58be66afa4a465cf84868f612	no	2
Warnings:					
Information:					
3	Fee Worksheet (SB06)	fee-info.pdf	30496 1574eff44c7ba9fc4ea4692095c4a10dc3e75e3e	no	2
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If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.
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If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.
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If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Doc Code: REIS.DECL
Document Description: Reissue Declaration Filed In Accordance With MPEP 1414

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REISSUE APPLICATION DECLARATION BY THE INVENTOR	Docket Number (Optional) 135576.010627/REI/CON

I hereby declare that:
Each inventor's residence and mailing address are stated below next to their name.
I believe I am the original inventor or an original joint inventor of the subject matter which is described and claimed in patent number 8,598,121, granted December 3, 2013 and for which a reissue patent is sought on the invention titled VARIANTS OF C-TYPE NATRIURETIC PEPTIDE,

the specification of which

☐ is attached hereto.

☑ was filed on July 11, 2017 as reissue application number 15/646,822.

The above-identified application was made or authorized to be made by me.

I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

I believe the original patent to be wholly or partly inoperative or invalid, for the reasons described below. (Check all boxes that apply.)

☐ by reason of a defective specification or drawing.

☑ by reason of the patentee claiming more or less than he had the right to claim in the patent.

☐ by reason of other errors.

At least one error upon which reissue is based is described below. If the reissue is a broadening reissue, a claim that the application seeks to broaden must be identified:

Patentee claimed less than Patentee had a right to claim in that claim 1 of U.S. Patent No. 8,598,121 is broadened by the presentation of new claim 15 in the Preliminary Amendment filed in the subject continuation reissue application, USSN 15/646,822, on July 11, 2017. New claim 15, directed to a macromolecule capable of releasing a CNP variant, is broadened to add additional, alternative amino acid sequences of the CNP variants of the macromolecule, such as, for example, the CNP variants set forth in SEQ ID NOs: 145-178.

This collection of information is required by 37 CFR 1.175. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 30 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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(REISSUE APPLICATION DECLARATION BY THE INVENTOR, page 2)

Docket Number (Optional): 135576.010627/REI/CON

Note: To appoint a power of attorney, use form PTO/AIA/81.

Correspondence Address: Direct all communications about the application to:

☑ The address associated with Customer Number: 35893

OR

☐ Firm or Individual Name					
Address					
City		State		Zip	
Country					
Telephone		Email			

WARNING:

Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.

Legal name of sole or first inventor (*E.g.*, Given Name (first and middle (if any) and Family Name or Surname)

Daniel J. Wendt

Inventor's Signature	Date (Optional)
Daniel J Wendt	12-6-2017

Residence: City	State	Country
Novato	CA	USA

Mailing Address

City	State	Zip	Country
Novato	CA	94949	USA

[■] Additional joint inventors are named on the 2 supplemental sheet(s) PTO/AIA/10 attached hereto.

Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

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SUPPLEMENTAL SHEET FOR DECLARATION | **ADDITIONAL INVENTOR(S)**
Supplemental Sheet (for PTO/AIA/08,09) Page 1 of 2

Legal Name of Additional Joint Inventor, if any:

(*E.g.*, Given Name (first and middle (if any)) and Family Name or Surname)

Shinong Long

Inventor's Signature | Date (Optional) 10/05/2017

Residence: City: Novato | State: CA | Country: USA

Mailing Address: 105 Digital Drive

City: Novato | State: CA | Zip: 94949 | Country: USA

Legal Name of Additional Joint Inventor, if any:

(*E.g.*, Given Name (first and middle (if any)) and Family Name or Surname)

Sianna Castillo

Inventor's Signature | Date (Optional)

Residence: City: Novato | State: CA | Country: USA

Mailing Address: 105 Digital Drive

City: Novato | State: CA | Zip: 94949 | Country: USA

Legal Name of Additional Joint Inventor, if any:

(*E.g.*, Given Name (first and middle (if any)) and Family Name or Surname)

Christopher P. Price

Inventor's Signature | Date (Optional)

Residence: City: Aachen | State: | Country: Germany

Mailing Address: Papiermuehlenweg 6

City: Aachen | State: | Zip: 52070 | Country: Germany

This collection of information is required by 35 U.S.C. 115 and 37 CFR 1.63. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 21 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: **Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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SUPPLEMENTAL SHEET FOR DECLARATION | **ADDITIONAL INVENTOR(S)**
Supplemental Sheet (for PTO/AIA/08,09) Page 1 of 2

Legal Name of Additional Joint Inventor, if any:

(*E.g.*, Given Name (first and middle (if any)) and Family Name or Surname)

Shinong Long

Inventor's Signature	Date (Optional)

Residence: City	State	Country
Novato	CA	USA

Mailing Address: 105 Digital Drive

City	State	Zip	Country
Novato	CA	94949	USA

Legal Name of Additional Joint Inventor, if any:

(*E.g.*, Given Name (first and middle (if any)) and Family Name or Surname)

Sianna Castillo

Inventor's Signature	Date (Optional)
Sianna Castillo	Oct 4, 2017

Residence: City	State	Country
Novato	CA	USA

Mailing Address: 105 Digital Drive

City	State	Zip	Country
Novato	CA	94949	USA

Legal Name of Additional Joint Inventor, if any:

(*E.g.*, Given Name (first and middle (if any)) and Family Name or Surname)

Christopher P. Price

Inventor's Signature	Date (Optional)

Residence: City	State	Country
Aachen		Germany

Mailing Address: Papiermuehlenweg 6

City	State	Zip	Country
Aachen		52070	Germany

This collection of information is required by 35 U.S.C. 115 and 37 CFR 1.63. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 21 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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SUPPLEMENTAL SHEET FOR DECLARATION | **ADDITIONAL INVENTOR(S)** Supplemental Sheet (for PTO/AIA/08,09) Page 1 of 2

Legal Name of Additional Joint Inventor, if any:

(E.g., Given Name (first and middle (if any)) and Family Name or Surname)

Shinong Long

Inventor's Signature | Date (Optional)

Residence: City: Novato | State: CA | Country: USA

Mailing Address: 105 Digital Drive

City: Novato | State: CA | Zip: 94949 | Country: USA

Legal Name of Additional Joint Inventor, if any:

(E.g., Given Name (first and middle (if any)) and Family Name or Surname)

Sianna Castillo

Inventor's Signature | Date (Optional)

Residence: City: Novato | State: CA | Country: USA

Mailing Address: 105 Digital Drive

City: Novato | State: CA | Zip: 94949 | Country: USA

Legal Name of Additional Joint Inventor, if any:

(E.g., Given Name (first and middle (if any)) and Family Name or Surname)

Christopher P. Price

Inventor's Signature | Date (Optional): Oct. 5, 2017

Residence: City: ~~Aachen~~ Munich CP | State | Country: Germany

Mailing Address: ~~Papiermuehlenweg 6~~ Rugendasstrasse 1 CP

City: ~~Aachen~~ Munich CP | State | Zip: ~~52070~~ 81479 CP | Country: Germany

This collection of information is required by 35 U.S.C. 115 and 37 CFR 1.63. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 21 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: **Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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SUPPLEMENTAL SHEET FOR DECLARATION	ADDITIONAL INVENTOR(S) Supplemental Sheet (for PTO/AIA/08,09) Page 2 of 2

Legal Name of Additional Joint Inventor, if any:

(*E.g.*, Given Name (first and middle (if any)) and Family Name or Surname)

Mika Aoyagi-Scharber

Inventor's Signature	Date (Optional)

Residence: City	State	Country
Novato	CA	USA

105 Digital Drive
Mailing Address

City	State	Zip	Country
Novato	CA	94949	USA

Legal Name of Additional Joint Inventor, if any:

(*E.g.*, Given Name (first and middle (if any)) and Family Name or Surname)

Michel Claude Vellard

Inventor's Signature	Date (Optional)

Residence: City	State	Country
Novato	CA	USA

105 Digital Drive
Mailing Address

City	State	Zip	Country
Novato	CA	94949	USA

Legal Name of Additional Joint Inventor, if any:

(*E.g.*, Given Name (first and middle (if any)) and Family Name or Surname)

Inventor's Signature	Date (Optional)

Residence: City	State	Country

Mailing Address

City	State	Zip	Country

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SUPPLEMENTAL SHEET FOR DECLARATION | **ADDITIONAL INVENTOR(S)**
Supplemental Sheet (for PTO/AIA/08,09) Page 2 of 2

Legal Name of Additional Joint Inventor, if any:

(*E.g.*, Given Name (first and middle (if any)) and Family Name or Surname)

Mika Aoyagi-Scharber

Inventor's Signature | Date (Optional)

Residence: City: Novato | State: CA | Country: USA

Mailing Address: 105 Digital Drive

City: Novato | State: CA | Zip: 94949 | Country: USA

Legal Name of Additional Joint Inventor, if any:

(*E.g.*, Given Name (first and middle (if any)) and Family Name or Surname)

Michel Claude Vellard

Inventor's Signature | Date (Optional): 1.3.2018

Residence: City: 1.3.18 mu ~~Novato~~ San Rafael | State: CA | Country: USA

Mailing Address: 1.3.18 mu ~~105 Digital Drive~~ 2 San Marino Ct

City: mu 1.3.18 ~~Novato~~ San Rafael | State: CA | Zip: 1.3.18 mu ~~94949~~ 94901 | Country: USA

Legal Name of Additional Joint Inventor, if any:

(*E.g.*, Given Name (first and middle (if any)) and Family Name or Surname)

Inventor's Signature | Date (Optional)

Residence: City | State | Country

Mailing Address

City | State | Zip | Country

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Doc code: REIS.DECL
Document Description: Reissue Declaration Filed In Accordance With MPEP 1414

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SUBSTITUTE STATEMENT IN LIEU OF AN OATH OR DECLARATION FOR REISSUE PATENT APPLICATION (35 U.S.C. 115(d) AND 37 CFR 1.64)

Title of Invention	Variants of C-Type Natriuretic Peptide

This statement is directed to:

☐ The attached application,

OR

☒ was filed on July 11, 2017 as reissue application number 15/646,822.

LEGAL NAME of inventor to whom this substitute statement applies:

(*E.g.*, Given Name (first and middle (if any)) and Family Name or Surname)

Augustus O. Okhamafe

Residence (except for a deceased or legally incapacitated inventor):

City	State	Country
Concord	CA	USA

Mailing Address (except for a deceased or legally incapacitated inventor):

City	State	Zip	Country
Concord	CA	94521	USA

I believe the above-named inventor or joint inventor to be the original inventor or an original joint inventor of a claimed invention in the application.

The above-identified application was made or authorized to be made by me.

I hereby acknowledge that any willful false statement made in this statement is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

Relationship to the inventor to whom this substitute statement applies:

☐ Legal Representative (for deceased or legally incapacitated inventor only),

☒ Assignee, or

☐ Joint Inventor.

Circumstances permitting execution of this substitute statement:

☐ Inventor is deceased,

☐ Inventor is under legal incapacity,

☒ Inventor cannot be found or reached after diligent effort, or

☐ Inventor has refused to execute the oath or declaration under 37 CFR 1.175.

This collection of information is required by 35 U.S.C. 115 and 37 CFR 1.63. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 1 minute to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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SUBSTITUTE STATEMENT IN LIEU OF AN OATH OR DECLARATION FOR REISSUE PATENT APPLICATION (35 U.S.C. 115(d) AND 37 CFR 1.64)

If there are joint inventors, please check the appropriate box below:

☒ An application data sheet under 37 CFR 1.76 (PTO/AIA/14 or equivalent) naming the entire inventive entity has been or is currently submitted.

OR

☐ An application data sheet under 37 CFR 1.76 (PTO/AIA/14 or equivalent) has not been submitted. Thus, a Substitute Statement Supplemental Sheet (PTO/AIA/11 or equivalent) naming the entire inventive entity and providing inventor information is attached. See 37 CFR 1.64(b).

I believe the original patent to be wholly or partly inoperative or invalid, for the reasons described below.
(Check all boxes that apply.)

☐ by reason of a defective specification or drawing.

☒ by reason of the patentee claiming more or less than he had the right to claim in the patent.

☐ by reason of other errors.

At least one error upon which reissue is based is described below. If the reissue is a broadening reissue, a claim that the application seeks to broaden must be identified:

Patentee claimed less than Patentee had a right to claim in that claim 1 of U.S. Patent No. 8,598,121 is broadened by the presentation of new claim 15 in the Preliminary Amendment filed in the subject continuation reissue application, USSN 15/646,822, on July 11, 2017. New claim 15, directed to a macromolecule capable of releasing a CNP variant, is broadened to add additional, alternative amino acid sequences of the CNP variants of the macromolecule, such as, for example, the CNP variants set forth in SEQ ID NOs: 145-178.

WARNING:

Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.

PERSON EXECUTING THIS SUBSTITUTE STATEMENT:

Name: Luisa Bigornia

Date (Optional):

Signature:

APPLICANT NAME AND TITLE OF PERSON EXECUTING THIS SUBSTITUTE STATEMENT:

If the applicant is a juristic entity, list the applicant name and the title of the signer.

Applicant Name: BioMarin Pharmaceutical Inc.

Title of Person Executing This Substitute Statement: Vice President, Intellectual Property

The signer, whose title is supplied above, is authorized to act on behalf of the applicant.

PTO/AIA/07 (09-13)
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SUBSTITUTE STATEMENT IN LIEU OF AN OATH OR DECLARATION FOR REISSUE PATENT APPLICATION (35 U.S.C. 115(d) AND 37 CFR 1.64)

Residence of the signer (unless provided in an application data sheet, PTO/SB/14 or equivalent):			
Novato City	CA State	USA County	
Mailing Address of the signer (unless provided in an application data sheet, PTO/SB/14 or equivalent): 105 Digital Drive			
Novato City	CA State	94949 Zip	USA Country
Note: Use an additional PTO/AIA/07 form for each inventor who is deceased, legally incapacitated, cannot be found or reached after diligent effort, or has refused to execute the oath or declaration under 37 CFR 1.63.			

Privacy Act Statement

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The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Docket No.: 135576.010627/REI/CON
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:	Daniel J. Wendt et al.	Group Art Unit:	1629
U.S. Serial No.:	15/646,822	Confirmation No.:	7727
Filed:	July 11, 2017		
Title:	*VARIANTS OF C-TYPE NATRIURETIC PEPTIDE*	Examiner:	To Be Assigned

SUBMITTED VIA EFS-Web
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST FOR STATUS INQUIRY RELATED TO POWER OF ATTORNEY

Applicants hereby inquire as to the status of the Power of Attorney document that was filed on September 27, 2017 in the above-identified patent application.

Based on the information set forth in USPTO PAIR, a change in the Power of Attorney in this application has not been effected as of the date of this inquiry. Accordingly, applicants respectfully request that a change in Power of Attorney be made in this application.

Applicants believe that no fee is required in connection with this paper. However, the Commissioner is hereby authorized to charge any fee(s) that may be due to Deposit Account No. 50-2678, Reference No. 135576.010627/REI/CON.

Respectfully submitted,

Greenberg Traurig LLP

Date: January 12, 2018 By: /Leslie Serunian/

Leslie Serunian
Registration No.: 35,353
Greenberg Traurig
One International Place
Boston, MA 02110
Tel: 617-310-6000
Customer No.: 35893

Electronic Acknowledgement Receipt

EFS ID:	31489409
Application Number:	15646822
International Application Number:	
Confirmation Number:	7727
Title of Invention:	Variants of C-Type Natriuretic Peptide
First Named Inventor/Applicant Name:	Daniel J. Wendt
Customer Number:	90849
Filer:	Leslie A. Serunian
Filer Authorized By:	
Attorney Docket Number:	30610/44556C1-R2
Receipt Date:	12-JAN-2018
Filing Date:	11-JUL-2017
Time Stamp:	15:00:40
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Request for status of Application	StatusInquiry.pdf	93926 0e3a37c57d7569dbd4d2ec2130fc6ee0baf02308	no	1

Warnings:

Information:	
Total Files Size (in bytes):	93926

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
15/646,822	07/11/2017	Daniel J. Wendt	30610/44556C1-R2

CONFIRMATION NO. 7727

90849
Marshall, Gerstein & Borun LLP (Biomarin)
233 South Wacker Drive
6300 Willis Tower
Chicago, IL 60606

FORMALITIES LETTER

OC000000096697013

Date Mailed: 01/17/2018

NOTICE OF INCOMPLETE REPLY (REISSUE)

Filing Date Granted

The U.S. Patent and Trademark Office has received your reply on 01/05/2018 to the Notice to File Missing Parts (Notice) mailed 08/10/2017 and it has been entered into the reissue application. The reply, however, does not include the following items required in the Notice.

The period of reply remains as set forth in the Notice. You may, however, obtain EXTENSIONS OF TIME under the provisions of 37 CFR 1.136 (a) accompanied by the appropriate fee (37 CFR 1.17(a)).

A complete reply must be timely filed to prevent ABANDONMENT of the above-identified application.

The required items noted below SHOULD be filed along with any items required above. The filing date of this reissue application will be the date of receipt of the items required above.

- The inventor's oath or declaration has not been submitted.

  An inventor's oath or declaration in compliance with 37 CFR 1.63 or 1.64 (and 37 CFR 1.175) executed by or with respect to each inventor is required. For a reissue application, the inventor's oath or declaration must be submitted prior to examination and cannot be postponed. See 37 CFR 1.175(e). An assignee of the entire interest can execute the oath or declaration if: (1) the application does not seek to enlarge the scope of the claims of the original patent; or (2) the application for the original patent was filed under 37 CFR 1.46 by the assignee of the entire interest. A substitute statement under 37 CFR 1.64 in lieu of an oath or declaration may be submitted in the circumstances provided for in 35 U.S.C. 115(d) and 37 CFR 1.64.

Replies must be received in the USPTO within the set time period or must include a proper Certificate of Mailing or Transmission under 37 CFR 1.8 with a mailing or transmission date within the set time period. For more information and a suggested format, see Form PTO/SB/92 and MPEP 512.

Replies should be mailed to:

Mail Stop Missing Parts
Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

Registered users of EFS-Web may alternatively submit their reply to this notice via EFS-Web, including a copy of this Notice and selecting the document description "Applicant response to Pre-Exam Formalities Notice". https://sportal.uspto.gov/authenticate/AuthenticateUserLocalEPF.html

For more information about EFS-Web please call the USPTO Electronic Business Center at 1-866-217-9197 or visit our website at http://www.uspto.gov/ebc.

If you are not using EFS-Web to submit your reply, you must include a copy of this notice.

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at **(571) 272-4000** or **(571) 272-4200** or **1-888-786-0101**.

/ddfelix/

PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 15/646,822

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	280
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	600
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	2160
TOTAL CLAIMS (37 CFR 1.16(i))	16 minus 20=	*			OR	x 80 =	0.00
INDEPENDENT CLAIMS (37 CFR 1.16(h))	6 minus 3 =	* 3				x 420 =	1260
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						1200
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							0.00
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	5500

APPLICATION AS AMENDED - PART II

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT A	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT B	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.

** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".

*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".

The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.

Doc Code: REIS.DECL
Document Description: Reissue Declaration Filed In Accordance With MPEP 1414

PTO/AIA/05 (06-12)
Approved for use through 01/31/2020. OMB 0651-0033
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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REISSUE APPLICATION DECLARATION BY THE INVENTOR	Docket Number (Optional) 135576.010627/REI/CON

I hereby declare that:
Each inventor's residence and mailing address are stated below next to their name.
I believe I am the original inventor or an original joint inventor of the subject matter which is described and claimed in patent number 8,598,121, granted December 3, 2013 and for which a reissue patent is sought on the invention titled VARIANTS OF C-TYPE NATRIURETIC PEPTIDE,

the specification of which

☐ is attached hereto.

☑ was filed on July 11, 2017 as reissue application number 15/646,822.

The above-identified application was made or authorized to be made by me.

I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

I believe the original patent to be wholly or partly inoperative or invalid, for the reasons described below. (Check all boxes that apply.)

☐ by reason of a defective specification or drawing.

☑ by reason of the patentee claiming more or less than he had the right to claim in the patent.

☐ by reason of other errors.

At least one error upon which reissue is based is described below. If the reissue is a broadening reissue, a claim that the application seeks to broaden must be identified:

Patentee claimed less than Patentee had a right to claim in that claim 1 of U.S. Patent No. 8,598,121 is broadened by the presentation of new claim 15 in the Preliminary Amendment filed in the subject continuation reissue application, USSN 15/646,822, on July 11, 2017. New claim 15, directed to a macromolecule capable of releasing a CNP variant, is broadened to add additional, alternative amino acid sequences of the CNP variants of the macromolecule, such as, for example, the CNP variants set forth in SEQ ID NOs: 145-178.

This collection of information is required by 37 CFR 1.175. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 30 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

PTO/AIA/05 (06-12)
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(REISSUE APPLICATION DECLARATION BY THE INVENTOR, page 2)

Docket Number (Optional): 135576.010627/REI/CON

Note: To appoint a power of attorney, use form PTO/AIA/81.

Correspondence Address: Direct all communications about the application to:

☑ The address associated with Customer Number: 35893

OR

☐ Firm or Individual Name					
Address					
City		State		Zip	
Country					
Telephone		Email			

WARNING:

Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.

Legal name of sole or first inventor (*E.g.*, Given Name (first and middle (if any) and Family Name or Surname)

Daniel J. Wendt

Inventor's Signature	Date (Optional)
Daniel J Wendt	12-6-2017

Residence: City	State	Country
Novato	CA	USA

Mailing Address

105 Digitial Drive

City	State	Zip	Country
Novato	CA	94949	USA

|■| Additional joint inventors are named on the 2 supplemental sheet(s) PTO/AIA/10 attached hereto.

Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

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SUPPLEMENTAL SHEET FOR DECLARATION | **ADDITIONAL INVENTOR(S)**
Supplemental Sheet (for PTO/AIA/08,09) Page 1 of 2

Legal Name of Additional Joint Inventor, if any:

(*E.g.*, Given Name (first and middle (if any)) and Family Name or Surname)

Shinong Long

Inventor's Signature | Date (Optional) 10/05/2017

Residence: City: Novato | State: CA | Country: USA

Mailing Address: 105 Digital Drive

City: Novato | State: CA | Zip: 94949 | Country: USA

Legal Name of Additional Joint Inventor, if any:

(*E.g.*, Given Name (first and middle (if any)) and Family Name or Surname)

Sianna Castillo

Inventor's Signature | Date (Optional)

Residence: City: Novato | State: CA | Country: USA

Mailing Address: 105 Digital Drive

City: Novato | State: CA | Zip: 94949 | Country: USA

Legal Name of Additional Joint Inventor, if any:

(*E.g.*, Given Name (first and middle (if any)) and Family Name or Surname)

Christopher P. Price

Inventor's Signature | Date (Optional)

Residence: City: Aachen | State | Country: Germany

Mailing Address: Papiermuehlenweg 6

City: Aachen | State | Zip: 52070 | Country: Germany

This collection of information is required by 35 U.S.C. 115 and 37 CFR 1.63. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 21 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: **Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

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SUPPLEMENTAL SHEET FOR DECLARATION | **ADDITIONAL INVENTOR(S)**
Supplemental Sheet (for PTO/AIA/08,09) Page 1 of 2

Legal Name of Additional Joint Inventor, if any:

(*E.g.*, Given Name (first and middle (if any)) and Family Name or Surname)

Shinong Long

Inventor's Signature	Date (Optional)

Residence: City	State	Country
Novato	CA	USA

Mailing Address: 105 Digital Drive

City	State	Zip	Country
Novato	CA	94949	USA

Legal Name of Additional Joint Inventor, if any:

(*E.g.*, Given Name (first and middle (if any)) and Family Name or Surname)

Sianna Castillo

Inventor's Signature	Date (Optional)
Sianna Castillo	Oct 4, 2017

Residence: City	State	Country
Novato	CA	USA

Mailing Address: 105 Digital Drive

City	State	Zip	Country
Novato	CA	94949	USA

Legal Name of Additional Joint Inventor, if any:

(*E.g.*, Given Name (first and middle (if any)) and Family Name or Surname)

Christopher P. Price

Inventor's Signature	Date (Optional)

Residence: City	State	Country
Aachen		Germany

Mailing Address: Papiermuehlenweg 6

City	State	Zip	Country
Aachen		52070	Germany

This collection of information is required by 35 U.S.C. 115 and 37 CFR 1.63. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 21 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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SUPPLEMENTAL SHEET FOR DECLARATION | **ADDITIONAL INVENTOR(S)** Supplemental Sheet (for PTO/AIA/08,09) Page 1 of 2

Legal Name of Additional Joint Inventor, if any:

(E.g., Given Name (first and middle (if any)) and Family Name or Surname)

Shinong Long

Inventor's Signature | Date (Optional)

Residence: City: Novato | State: CA | Country: USA

Mailing Address: 105 Digital Drive

City: Novato | State: CA | Zip: 94949 | Country: USA

Legal Name of Additional Joint Inventor, if any:

(E.g., Given Name (first and middle (if any)) and Family Name or Surname)

Sianna Castillo

Inventor's Signature | Date (Optional)

Residence: City: Novato | State: CA | Country: USA

Mailing Address: 105 Digital Drive

City: Novato | State: CA | Zip: 94949 | Country: USA

Legal Name of Additional Joint Inventor, if any:

(E.g., Given Name (first and middle (if any)) and Family Name or Surname)

Christopher P. Price

Inventor's Signature | Date (Optional): Oct. 5, 2017

Residence: City: ~~Aachen~~ Munich CP | State | Country: Germany

Mailing Address: ~~Papiermuehlenweg 6~~ Rugendasstrasse 1 CP

City: ~~Aachen~~ Munich CP | State | Zip: ~~52070~~ 81479 CP | Country: Germany

This collection of information is required by 35 U.S.C. 115 and 37 CFR 1.63. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 21 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: **Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

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SUPPLEMENTAL SHEET FOR DECLARATION	ADDITIONAL INVENTOR(S) Supplemental Sheet (for PTO/AIA/08,09) Page 2 of 2

Legal Name of Additional Joint Inventor, if any:

(*E.g.*, Given Name (first and middle (if any)) and Family Name or Surname)

Mika Aoyagi-Scharber

Inventor's Signature	Date (Optional)

Residence: City	State	Country
Novato	CA	USA

Mailing Address: 105 Digital Drive

City	State	Zip	Country
Novato	CA	94949	USA

Legal Name of Additional Joint Inventor, if any:

(*E.g.*, Given Name (first and middle (if any)) and Family Name or Surname)

Michel Claude Vellard

Inventor's Signature	Date (Optional)

Residence: City	State	Country
Novato	CA	USA

Mailing Address: 105 Digital Drive

City	State	Zip	Country
Novato	CA	94949	USA

Legal Name of Additional Joint Inventor, if any:

(*E.g.*, Given Name (first and middle (if any)) and Family Name or Surname)

Inventor's Signature	Date (Optional)

Residence: City	State	Country

Mailing Address

City	State	Zip	Country

This collection of information is required by 35 U.S.C. 115 and 37 CFR 1.63. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 21 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: **Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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SUPPLEMENTAL SHEET FOR DECLARATION | **ADDITIONAL INVENTOR(S)**
Supplemental Sheet (for PTO/AIA/08,09) Page 2 of 2

Legal Name of Additional Joint Inventor, if any:

(*E.g.*, Given Name (first and middle (if any)) and Family Name or Surname)

Mika Aoyagi-Scharber

Inventor's Signature | Date (Optional)

Residence: City: Novato | State: CA | Country: USA

Mailing Address: 105 Digital Drive

City: Novato | State: CA | Zip: 94949 | Country: USA

Legal Name of Additional Joint Inventor, if any:

(*E.g.*, Given Name (first and middle (if any)) and Family Name or Surname)

Michel Claude Vellard

Inventor's Signature | Date (Optional): 1.3.2018

Residence: City: 1.3.18 mu ~~Novato~~ San Rafael | State: CA | Country: USA

Mailing Address: 1.3.18 mu ~~105 Digital Drive~~ 2 San Marino Ct

City: mu 1.3.18 ~~Novato~~ San Rafael | State: CA | Zip: 1.3.18 mu ~~94949~~ 94901 | Country: USA

Legal Name of Additional Joint Inventor, if any:

(*E.g.*, Given Name (first and middle (if any)) and Family Name or Surname)

Inventor's Signature | Date (Optional)

Residence: City | State | Country

Mailing Address

City | State | Zip | Country

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Doc code: REIS.DECL
Document Description: Reissue Declaration Filed In Accordance With MPEP 1414

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SUBSTITUTE STATEMENT IN LIEU OF AN OATH OR DECLARATION FOR REISSUE PATENT APPLICATION (35 U.S.C. 115(d) AND 37 CFR 1.64)

Title of Invention	Variants of C-Type Natriuretic Peptide

This statement is directed to:

☐ The attached application,

OR

☒ was filed on July 11, 2017 as reissue application number 15/646,822.

LEGAL NAME of inventor to whom this substitute statement applies:

(*E.g.*, Given Name (first and middle (if any)) and Family Name or Surname)

Augustus O. Okhamafe

Residence (except for a deceased or legally incapacitated inventor):

City	State	Country
Concord	CA	USA

Mailing Address (except for a deceased or legally incapacitated inventor):

City	State	Zip	Country
Concord	CA	94521	USA

I believe the above-named inventor or joint inventor to be the original inventor or an original joint inventor of a claimed invention in the application.

The above-identified application was made or authorized to be made by me.

I hereby acknowledge that any willful false statement made in this statement is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

Relationship to the inventor to whom this substitute statement applies:

☐ Legal Representative (for deceased or legally incapacitated inventor only),

☒ Assignee, or

☐ Joint Inventor.

Circumstances permitting execution of this substitute statement:

☐ Inventor is deceased,

☐ Inventor is under legal incapacity,

☒ Inventor cannot be found or reached after diligent effort, or

☐ Inventor has refused to execute the oath or declaration under 37 CFR 1.175.

This collection of information is required by 35 U.S.C. 115 and 37 CFR 1.63. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 1 minute to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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SUBSTITUTE STATEMENT IN LIEU OF AN OATH OR DECLARATION FOR REISSUE PATENT APPLICATION (35 U.S.C. 115(d) AND 37 CFR 1.64)

If there are joint inventors, please check the appropriate box below:

☒ An application data sheet under 37 CFR 1.76 (PTO/AIA/14 or equivalent) naming the entire inventive entity has been or is currently submitted.

OR

☐ An application data sheet under 37 CFR 1.76 (PTO/AIA/14 or equivalent) has not been submitted. Thus, a Substitute Statement Supplemental Sheet (PTO/AIA/11 or equivalent) naming the entire inventive entity and providing inventor information is attached. See 37 CFR 1.64(b).

I believe the original patent to be wholly or partly inoperative or invalid, for the reasons described below.
(Check all boxes that apply.)

☐ by reason of a defective specification or drawing.

☒ by reason of the patentee claiming more or less than he had the right to claim in the patent.

☐ by reason of other errors.

At least one error upon which reissue is based is described below. If the reissue is a broadening reissue, a claim that the application seeks to broaden must be identified:

Patentee claimed less than Patentee had a right to claim in that claim 1 of U.S. Patent No. 8,598,121 is broadened by the presentation of new claim 15 in the Preliminary Amendment filed in the subject continuation reissue application, USSN 15/646,822, on July 11, 2017. New claim 15, directed to a macromolecule capable of releasing a CNP variant, is broadened to add additional, alternative amino acid sequences of the CNP variants of the macromolecule, such as, for example, the CNP variants set forth in SEQ ID NOs: 145-178.

WARNING:

Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.

PERSON EXECUTING THIS SUBSTITUTE STATEMENT:

Name: Luisa Bigornia

Date (Optional):

Signature:

APPLICANT NAME AND TITLE OF PERSON EXECUTING THIS SUBSTITUTE STATEMENT:

If the applicant is a juristic entity, list the applicant name and the title of the signer.

Applicant Name: BioMarin Pharmaceutical Inc.

Title of Person Executing This Substitute Statement: Vice President, Intellectual Property

The signer, whose title is supplied above, is authorized to act on behalf of the applicant.

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SUBSTITUTE STATEMENT IN LIEU OF AN OATH OR DECLARATION FOR REISSUE PATENT APPLICATION (35 U.S.C. 115(d) AND 37 CFR 1.64)

Residence of the signer (unless provided in an application data sheet, PTO/SB/14 or equivalent):

Novato	CA	USA
City	State	County

Mailing Address of the signer (unless provided in an application data sheet, PTO/SB/14 or equivalent):

105 Digital Drive

Novato	CA	94949	USA
City	State	Zip	Country

Note: Use an additional PTO/AIA/07 form for each inventor who is deceased, legally incapacitated, cannot be found or reached after diligent effort, or has refused to execute the oath or declaration under 37 CFR 1.63.

Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronic Acknowledgement Receipt

EFS ID:	31521430
Application Number:	15646822
International Application Number:	
Confirmation Number:	7727
Title of Invention:	Variants of C-Type Natriuretic Peptide
First Named Inventor/Applicant Name:	Daniel J. Wendt
Customer Number:	90849
Filer:	Leslie A. Serunian
Filer Authorized By:	
Attorney Docket Number:	30610/44556C1-R2
Receipt Date:	17-JAN-2018
Filing Date:	11-JUL-2017
Time Stamp:	11:54:26
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Oath or Declaration filed	135576_010627REIUSOath.pdf	782919 4e2349c338109ed362a7de90c2017881c4da5364	no	12
Warnings:					

Information:	
Total Files Size (in bytes):	782919

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New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Doc Code: REIS.DECL
Document Description: Reissue Declaration Filed In Accordance With MPEP 1414

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REISSUE APPLICATION DECLARATION BY THE INVENTOR	Docket Number (Optional) 135576.010627/REI/CON

I hereby declare that:
Each inventor's residence and mailing address are stated below next to their name.
I believe I am the original inventor or an original joint inventor of the subject matter which is described and claimed in patent number 8,598,121, granted December 3, 2013 and for which a reissue patent is sought on the invention titled VARIANTS OF C-TYPE NATRIURETIC PEPTIDE,

the specification of which

☐ is attached hereto.

☑ was filed on July 11, 2017 as reissue application number 15/646,822.

The above-identified application was made or authorized to be made by me.

I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

I believe the original patent to be wholly or partly inoperative or invalid, for the reasons described below. (Check all boxes that apply.)

☐ by reason of a defective specification or drawing.

☑ by reason of the patentee claiming more or less than he had the right to claim in the patent.

☐ by reason of other errors.

At least one error upon which reissue is based is described below. If the reissue is a broadening reissue, a claim that the application seeks to broaden must be identified:

Patentee claimed less than Patentee had a right to claim in that claim 1 of U.S. Patent No. 8,598,121 is broadened by the presentation of new claim 15 in the Preliminary Amendment filed in the subject continuation reissue application, USSN 15/646,822, on July 11, 2017. New claim 15, directed to a macromolecule capable of releasing a CNP variant, is broadened to add additional, alternative amino acid sequences of the CNP variants of the macromolecule, such as, for example, the CNP variants set forth in SEQ ID NOs: 145-178.

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(REISSUE APPLICATION DECLARATION BY THE INVENTOR, page 2)

Docket Number (Optional): 135576.010627/REI/CON

Note: To appoint a power of attorney, use form PTO/AIA/81.

Correspondence Address: Direct all communications about the application to:

☑ The address associated with Customer Number: 35893

OR

☐ Firm or Individual Name					
Address					
City		State		Zip	
Country					
Telephone		Email			

WARNING:

Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.

Legal name of sole or first inventor (*E.g.*, Given Name (first and middle (if any) and Family Name or Surname)

Daniel J. Wendt

Inventor's Signature	Date (Optional)
Daniel J Wendt	12-6-2017

Residence: City	State	Country
Novato	CA	USA

Mailing Address

105 Digitial Drive

City	State	Zip	Country
Novato	CA	94949	USA

|■| Additional joint inventors are named on the 2 supplemental sheet(s) PTO/AIA/10 attached hereto.

Privacy Act Statement

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2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
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6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
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SUPPLEMENTAL SHEET FOR DECLARATION | **ADDITIONAL INVENTOR(S)**
Supplemental Sheet (for PTO/AIA/08,09) Page 1 of 2

Legal Name of Additional Joint Inventor, if any:

(*E.g.*, Given Name (first and middle (if any)) and Family Name or Surname)

Shinong Long

Inventor's Signature | Date (Optional) 10/05/2017

Residence: City: Novato | State: CA | Country: USA

Mailing Address: 105 Digital Drive

City: Novato | State: CA | Zip: 94949 | Country: USA

Legal Name of Additional Joint Inventor, if any:

(*E.g.*, Given Name (first and middle (if any)) and Family Name or Surname)

Sianna Castillo

Inventor's Signature | Date (Optional)

Residence: City: Novato | State: CA | Country: USA

Mailing Address: 105 Digital Drive

City: Novato | State: CA | Zip: 94949 | Country: USA

Legal Name of Additional Joint Inventor, if any:

(*E.g.*, Given Name (first and middle (if any)) and Family Name or Surname)

Christopher P. Price

Inventor's Signature | Date (Optional)

Residence: City: Aachen | State: | Country: Germany

Mailing Address: Papiermuehlenweg 6

City: Aachen | State: | Zip: 52070 | Country: Germany

This collection of information is required by 35 U.S.C. 115 and 37 CFR 1.63. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 21 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: **Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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Supplemental Sheet (for PTO/AIA/08,09) Page 1 of 2

Legal Name of Additional Joint Inventor, if any:

(*E.g.*, Given Name (first and middle (if any)) and Family Name or Surname)

Shinong Long

Inventor's Signature		Date (Optional)

Residence: City	State	Country
Novato	CA	USA

Mailing Address: 105 Digital Drive

City	State	Zip	Country
Novato	CA	94949	USA

Legal Name of Additional Joint Inventor, if any:

(*E.g.*, Given Name (first and middle (if any)) and Family Name or Surname)

Sianna Castillo

Inventor's Signature	Sianna Castillo	Date (Optional)
		Oct 4, 2017

Residence: City	State	Country
Novato	CA	USA

Mailing Address: 105 Digital Drive

City	State	Zip	Country
Novato	CA	94949	USA

Legal Name of Additional Joint Inventor, if any:

(*E.g.*, Given Name (first and middle (if any)) and Family Name or Surname)

Christopher P. Price

Inventor's Signature		Date (Optional)

Residence: City	State	Country
Aachen		Germany

Mailing Address: Papiermuehlenweg 6

City	State	Zip	Country
Aachen		52070	Germany

This collection of information is required by 35 U.S.C. 115 and 37 CFR 1.63. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 21 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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SUPPLEMENTAL SHEET FOR DECLARATION | **ADDITIONAL INVENTOR(S)** Supplemental Sheet (for PTO/AIA/08,09) Page 1 of 2

Legal Name of Additional Joint Inventor, if any:

(E.g., Given Name (first and middle (if any)) and Family Name or Surname)

Shinong Long

Inventor's Signature | Date (Optional)

Residence: City: Novato | State: CA | Country: USA

Mailing Address: 105 Digital Drive

City: Novato | State: CA | Zip: 94949 | Country: USA

Legal Name of Additional Joint Inventor, if any:

(E.g., Given Name (first and middle (if any)) and Family Name or Surname)

Sianna Castillo

Inventor's Signature | Date (Optional)

Residence: City: Novato | State: CA | Country: USA

Mailing Address: 105 Digital Drive

City: Novato | State: CA | Zip: 94949 | Country: USA

Legal Name of Additional Joint Inventor, if any:

(E.g., Given Name (first and middle (if any)) and Family Name or Surname)

Christopher P. Price

Inventor's Signature | Date (Optional): Oct. 5, 2017

Residence: City: ~~Aachen~~ Munich CP | State | Country: Germany

Mailing Address: ~~Papiermuehlenweg 6~~ Rugendasstrasse 1 CP

City: ~~Aachen~~ Munich CP | State | Zip: ~~52070~~ 81479 CP | Country: Germany

This collection of information is required by 35 U.S.C. 115 and 37 CFR 1.63. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 21 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: **Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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SUPPLEMENTAL SHEET FOR DECLARATION	ADDITIONAL INVENTOR(S) Supplemental Sheet (for PTO/AIA/08,09) Page 2 of 2

Legal Name of Additional Joint Inventor, if any:

(*E.g.*, Given Name (first and middle (if any)) and Family Name or Surname)

Mika Aoyagi-Scharber

Inventor's Signature	Date (Optional)

Residence: City	State	Country
Novato	CA	USA

Mailing Address: 105 Digital Drive

City	State	Zip	Country
Novato	CA	94949	USA

Legal Name of Additional Joint Inventor, if any:

(*E.g.*, Given Name (first and middle (if any)) and Family Name or Surname)

Michel Claude Vellard

Inventor's Signature	Date (Optional)

Residence: City	State	Country
Novato	CA	USA

Mailing Address: 105 Digital Drive

City	State	Zip	Country
Novato	CA	94949	USA

Legal Name of Additional Joint Inventor, if any:

(*E.g.*, Given Name (first and middle (if any)) and Family Name or Surname)

Inventor's Signature	Date (Optional)

Residence: City	State	Country

Mailing Address

City	State	Zip	Country

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PTO/AIA/10 (06-12)
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SUPPLEMENTAL SHEET FOR DECLARATION | **ADDITIONAL INVENTOR(S)**
Supplemental Sheet (for PTO/AIA/08,09) Page 2 of 2

Legal Name of Additional Joint Inventor, if any:

(*E.g.*, Given Name (first and middle (if any)) and Family Name or Surname)

Mika Aoyagi-Scharber

Inventor's Signature | Date (Optional)

Residence: City: Novato | State: CA | Country: USA

Mailing Address: 105 Digital Drive

City: Novato | State: CA | Zip: 94949 | Country: USA

Legal Name of Additional Joint Inventor, if any:

(*E.g.*, Given Name (first and middle (if any)) and Family Name or Surname)

Michel Claude Vellard

Inventor's Signature | Date (Optional): 1.3.2018

Residence: City: 1.3.18 mu ~~Novato~~ San Rafael | State: CA | Country: USA

Mailing Address: 1.3.18 mu ~~105 Digital Drive~~ 2 San Marino Ct

City: mu 1.3.18 ~~Novato~~ San Rafael | State: CA | Zip: 1.3.18 mu ~~94949~~ 94901 | Country: USA

Legal Name of Additional Joint Inventor, if any:

(*E.g.*, Given Name (first and middle (if any)) and Family Name or Surname)

Inventor's Signature | Date (Optional)

Residence: City | State | Country

Mailing Address

City | State | Zip | Country

This collection of information is required by 35 U.S.C. 115 and 37 CFR 1.63. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 21 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

Doc code: REIS.DECL
Document Description: Reissue Declaration Filed In Accordance With MPEP 1414

PTO/AIA/07 (09-13)
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SUBSTITUTE STATEMENT IN LIEU OF AN OATH OR DECLARATION FOR REISSUE PATENT APPLICATION (35 U.S.C. 115(d) AND 37 CFR 1.64)

Title of Invention	Variants of C-Type Natriuretic Peptide

This statement is directed to:

☐ The attached application,

OR

☒ was filed on July 11, 2017 as reissue application number 15/646,822.

LEGAL NAME of inventor to whom this substitute statement applies:

(*E.g.*, Given Name (first and middle (if any)) and Family Name or Surname)

Augustus O. Okhamafe

Residence (except for a deceased or legally incapacitated inventor):

City	State	Country
Concord	CA	USA

Mailing Address (except for a deceased or legally incapacitated inventor):

5310 Crystyl Ranch Drive

City	State	Zip	Country
Concord	CA	94521	USA

I believe the above-named inventor or joint inventor to be the original inventor or an original joint inventor of a claimed invention in the application.

The above-identified application was made or authorized to be made by me.

I hereby acknowledge that any willful false statement made in this statement is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

Relationship to the inventor to whom this substitute statement applies:

☐ Legal Representative (for deceased or legally incapacitated inventor only),

☒ Assignee, or

☐ Joint Inventor.

Circumstances permitting execution of this substitute statement:

☐ Inventor is deceased,

☐ Inventor is under legal incapacity,

☒ Inventor cannot be found or reached after diligent effort, or

☐ Inventor has refused to execute the oath or declaration under 37 CFR 1.175.

This collection of information is required by 35 U.S.C. 115 and 37 CFR 1.63. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 1 minute to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

PTO/AIA/07 (09-13)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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SUBSTITUTE STATEMENT IN LIEU OF AN OATH OR DECLARATION FOR REISSUE PATENT APPLICATION (35 U.S.C. 115(d) AND 37 CFR 1.64)

If there are joint inventors, please check the appropriate box below:

☒ An application data sheet under 37 CFR 1.76 (PTO/AIA/14 or equivalent) naming the entire inventive entity has been or is currently submitted.

OR

☐ An application data sheet under 37 CFR 1.76 (PTO/AIA/14 or equivalent) has not been submitted. Thus, a Substitute Statement Supplemental Sheet (PTO/AIA/11 or equivalent) naming the entire inventive entity and providing inventor information is attached. See 37 CFR 1.64(b).

I believe the original patent to be wholly or partly inoperative or invalid, for the reasons described below.
(Check all boxes that apply.)

☐ by reason of a defective specification or drawing.

☒ by reason of the patentee claiming more or less than he had the right to claim in the patent.

☐ by reason of other errors.

At least one error upon which reissue is based is described below. If the reissue is a broadening reissue, a claim that the application seeks to broaden must be identified:

Patentee claimed less than Patentee had a right to claim in that claim 1 of U.S. Patent No. 8,598,121 is broadened by the presentation of new claim 15 in the Preliminary Amendment filed in the subject continuation reissue application, USSN 15/646,822, on July 11, 2017. New claim 15, directed to a macromolecule capable of releasing a CNP variant, is broadened to add additional, alternative amino acid sequences of the CNP variants of the macromolecule, such as, for example, the CNP variants set forth in SEQ ID NOs: 145-178.

WARNING:

Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.

PERSON EXECUTING THIS SUBSTITUTE STATEMENT:

Name: Luisa Bigornia

Date (Optional):

Signature:

APPLICANT NAME AND TITLE OF PERSON EXECUTING THIS SUBSTITUTE STATEMENT:

If the applicant is a juristic entity, list the applicant name and the title of the signer.

Applicant Name: BioMarin Pharmaceutical Inc.

Title of Person Executing This Substitute Statement: Vice President, Intellectual Property

The signer, whose title is supplied above, is authorized to act on behalf of the applicant.

PTO/AIA/07 (09-13)
Approved for use through 01/31/2020. OMB 0651-0033
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

SUBSTITUTE STATEMENT IN LIEU OF AN OATH OR DECLARATION FOR REISSUE PATENT APPLICATION (35 U.S.C. 115(d) AND 37 CFR 1.64)

Residence of the signer (unless provided in an application data sheet, PTO/SB/14 or equivalent):			
Novato City	CA State	USA County	
Mailing Address of the signer (unless provided in an application data sheet, PTO/SB/14 or equivalent): 105 Digital Drive			
Novato City	CA State	94949 Zip	USA Country
Note: Use an additional PTO/AIA/07 form for each inventor who is deceased, legally incapacitated, cannot be found or reached after diligent effort, or has refused to execute the oath or declaration under 37 CFR 1.63.			

Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronic Acknowledgement Receipt

EFS ID:	31523218
Application Number:	15646822
International Application Number:	
Confirmation Number:	7727
Title of Invention:	Variants of C-Type Natriuretic Peptide
First Named Inventor/Applicant Name:	Daniel J. Wendt
Customer Number:	90849
Filer:	Leslie A. Serunian/Nancy Bechet
Filer Authorized By:	Leslie A. Serunian
Attorney Docket Number:	30610/44556C1-R2
Receipt Date:	17-JAN-2018
Filing Date:	11-JUL-2017
Time Stamp:	13:21:01
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Oath or Declaration filed	135576_010627REIUSOath.pdf	797101 e7760aa61c37fb728f942c5b9e52ad0833139218	no	12

Warnings:

Information:	
Total Files Size (in bytes):	797101

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 15/646,822

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	300
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	660
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	2200
TOTAL CLAIMS (37 CFR 1.16(i))	16 minus 20=	*			OR	x 100 =	0.00
INDEPENDENT CLAIMS (37 CFR 1.16(h))	6 minus 3 =	* 3				x 460 =	1380
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						1200
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							0.00
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	5740

APPLICATION AS AMENDED - PART II

AMENDMENT A

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
Application Size Fee (37 CFR 1.16(s))									
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
					TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

AMENDMENT B

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE($)	ADDITIONAL FEE($)		RATE($)	ADDITIONAL FEE($)
Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
Application Size Fee (37 CFR 1.16(s))									
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
					TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
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Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
15/646,822	07/11/2017	1629	5640	30610/44556C1-R2	16	6

CONFIRMATION NO. 7727

UPDATED FILING RECEIPT

90849
Marshall, Gerstein & Borun LLP (Biomarin)
233 South Wacker Drive
6300 Willis Tower
Chicago, IL 60606

OC000000098792021

Date Mailed: 01/19/2018

Receipt is acknowledged of this reissue patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

Inventor(s)

Daniel J. Wendt, Novato, CA;
Shinong Long, Novato, CA;
Sianna Castillo, Novato, CA;
Christopher P. Price, Aachen, GERMANY;
Mika Aoyagi-Scharber, Novato, CA;
Michel Claude Vellard, Novato, CA;
Augustus O. Okamafe, Concord, CA;

Applicant(s)

BioMarin Pharmaceutical Inc., Novato, CA, Assignee (with 37 CFR 1.172 Interest);

Assignment For Published Patent Application

BioMarin Pharmaceutical Inc., Novato, CA

Power of Attorney: The patent practitioners associated with Customer Number 90849

Domestic Priority data as claimed by applicant

This application is a CON of 14/604,262 01/23/2015
which is a REI of 13/466,672 05/08/2012 PAT 8598121
which is a CON of 12/784,117 05/20/2010 PAT 8198242
which claims benefit of 61/180,112 05/20/2009
and claims benefit of 61/254,563 10/23/2009

Foreign Applications for which priority is claimed (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.) - None.
Foreign application information must be provided in an Application Data Sheet in order to constitute a claim to foreign priority. See 37 CFR 1.55 and 1.76.

Permission to Access Application via Priority Document Exchange: Yes

Permission to Access Search Results: Yes

Applicant may provide or rescind an authorization for access using Form PTO/SB/39 or Form PTO/SB/69 as appropriate.

If Required, Foreign Filing License Granted: 07/12/2017

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 15/646,822**

Projected Publication Date: None, application is not eligible for pre-grant publication

Non-Publication Request: No

Early Publication Request: No

Title

Variants of C-Type Natriuretic Peptide

Preliminary Class

514

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

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For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4258).

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Title 37, Code of Federal Regulations, 5.11 & 5.15

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Electronic Patent Application Fee Transmittal

Application Number:	15646822
Filing Date:	11-Jul-2017
Title of Invention:	Variants of C-Type Natriuretic Peptide
First Named Inventor/Applicant Name:	Daniel J. Wendt
Filer:	Leslie A. Serunian
Attorney Docket Number:	30610/44556C1-R2

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
UTILITY APPL SIZE FEE PER 50 SHEETS >100	1081	3	400	1200
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				

Adjustment date: 01/04/2018 SDIRETA1
10/05/2017 INTEFSW 00000167 15646822
01 FC:1081 1200.00 CR

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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
15/646,822	07/11/2017	Daniel J. Wendt	30610/44556C1-R2

CONFIRMATION NO. 7727

POWER OF ATTORNEY NOTICE

90849
Marshall, Gerstein & Borun LLP (Biomarin)
233 South Wacker Drive
6300 Willis Tower
Chicago, IL 60606

OC000000097071417

Date Mailed: 01/31/2018

NOTICE REGARDING CHANGE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 09/27/2017.

- The Power of Attorney to you in this application has been revoked by the assignee who has intervened as provided by 37 CFR 3.71. Future correspondence will be mailed to the new address of record(37 CFR 1.33).

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at **(571) 272-4000** or **(571) 272-4200** or **1-888-786-0101**.

/kesmith/

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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
15/646,822	07/11/2017	Daniel J. Wendt	30610/44556C1-R2

CONFIRMATION NO. 7727

35893
GREENBERG TRAURIG (BOS)
ONE INTERNATIONAL PLACE, 20th FL
ATTN: PATENT ADMINISTRATOR
BOSTON, MA 02110

POA ACCEPTANCE LETTER

OC000000097071451

Date Mailed: 01/31/2018

NOTICE OF ACCEPTANCE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 09/27/2017.

The Power of Attorney in this application is accepted. Correspondence in this application will be mailed to the above address as provided by 37 CFR 1.33.

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at **(571) 272-4000** or **(571) 272-4200** or **1-888-786-0101**.

/kesmith/

Litigation Search Report CRU 3999

Reissue Serial No. 15/646,822

To: Examiner Location: CRU Art Unit: 3991 Date: 02/13/2018	From: Renee Preston Location: CRU 3999 MDE 4B15 Phone: (571) 272-1607 Renee.preston@uspto.gov

Search Notes

U.S. Patent No. 8,598,121

1) I performed a KeyCite Search in Westlaw, which retrieves all history on the patent including any litigation.

2) I performed a search on the patent in Lexis CourtLink for any open dockets or closed cases.

3) I performed a search in Lexis in the Federal Courts and Administrative Materials databases for any cases found.

4) I performed a search in Lexis in the IP Journal and Periodicals database for any articles on the patent.

5) I performed a search in Lexis in the news databases for any articles about the patent or any articles about litigation on this patent.

Litigation: No cases found

References Cited Items (32)

Title	Date	Type
1. RECEPTOR SPECIFIC ATRIAL NATRIURETIC PEPTIDES Out Of Print US PAT 5846932	Jun 06, 1995	Patents
2. THERAPEUTIC AGENTS FOR ACHONDROPLASIA Out Of Print US PAT 6743425	Aug 14, 2002	Patents
3. PORCINE CNP GENE AND PRECURSOR PROTEIN Out Of Print US PAT 6020168	Jul 12, 1991	Patents
4. HUMAN CNP GENE AND PRECURSOR PROTEIN Out Of Print US PAT 6034231	Sep 26, 1991	Patents
5. PROTECTION OF ENDOGENOUS THERAPEUTIC PEPTIDES FROM PEPTIDASE ACTIVITY THROUGH CONJUGATION TO BLOOD COMPONENTS Out Of Print US PAT 6849714	Sep 05, 2000	Patents
6. ANIMAL MODEL WITH DISRUPTED FGF-9 GENE Out Of Print US PAT 6136040	Feb 24, 1999	Patents
7. EXPORT AND MODIFICATION OF (POLY)PEPTIDES IN THE LANTIBIOTIC WAY Out Of Print US PAT 6861236	Feb 07, 2003	Patents
8. ANIMAL MODEL FOR FIBROBLAST GROWTH FACTOR RECEPTOR ASSOCIATED CHONDRODYSPLASIA Out Of Print US PAT 6265632	Aug 26, 1999	Patents
9. TRICYCLIC QUINOXALINE DERIVATIVES AS PROTEIN TYROSINE KINASE INHIBITORS Out Of Print US PAT 6329375	Aug 04, 1998	Patents
10. N-TERMINALLY CHEMICALLY MODIFIED PROTEIN COMPOSITIONS AND METHODS Out Of Print US PAT 5824784	Oct 12, 1994	Patents
11. IRREVERSIBLE INHIBITORS OF TYROSINE KINASES Out Of Print US PAT 6344459	Jun 08, 1999	Patents
12. METHOD AND COMPOSITION FOR TREATMENT OF SKELETAL DYSPLASIAS Out Of Print US PAT APP 20080194682	Aug 17, 2007	Patents
13. VARIANTS OF C-TYPE NATRIURETIC PEPTIDE Out Of Print US PAT APP 20100297021	May 20, 2010	Patents
14. METHOD AND COMPOSITION FOR TREATMENT OF SKELETAL DYSPLASIAS Out Of Print US PAT APP 20040138134	Sep 15, 2003	Patents
15. THEURAPEUTIC OR PROPHYIACTIC AGENT FOR ARTHRITIS Out Of Print US PAT APP 20070197434	Mar 31, 2005	Patents
16. ASSESSMENT OF SKELETAL GROWTH USING MEASUREMENTS OF NT-CNP PEPTIDES Out Of Print US PAT APP 20070292966	Jun 17, 2004	Patents
17. PREPARATION AND USE OF POLYETHYLENE GLYCOL PROPIONALDEHYDE Out Of Print US PAT 5252714	Nov 28, 1990	Patents
18. PORCINE DERIVED NOVEL PHYSIOLOGICALLY ACTIVE PEPTIDE Out Of Print US PAT 5352770	Apr 20, 1991	Patents

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Title	Date	Type
19. CNP ANALOG PEPTIDES AND THEIR USE Out Of Plan US PAT 5434133	Jan 31, 1992	Patents
20. METHOD AND COMPOSITION FOR TREATMENT OF SKELETAL DYSPLASIAS Out Of Plan US PAT 7276481	Sep 15, 2003	Patents
21. VARIANTS OF C-TYPE NATRIURETIC PEPTIDE Out Of Plan US PAT 8198242	May 20, 2010	Patents
22. VARIANTS OF C-TYPE NATRIURETIC PEPTIDE Out Of Plan WO 2010135541 A3	May 20, 2010	Patents
23. CNP analog peptides and their use Out Of Plan EP PAT 0497368 B1	Jan 31, 1992	Patents
24. Novel physiologically active porcine peptide (CNP-53) Out Of Plan EP PAT 0466174 B1	Jul 12, 1991	Patents
25. MODIFIED NATURETIC COMPOUNDS, CONJUGATES, AND USES THEREOF Out Of Plan WO 2004047871 A3	Nov 12, 2003	Patents
26. SUBSTRATE TRANSFER METHOD Out Of Plan WO 2002047871 A1	Dec 03, 2001	Patents
27. OPTICAL WAVEGUIDE, OPTICAL WAVEGUIDE MODULE AND METHOD FOR FORMING OPTICAL WAVEGUIDE Out Of Plan WO 2005098490 A1	Mar 30, 2005	Patents
28. COMPOSITION FOR INCREASING BODY HEIGHT Out Of Plan EP PAT APP 1743653 A1	Mar 31, 2005	Patents
29. METHOD AND COMPOSITION FOR TREATMENT OF SKELETAL DYSPLASIAS Out Of Plan WO 2002074234 A3	Mar 20, 2002	Patents
30. VARIANTS OF C-TYPE NATRIURETIC PEPTIDE Out Of Plan WO 2009067639 A4	Nov 21, 2008	Patents
31. VASONATRIN PEPTIDE AND ANALOGS THEREOF Out Of Plan WO 1994020534 A1	Mar 02, 1994	Patents
32. MODIFIED PENTAPEPTIDE ANTAGONISTS OF THE ATRIAL NATRIURETIC PEPTIDE CLEARANCE RECEPTOR Out Of Plan WO 2000061631 A1	Apr 07, 2000	Patents

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Citing References (8)

Treatment	Title	Date	Type	Depth	Headnote(s)
—	1. NEW C-TYPE NATRIURETIC PEPTIDE VARIANTS FOR TREATING BONE-RELATED AND VASCULAR SMOOTH MUSCLE DISORDERS E.G. OSTEOARTHRITIS, HYPOPHOSPHATEMIC RICKETS, ACHONDROPLASIA, SHORT STATURE, HYPERTENSION, RESTENOSIS, NEPHREDEMA AND ARTERIOSCLEROSIS Out Of Plan DWPI 2010-P62526+	May 20, 2009	DWPI	—	—
—	2. RF 028206/0517 Out Of Plan	May 14, 2012	Assignments	—	—
—	3. USE OF C-TYPE NATRIURETIC PEPTIDE VARIANTS TO TREAT SKELETAL DYSPLASIA Out Of Plan WO 2017020034 A1 , WIPO PCT Application	Feb. 02, 2017	Patents	—	—
—	4. PatStat 8598121	Mar. 31, 2015	Patent Status Files	—	—
—	5. USE OF C-TYPE NATRIURETIC PEPTIDE VARIANTS TO TREAT OSTEOARTHRITIS Out Of Plan WO 2017100400 A2 , WIPO PCT Application	June 15, 2017	Patents	—	—
—	6. REPLACEMENT THERAPY FOR NATRIURETIC PEPTIDE DEFICIENCIES Out Of Plan US PAT APP 20170087205 , U.S. PTO Application	Mar. 30, 2017	Patents	—	—
—	7. USE OF C-TYPE NATRIURETIC PEPTIDE VARIANTS TO TREAT SKELETAL DYSPLASIA Out Of Plan US PAT APP 20170028023+ , U.S. PTO Application	Feb. 02, 2017	Patents	—	—
—	8. REPLACEMENT THERAPY FOR NATRIURETIC PEPTIDE DEFICIENCIES Out Of Plan US PAT APP 20160354443 , U.S. PTO Application	Dec. 08, 2016	Patents	—	—

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Patent Search 8598121 2/13/2018

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Date and Time: Monday, February 12, 2018 9:44:00 AM EST
Job Number: 61205419

Documents (10)

1. *Biomarin Pharmaceutical Assigned Patent*
   Client/Matter: -None-
   Search Terms: 8598121 or 8,598,121
   Search Type: Terms and Connectors
   Narrowed by:

Content Type	Narrowed by
News	-None-

2. *U.S. Patents Awarded to Inventors in California (Dec. 12)*
   Client/Matter: -None-
   Search Terms: 8598121 or 8,598,121
   Search Type: Terms and Connectors
   Narrowed by:

Content Type	Narrowed by
News	-None-

3. *US Patent Issued to Biomarin Pharmaceutical on Dec. 3 for "Variants of C-type natriuretic peptide" (California Inventors)*
   Client/Matter: -None-
   Search Terms: 8598121 or 8,598,121
   Search Type: Terms and Connectors
   Narrowed by:

Content Type	Narrowed by
News	-None-

4. *Biomarin Pharmaceutical Inc Granted United States Patent for Variants of C-Type Natriuretic Peptide*
   Client/Matter: -None-
   Search Terms: 8598121 or 8,598,121
   Search Type: Terms and Connectors
   Narrowed by:

Content Type	Narrowed by
News	-None-

5. *US Patent granted to Biomarin Pharmaceutical Inc (California) on December 03 titled as "Variants of C-type natriuretic peptide"*
   Client/Matter: -None-

Search Terms: 8598121 or 8,598,121
Search Type: Terms and Connectors
Narrowed by:

Content Type	Narrowed by
News	-None-

6. *US Patent granted to Biomarin Pharmaceutical Inc (California) on December 03 titled as "Variants of C-type natriuretic peptide"*
Client/Matter: -None-
Search Terms: 8598121 or 8,598,121
Search Type: Terms and Connectors
Narrowed by:

Content Type	Narrowed by
News	-None-

7. *Tabla / Mercados Financieros*
Client/Matter: -None-
Search Terms: 8598121 or 8,598,121
Search Type: Terms and Connectors
Narrowed by:

Content Type	Narrowed by
News	-None-

8. *Tabla / Mercados Financieros*
Client/Matter: -None-
Search Terms: 8598121 or 8,598,121
Search Type: Terms and Connectors
Narrowed by:

Content Type	Narrowed by
News	-None-

9. *Tabla / Mercados Financieros*
Client/Matter: -None-
Search Terms: 8598121 or 8,598,121
Search Type: Terms and Connectors
Narrowed by:

Content Type	Narrowed by
News	-None-

10. *InVision Technologies Reports Strong Second-quarter Results; Company Earns $0.52 Per Share, Revenues Double From First Quarter*
Client/Matter: -None-
Search Terms: 8598121 or 8,598,121
Search Type: Terms and Connectors
Narrowed by:

Content Type	Narrowed by

News	-None-

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Biomarin Pharmaceutical Assigned Patent

Targeted News Service

December 12, 2013 Thursday 11:21 PM EST

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Length: 166 words

Byline: Targeted News Service

Dateline: Alexandria, Va.

Body

ALEXANDRIA, Va., Dec. 12 -- Biomarin Pharmaceutical, Novato, Calif., has been assigned a patent (***8,598,121***) developed by seven co-inventors for "variants of C-type natriuretic peptide." The co-inventors are Daniel J. Wendt, Walnut Creek, Calif., Shinong Long, Milpitas, Calif., Sianna Castillo, San Francisco, Christopher P. Price, Kentfield, Calif., Mika Aoyagi-Scharber, Mill Valley, Calif., Michel Claude Vellard, San Rafael, Calif., and Augustus O. Okhamafe, Concord, Calif.

The patent application was filed on May 8, 2012 (13/466,672). The full-text of the patent can be found at *http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=8598121.PN.&OS=PN/8598121&RS=PN/8598121*

Written by Jude Bautista; edited by Marlyn Vitin.

For more information about Targeted News Service federal patent awards please contact: Myron Struck, Editor, Direct: 703/866-4708, Cell: 703/304-1897, *Myron@targetednews.com*

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End of Document

U.S. Patents Awarded to Inventors in California (Dec. 12)

Targeted News Service

December 12, 2013 Thursday 2:03 PM EST

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Length: 10788 words

Byline: Targeted News Service Targeted News Service

Dateline: Alexandria, VA.

Body

ALEXANDRIA, Va., Dec. 12 -- The following federal patents were awarded to inventors in California.

***

Broadcom Assigned Patent for Network Transmitting Signal

ALEXANDRIA, Va., Dec. 11 -- Broadcom, Irvine, Calif., has been assigned a patent (8,600,322) developed by Kevin T. Chan, Pasadena, Calif., for a method "for transmitting a signal below a current transmit power in a network."

The patent application was filed on Nov. 29, 2012 (13/689,028). The full text of the patent can be found at http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=8600322.PN.&OS=PN/8600322&RS=PN/8600322

Written by Eloisa Asedillo; edited by Vessie Ann Abalos.

***

Broadcom Assigned Patent for Transmission Device Power Management

ALEXANDRIA, Va., Dec. 11 -- Broadcom, Irvine, Calif., has been assigned a patent (8,600,318) developed by Ahmadreza Rofougaran, Newport Coast, Calif., for "transmit power management for a communication device and method for use therewith."

The patent application was filed on Feb. 18, 2013 (13/769,476). The full text of the patent can be found at http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=8600318.PN.&OS=PN/8600318&RS=PN/8600318

Written by Eloisa Asedillo; edited by Vessie Ann Abalos.

***

Fleetcor Technologies Operating Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- Fleetcor Technologies Operating, Norcross, Ga., has been assigned a patent (8,606,458) developed by Jinsong Hu, San Jose, Calif., for a "navigation system having mileage mechanism and method of operation thereof."

RENEE PRESTON

The patent application was filed on Dec. 12, 2008 (12/334,350). The full-text of the patent can be found at http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO2&Sect2=HITOFF&p=1&u=%2Fnetahtml%2FPTO%2Fsearch-bool.html&r=29&f=G&l=50&co1=AND&d=PTXT&s1=20131210.PD.&s2=%28GA.ASST.%29&OS=ISD/12/10/2013+AND+AS/GA&RS=ISD/12/10/2013+AND+AS/GA

Written by Sudarshan Harpal; edited by Jaya Anand.

***

International Business Machines Assigned Patent for Automatically Segmenting and Populating Distributed Computing Problem

ALEXANDRIA, Va., Dec. 12 -- International Business Machines, Armonk, N.Y., has been assigned a patent (8,606,844) developed by James H. Kaufman, San Jose, Calif., and Tobin Jon Lehman, Los Gatos, Calif., for a "system and method for automatically segmenting and populating a distributed computing problem."

The patent application was filed on Aug. 14, 2008 (12/191,483). The full-text of the patent can be found at http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO2&Sect2=HITOFF&p=2&u=%2Fnetahtml%2FPTO%2Fsearch-bool.html&r=90&f=G&l=50&co1=AND&d=PTXT&s1=20131210.PD.&s2=%28NY.ASST.%29&OS=ISD/12/10/2013+AND+AS/NY&RS=ISD/12/10/2013+AND+AS/NY

Written by Sudarshan Harpal; edited by Jaya Anand.

***

Hipcricket Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- Hipcricket, New York, has been assigned a patent (8,606,874) developed by four co-inventors for a "system and method of binding a client to a server." The co-inventors are John M. Cooney, San Carlos, Calif., Awais Ali, Santa Clara, Calif., Fabrizio Talucci, San Jose, Calif., and Charlie Zimmerman, San Carlos, Calif.

The patent application was filed on Aug. 31, 2009 (12/551,299). The full-text of the patent can be found at http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO2&Sect2=HITOFF&p=2&u=%2Fnetahtml%2FPTO%2Fsearch-bool.html&r=85&f=G&l=50&co1=AND&d=PTXT&s1=20131210.PD.&s2=%28NY.ASST.%29&OS=ISD/12/10/2013+AND+AS/NY&RS=ISD/12/10/2013+AND+AS/NY

Written by Sudarshan Harpal; edited by Jaya Anand.

***

International Business Machines Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- International Business Machines, Armonk, N.Y., has been assigned a patent (8,607,005) developed by four co-inventors for "monitoring program execution to learn data blocks accessed by software process for facilitating efficient prefetching." The co-inventors are Kenneth Wayne Boyd, Tucson, Ariz., Kenneth Fairclough Day III, Tucson, Ariz., David Allan Pease, Redwood Estates, Calif., and John Jay Wolfgang, Winston-Salem, N.C.

The patent application was filed on Feb. 17, 2006 (11/357,473). The full-text of the patent can be found at http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO2&Sect2=HITOFF&p=2&u=%2Fnetahtml%2FPTO%2Fsearch-bool.html&r=71&f=G&l=50&co1=AND&d=PTXT&s1=20131210.PD.&s2=%28NY.ASST.%29&OS=ISD/12/10/2013+AND+AS/NY&RS=ISD/12/10/2013+AND+AS/NY

Written by Sudarshan Harpal; edited by Jaya Anand.

RENEE PRESTON

***

International Business Machines Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- International Business Machines, Armonk, N.Y., has been assigned a patent (8,606,927) developed by Jeffrey Scott Pierce, San Jose, Calif., for a "multi-device communication method and system."

The patent application was filed on March 19, 2008 (12/050,986). The full-text of the patent can be found at http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO2&Sect2=HITOFF&p=2&u=%2Fnetahtml%2FPTO%2Fsearch-bool.html&r=81&f=G&l=50&co1=AND&d=PTXT&s1=20131210.PD.&s2=%28NY.ASST.%29&OS=ISD/12/10/2013+AND+AS/NY&RS=ISD/12/10/2013+AND+AS/NY

Written by Sudarshan Harpal; edited by Jaya Anand.

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International Business Machines Assigned Patent for Combining and Processing Web Data

ALEXANDRIA, Va., Dec. 12 -- International Business Machines, Armonk, N.Y., has been assigned a patent (8,606,773) developed by five co-inventors for a "method for combining and processing web data using tables and programming-by-demonstration." The co-inventors are Allen Cypher, Aptos, Calif., Tessa A. Lau, Mountain View, Calif., James Lin, Cupertino, Calif., Jeffrey W. Nichols, San Jose, Calif., and Jeffrey Wong, Pittsburg.

The patent application was filed on Jan. 18, 2010 (12/689,216). The full-text of the patent can be found at http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO2&Sect2=HITOFF&p=3&u=%2Fnetahtml%2FPTO%2Fsearch-bool.html&r=101&f=G&l=50&co1=AND&d=PTXT&s1=20131210.PD.&s2=%28NY.ASST.%29&OS=ISD/12/10/2013+AND+AS/NY&RS=ISD/12/10/2013+AND+AS/NY

Written by Sudarshan Harpal; edited by Jaya Anand.

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International Business Machines Assigned Patent for Analyzing Electronic Text

ALEXANDRIA, Va., Dec. 12 -- International Business Machines, Armonk, N.Y., has been assigned a patent (8,606,815) developed by Ying Chen, San Jose, Calif., Larry Proctor, Coppell, Texas, and William Scott Spangler, San Martin, Calif., for "systems and methods for analyzing electronic text."

The patent application was filed on Dec. 9, 2008 (12/331,271). The full-text of the patent can be found at http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO2&Sect2=HITOFF&p=2&u=%2Fnetahtml%2FPTO%2Fsearch-bool.html&r=96&f=G&l=50&co1=AND&d=PTXT&s1=20131210.PD.&s2=%28NY.ASST.%29&OS=ISD/12/10/2013+AND+AS/NY&RS=ISD/12/10/2013+AND+AS/NY

Written by Sudarshan Harpal; edited by Jaya Anand.

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Autonet Mobile Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- Autonet Mobile, San Francisco, has been assigned a patent (8,605,698) developed by Douglas S. Moeller, Santa Rosa, Calif., and Ronald W. Pashby, San Francisco, for a "vehicle with mobile router."

The patent application was filed on Dec. 5, 2011 (13/373,932). The full-text of the patent can be found at http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO2&Sect2=HITOFF&p=1&u=%2Fnetahtml%2FPTO%2Fsearch-

bool.html&r=28&f=G&l=50&co1=AND&d=PTXT&s1=20131210.PD.&s2=%28CO.ASST.%29&OS=ISD/12/10/2013+AND+AS/CO&RS=ISD/12/10/2013+AND+AS/CO

Written by Satyaban Rath; edited by Jaya Anand.

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Wireless Seismic Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- Wireless Seismic, Louisville, Colo., has been assigned a patent (8,605,545) developed by Douglas Crice, Grass Valley, Calif., and Mihai Beffa, Cupertino, Calif., for a "wireless exploration seismic system."

The patent application was filed on July 5, 2012 (13/541,932). The full-text of the patent can be found at http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO2&Sect2=HITOFF&p=1&u=%2Fnetahtml%2FPTO%2Fsearch-bool.html&r=29&f=G&l=50&co1=AND&d=PTXT&s1=20131210.PD.&s2=%28CO.ASST.%29&OS=ISD/12/10/2013+AND+AS/CO&RS=ISD/12/10/2013+AND+AS/CO

Written by Satyaban Rath; edited by Jaya Anand.

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CFPH Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- CFPH, New York, has been assigned a patent (8,606,685) developed by Timothy M. Keiser, Los Angeles, and Michael R. Burns, Los Angeles, for a "computer-implemented securities trading system."

The patent application was filed on Sept. 4, 2003 (10/655,409). The full-text of the patent can be found at http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO2&Sect2=HITOFF&p=3&u=%2Fnetahtml%2FPTO%2Fsearch-bool.html&r=114&f=G&l=50&co1=AND&d=PTXT&s1=20131210.PD.&s2=%28NY.ASST.%29&OS=ISD/12/10/2013+AND+AS/NY&RS=ISD/12/10/2013+AND+AS/NY

Written by Sudarshan Harpal; edited by Jaya Anand.

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Ciena Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- Ciena, Hanover, Md., has been assigned a patent (8,606,105) developed by Lyndon Y. Ong, Sunnyvale, Calif., for a "virtual core router and switch systems and methods with a hybrid control architecture."

The patent application was filed on Sept. 15, 2011 (13/233,603). The full-text of the patent can be found at http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO2&Sect2=HITOFF&p=1&u=%2Fnetahtml%2FPTO%2Fsearch-bool.html&r=9&f=G&l=50&co1=AND&d=PTXT&s1=20131210.PD.&s2=%28MD.ASST.%29&OS=ISD/12/10/2013+AND+AS/MD&RS=ISD/12/10/2013+AND+AS/MD

Written by Satyaban Rath; edited by Jaya Anand.

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Verizon Patent and Licensing Assigned Patent for Network Aware Content Pre-delivery over Radio Access Network

ALEXANDRIA, Va., Dec. 12 -- Verizon Patent and Licensing, Basking Ridge, N.J., has been assigned a patent (8,605,640) developed by Arda Aksu, Martinez, Calif., and Yee Sin Chan, San Jose, Calif., for a "network aware content pre-delivery over a radio access network."

The patent application was filed on May 26, 2011 (13/116,471). The full-text of the patent can be found at *http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO2&Sect2=HITOFF&p=1&u=%2Fnetahtml%2FPTO%2Fsearch-bool.html&r=39&f=G&l=50&co1=AND&d=PTXT&s1=20131210.PD.&s2=%28NJ.ASST.%29&OS=ISD/12/10/2013+AND+AS/NJ&RS=ISD/12/10/2013+AND+AS/NJ*

Written by Satyaban Rath; edited by Jaya Anand.

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Avaya Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- Avaya, Basking Ridge, N.J., has been assigned a patent (8,605,863) developed by Nicholas Sauriol, Ottawa, Arn Hyndman, Ottawa, and Paul To, Menlo Park, Calif., for a "method and apparatus for providing state indication on a telephone call."

The patent application was filed on Dec. 27, 2008 (12/344,465). The full-text of the patent can be found at *http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO2&Sect2=HITOFF&p=1&u=%2Fnetahtml%2FPTO%2Fsearch-bool.html&r=34&f=G&l=50&co1=AND&d=PTXT&s1=20131210.PD.&s2=%28NJ.ASST.%29&OS=ISD/12/10/2013+AND+AS/NJ&RS=ISD/12/10/2013+AND+AS/NJ*

Written by Satyaban Rath; edited by Jaya Anand.

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Round Rock Research Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- Round Rock Research, Jersey City, N.J., has been assigned a patent (8,605,515) developed by Frankie F. Roohparvar, Monte Sereno, Calif., for "memory devices and their operation with different sets of logical erase blocks."

The patent application was filed on June 11, 2012 (13/493,317). The full-text of the patent can be found at *http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO2&Sect2=HITOFF&p=1&u=%2Fnetahtml%2FPTO%2Fsearch-bool.html&r=43&f=G&l=50&co1=AND&d=PTXT&s1=20131210.PD.&s2=%28NJ.ASST.%29&OS=ISD/12/10/2013+AND+AS/NJ&RS=ISD/12/10/2013+AND+AS/NJ*

Written by Satyaban Rath; edited by Jaya Anand.

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Sony Electronics Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- Sony Electronics, Park Ridge, N.J., and Sony, Tokyo, have been assigned a patent (8,606,074) developed by Brant L. Candelore, Escondido, Calif., for a "method and system for altering the presentation of broadcast content."

The patent application was filed on June 13, 2006 (11/453,123). The full-text of the patent can be found at *http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO2&Sect2=HITOFF&p=1&u=%2Fnetahtml%2FPTO%2Fsearch-bool.html&r=30&f=G&l=50&co1=AND&d=PTXT&s1=20131210.PD.&s2=%28NJ.ASST.%29&OS=ISD/12/10/2013+AND+AS/NJ&RS=ISD/12/10/2013+AND+AS/NJ*

Written by Satyaban Rath; edited by Jaya Anand.

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Sony Electronics Assigned Patent

U.S. Patents Awarded to Inventors in California (Dec. 12)

ALEXANDRIA, Va., Dec. 12 -- Sony Electronics, Park Ridge, N.J., and Sony, Tokyo, have been assigned a patent (8,605,219) developed by Victor Carvajal, San Marcos, Calif., Jaime Chee, San Diego, and Lin Jun Wong, Escondido, Calif., for "techniques for implementing a cursor for televisions."

The patent application was filed on Nov. 11, 2008 (12/269,028). The full-text of the patent can be found at *http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO2&Sect2=HITOFF&p=1&u=%2Fnetahtml%2FPTO%2Fsearch-bool.html&r=46&f=G&l=50&co1=AND&d=PTXT&s1=20131210.PD.&s2=%28NJ.ASST.%29&OS=ISD/12/10/2013+AND+AS/NJ&RS=ISD/12/10/2013+AND+AS/NJ*

Written by Satyaban Rath; edited by Jaya Anand.

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Hewlett-Packard Development Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- Hewlett-Packard Development, Houston, has been assigned a patent (8,606,058) developed by five co-inventors for a multi-channel optical coupler. The co-inventors are Huei Pei Kuo, Cupertino, Calif., Michael Renne Ty Tan, Menlo Park, Calif., Robert G. Walmsley, Palo Alto, Calif., Shih-Yuan (SY) Wang, Palo Alto, Calif., and Paul Kessler Rosenberg, Sunnyvale, Calif.

The patent application was filed on April 30, 2009 (12/433,794). The full-text of the patent can be found at *http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO2&Sect2=HITOFF&p=1&u=%2Fnetahtml%2FPTO%2Fsearch-bool.html&r=48&f=G&l=50&co1=AND&d=PTXT&s1=20131210.PD.&s2=%28TX.ASST.%29&OS=ISD/12/10/2013+AND+AS/TX&RS=ISD/12/10/2013+AND+AS/TX*

Written by Satyaban Rath; edited by Jaya Anand.

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Hewlett-Packard Development Assigned Patent for Electronic Inks and Displays and Image Displaying Methods

ALEXANDRIA, Va., Dec. 12 -- Hewlett-Packard Development, Houston, has been assigned a patent (8,605,352) developed by seven co-inventors for "electronic inks and displays and image displaying methods." The co-inventors are Yaron Grinwald, Meitar, Israel, Stella Stolin Roditi, Rehovot, Israel, Yigal Berson, Lod, Israel, Zhang-Lin Zhou, Palo Alto, Calif., Gregg Alan Combs, Monmouth, Ore., Jeffrey Todd Mabeck, Corvallis, Ore., and Albert Teishev, Rishon le-zion, Israel.

The patent application was filed on Dec. 30, 2008 (13/142,274). The full-text of the patent can be found at *http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO2&Sect2=HITOFF&p=2&u=%2Fnetahtml%2FPTO%2Fsearch-bool.html&r=78&f=G&l=50&co1=AND&d=PTXT&s1=20131210.PD.&s2=%28TX.ASST.%29&OS=ISD/12/10/2013+AND+AS/TX&RS=ISD/12/10/2013+AND+AS/TX*

Written by Satyaban Rath; edited by Jaya Anand.

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Hewlett-Packard Development Assigned Patent for Self-repairing Memristor and Method

ALEXANDRIA, Va., Dec. 12 -- Hewlett-Packard Development, Houston, has been assigned a patent (8,605,484) developed by Julien Borghetti, Mountain View, Calif., Alexandre M. Bratkovski, Mountain View, Calif., and Matthew D. Pickett, San Francisco, for a "self-repairing memristor and method."

The patent application was filed on Jan. 29, 2009 (13/130,822). The full-text of the patent can be found at *http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO2&Sect2=HITOFF&p=2&u=%2Fnetahtml%2FPTO%2Fsearch-bool.html&r=74&f=G&l=50&co1=AND&d=PTXT&s1=20131210.PD.&s2=%28TX.ASST.%29&OS=ISD/12/10/2013+AND+AS/TX&RS=ISD/12/10/2013+AND+AS/TX*

RENEE PRESTON

Written by Satyaban Rath; edited by Jaya Anand.

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Hewlett-Packard Development Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- Hewlett-Packard Development, Houston, has been assigned a patent (8,605,488) developed by Dmitri Borisovich Strukov, Mountain View, Calif., Gregory S. Snider, Los Altos, Calif., and R. Stanley Williams, Portola Valley, Calif., for capacitive crossbar arrays

The patent application was filed on June 12, 2009 (13/256,239). The full-text of the patent can be found at *http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO2&Sect2=HITOFF&p=2&u=%2Fnetahtml%2FPTO%2Fsearch-bool.html&r=73&f=G&l=50&co1=AND&d=PTXT&s1=20131210.PD.&s2=%28TX.ASST.%29&OS=ISD/12/10/2013+AND+AS/TX&RS=ISD/12/10/2013+AND+AS/TX*

Written by Satyaban Rath; edited by Jaya Anand.

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Hewlett-Packard Development Assigned Patent for Memristive Device and Methods of Making and Using the Same

ALEXANDRIA, Va., Dec. 12 -- Hewlett-Packard Development, Houston, has been assigned a patent (8,605,483) developed by R. Stanley Williams, Portola Valley, Calif., Dmitri Borisovich Strukov, Menlo Park, Calif., and Alexandre M. Bratkovski, Mountain View, Calif., for a "memristive device and methods of making and using the same."

The patent application was filed on Dec. 23, 2008 (13/120,905). The full-text of the patent can be found at *http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO2&Sect2=HITOFF&p=2&u=%2Fnetahtml%2FPTO%2Fsearch-bool.html&r=75&f=G&l=50&co1=AND&d=PTXT&s1=20131210.PD.&s2=%28TX.ASST.%29&OS=ISD/12/10/2013+AND+AS/TX&RS=ISD/12/10/2013+AND+AS/TX*

Written by Satyaban Rath; edited by Jaya Anand.

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SecureCare Technologies Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- SecureCare Technologies, Austin, Texas, has been assigned a patent (8,605,296) developed by four co-inventors for a "digital signature system and method." The co-inventors are Eugene L. Fry, Austin, Texas, Aleksander Roman Szymanski, Ipswich, United Kingdom, Dennis J. Nasto, San Diego, and David D. Vineyard, Bastrop, Texas.

The patent application was filed on May 28, 2010 (12/790,376). The full-text of the patent can be found at *http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO2&Sect2=HITOFF&p=2&u=%2Fnetahtml%2FPTO%2Fsearch-bool.html&r=81&f=G&l=50&co1=AND&d=PTXT&s1=20131210.PD.&s2=%28TX.ASST.%29&OS=ISD/12/10/2013+AND+AS/TX&RS=ISD/12/10/2013+AND+AS/TX*

Written by Satyaban Rath; edited by Jaya Anand.

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Hewlett-Packard Development Assigned Patent for Probe Having Nano-fingers

ALEXANDRIA, Va., Dec. 12 -- Hewlett-Packard Development, Houston, has been assigned a patent (8,605,281) developed by Zhiyong Li, Foster City, Calif., for a probe having nano-fingers.

The patent application was filed on Oct. 13, 2011 (13/272,987). The full-text of the patent can be found at *http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO2&Sect2=HITOFF&p=2&u=%2Fnetahtml%2FPTO%2Fsearch-bool.html&r=82&f=G&l=50&co1=AND&d=PTXT&s1=20131210.PD.&s2=%28TX.ASST.%29&OS=ISD/12/10/2013+AND+AS/TX&RS=ISD/12/10/2013+AND+AS/TX*

Written by Satyaban Rath; edited by Jaya Anand.

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U.S. Navy Assigned Patent for Transparent Interferometric Visible Spectrum Modulator

ALEXANDRIA, Va., Dec. 12 -- The U.S. Navy has been assigned a patent (8,605,351) developed by Andrew Wang, San Diego, for a "transparent interferometric visible spectrum modulator."

The patent application was filed on June 27, 2012 (13/534,049). The full-text of the patent can be found at *http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=86,05,351.PN.&OS=PN/86,05,351&RS=PN/86,05,351*

Written by Amal Ahmed; edited by Jaya Anand.

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Sequent Medical Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- Sequent Medical, Aliso Viejo, Calif., has been assigned a patent (8,597,333) developed by eight co-inventors for systems and methods "for vascular implants for delivery and detachment." The co-inventors are Daniel Welsh, Encinitas, Calif., Eileen Charlton, Garden Grove, Calif., Son Pham, Garden Grove, Calif., Claudio Plaza, Irvine, Calif., William R. Patterson, Irvine, Calif., Todd Hewitt, Laguna Niguel, Calif., Scott Hemmelgarn, Lake Forest, Calif., and Philippe Marchand, Munich.

The patent application was filed on March 15, 2013 (13/842,492). The full-text of the patent can be found at *http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=8597323.PN.&OS=PN/8597323&RS=PN/8597323*

Written by Lesley Gonzales; edited by Marlyn Vitin.

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UTC Fire & Security Americas Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- UTC Fire & Security Americas, Bradenton, Fla., has been assigned a patent (8,605,151) developed by seven co-inventors for "methods and systems for operating a video surveillance system." The co-inventors are Dirk Livingston Bellamy, Salem, Ore., Nery Manuel Moreno, Costa Mesa, Calif., Kenneth McCormack, Albany, Ore., Kenton Arthur St. John, Albany, Ore., Vikas Mohan Sharma, Irvine, Calif., R. Kenin Page, Salem, Ore., and Stephen Anthony Shelton, Encinitas, Calif.

The patent application was filed on Sept. 21, 2007 (11/859,531). The full-text of the patent can be found at *http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=86,05,151.PN.&OS=PN/86,05,151&RS=PN/86,05,151*

Written by Amal Ahmed; edited by Jaya Anand.

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Microsoft Assigned Patent for Management of Collections of Websites

ALEXANDRIA, Va., Dec. 12 -- Microsoft, Redmond, Wash., has been assigned a patent (8,606,816) developed by seven co-inventors for the "management of collections of websites." The co-inventors are Zachary D. Rosenfield, Seattle, William David Taylor, Redmond, Wash., Titus C. Miron, Seattle, Jason M. Cahill, Woodinville, Wash., Umesh Unnikrishnan, Sammamish, Wash., Harshal Ingole, San Jose, Calif., and Mark J. Walker, Redmond, Wash.

The patent application was filed on Oct. 25, 2011 (13/281,196). The full-text of the patent can be found at *http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=86,06,816.PN.&OS=PN/86,06,816&RS=PN/86,06,816*

Written by Amal Ahmed; edited by Jaya Anand.

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Innovative Spine Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- Innovative Spine, Colvis, Calif., has been assigned a patent (8,597,299) developed by three co-inventors for a system "for accessing a spine from a curved postero-lateral approach may include a curved cannula positioned along a curved path from an opening in the skin to a location proximate the spine." The co-inventors are Joshua Butters, Chandler, Ariz., Morteza Farr, Santa Cruz, Calif., and T. Wade Fallin, Hyde Park, Utah.

The patent application was filed on Nov. 2, 2007 (11/934,636). The full-text of the patent can be found at *http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=8597299.PN.&OS=PN/8597299&RS=PN/8597299*

Written by Lesley Gonzales; edited by Marlyn Vitin.

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Theravance Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- Theravance, South San Francisco, Calif., has been assigned a patent (8,598,170) developed by three co-inventors for "[d]ual-acting oxazole antihypertensive agents." The co-inventors are Paul R. Fatheree, San Francisco, Ryan Hudson, San Jose, Calif., and Robert Murray McKinnell, Millbrae, Calif.

The patent application was filed on Nov. 20, 2012 (13/682,299). The full-text of the patent can be found at *http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=8598170.PN.&OS=PN/8598170&RS=PN/8598170*

Written by Jude Bautista; edited by Marlyn Vitin.

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Cubist Pharmaceuticals Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- Cubist Pharmaceuticals, Lexington, Mass., has been assigned a patent (8,604,164) developed by six co-inventors for high purity lipopeptides. The co-inventors are Thomas J. Kelleher, Thousand Oaks, Calif., Jan-Ji Lai, Westborough, Mass., Joseph P. DeCourcey, Boston, Paul D. Lynch, Arlington, Mass., Maurizio Zenoni, Ferentino Frosinone, Italy, and Auro R. Tagliani, Pavia, Italy.

The patent application was filed on Feb. 16, 2012 (13/398,219). The full-text of the patent can be found at *http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=86,04,164.PN.&OS=PN/86,04,164&RS=PN/86,04,164*

Written by Amal Ahmed; edited by Jaya Anand.

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Westinghouse Electric Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- Westinghouse Electric, Cranberry Township, Pa., has been assigned a patent (8,605,852) developed by four co-inventors for a "wedge positioning apparatus for jet pump assemblies in nuclear reactors." The co-inventors are Michael A. Flanigan, Dutch Flat, Calif., Stephen J. Kaylor, San Jose, Calif., James M. DuBay, Louisville, Colo., and Rodney Lum, Dublin, Calif.

The patent application was filed on Nov. 1, 2010 (12/916,660). The full-text of the patent can be found at *http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=86,05,852.PN.&OS=PN/86,05,852&RS=PN/86,05,852*

Written by Amal Ahmed; edited by Jaya Anand.

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Neuropro Technologies Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- Neuropro Technologies, Modesto, Calif., has been assigned a patent (8,597,360) developed by five co-inventors "for a bone fusion device which provides stability to bones during a bone fusion period." The co-inventors are Benjamin J. Remington, Modesto, Calif., Joseph N. Logan, Trumbull, Conn., Daniel R. Baker, Seattle, Gary R. McLuen, Port Townsend, Wash., and Gregory C. Stalcup, Columbia City, Ind.

The patent application was filed on May 29, 2012 (13/482,778). The full-text of the patent can be found at *http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=8597360.PN.&OS=PN/8597360&RS=PN/8597360*

Written by Lesley Gonzales; edited by Marlyn Vitin.

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MIT Assigned Patent for Electrochemical Actuator

ALEXANDRIA, Va., Dec. 12 -- Massachusetts Institute of Technology, Cambridge, Mass., has been assigned a patent (8,604,664) developed by Yet-Ming Chiang, Framingham, Mass., Michael J. Cima, Winchester, Mass., and Timothy E. Chin, San Jose, Calif., for an electrochemical actuator.

The patent application was filed on July 5, 2012 (13/542,179). The full-text of the patent can be found at *http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=86,04,664.PN.&OS=PN/86,04,664&RS=PN/86,04,664*

Written by Amal Ahmed; edited by Jaya Anand.

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Boston University, Brigham and Women's Hospital, University of Southern California Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- Boston University, Boston, Brigham and Women's Hospital, Boston, and the University of Southern California, Los Angeles, have been assigned a patent (8,604,229) developed by Thomas E. Van Dyke, West Roxbury, Mass., Nicos A. Petasis, Hacienda Heights, Calif., and Charles N. Serhan, Needham, Mass., for "lipoxin analogs and methods for the treatment of periodontal disease."

The patent application was filed on Jan. 9, 2012 (13/346,543). The full-text of the patent can be found at *http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=86.04.229.PN.&OS=PN/86.04.229&RS=PN/86.04.229*

Written by Amal Ahmed; edited by Jaya Anand.

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Novellus Systems Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- Novellus Systems, Fremont, Calif., has been assigned a patent (8,597,448) developed by four co-inventors for methods "for extending electrostatic chuck lifetimes are provided." The co-inventors are Alisa Hart, Boulder Creek, Calif., Liza Palma, Manteca, Calif., Alasdair Dent, San Jose, Calif., and John C. Boyd, Crawford, Texas.

The patent application was filed on Dec. 29, 2009 (12/648,638). The full-text of the patent can be found at *http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=8597448.PN.&OS=PN/8597448&RS=PN/8597448*

Written by Lesley Gonzales; edited by Marlyn Vitin.

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Content Technologies Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- Content Technologies, Kirkland, Wash., has been assigned a patent (8,606,856) developed by Bryan Dunkeld, Sausalito, Calif., and Edward Lambert, Mercer Island, Wash., for a "digital media asset identification system and method."

The patent application was filed on April 15, 2008 (12/103,591). The full-text of the patent can be found at *http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=86.06.856.PN.&OS=PN/86.06.856&RS=PN/86.06.856*

Written by Amal Ahmed; edited by Jaya Anand.

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Vertex Pharmaceuticals Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- Vertex Pharmaceuticals, Cambridge, Mass., has been assigned a patent (8,604,203) developed by six co-inventors for "modulators of cystic fibrosis transmembrane conductance regulator." The co-inventors are Hayley Binch, Encinitas, Calif., Lev T. D. Fanning, San Marcos, Calif., Martyn Botfield, Concord, Mass., Peter D.J. Grootenhuis, San Diego, Fredrick Van Goor, San Diego, and Mehdi Michel Djamel Numa, San Diego.

The patent application was filed on Oct. 15, 2012 (13/652,056). The full-text of the patent can be found at http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=86,04,203.PN.&OS=PN/86,04,203&RS=PN/86,04,203

Written by Amal Ahmed; edited by Jaya Anand.

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Curis Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- Curis, Lexington, Mass., has been assigned a patent (8,604,044) developed by four co-inventors for "quinazoline based EGFR inhibitors containing a zinc binding moiety." The co-inventors are Changgeng Qian, Wayland, Mass., Xiong Cai, Belmont, Mass., Stephen Gould, San Carlos, Calif., and Haixiao Zhai, Bedford, Mass.

The patent application was filed on Sept. 10, 2007 (11/852,604). The full-text of the patent can be found at http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=86,04,044.PN.&OS=PN/86,04,044&RS=PN/86,04,044

Written by Amal Ahmed; edited by Jaya Anand.

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Lam Research Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- Lam Research, Fremont, Calif., has been assigned a patent (8,598,040) developed by four co-inventors for a method "for etching features in a plurality of silicon based bilayers." The co-inventors are Anne Le Gouil, Fremont, Calif., Jeffrey R. Lindain. Tracy, Calif., Yasushi Ishikawa, Fremont, Calif., and Yoko Yamaguchi-Adams, Union City, Calif.

The patent application was filed on Sept. 6, 2011 (13/226,087). The full-text of the patent can be found at http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=8598040.PN.&OS=PN/8598040&RS=PN/8598040

Written by Jude Bautista; edited by Marlyn Vitin.

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Method Products Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- Method Products, San Francisco, has been assigned a patent (8,598,109) developed by two co-inventors for a "fabric softener formulation." The co-inventors are Frederick W. Holzhauer, Novato, Calif., and Donald H. Frey, West Hollywood, Calif.

The patent application was filed on Sept. 13, 2010 (12/881,077). The full-text of the patent can be found at http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=8598109.PN.&OS=PN/8598109&RS=PN/8598109

Written by Jude Bautista; edited by Marlyn Vitin.

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Applied Materials Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- Applied Materials, Santa Clara, Calif., has been assigned a patent (8,598,020) developed by four co-inventors for a method of "depositing a crystalline germanium layer on a substrate." The co-inventors are Victor Nguyen, Novato, Calif., Mihaela Balseanu, Sunnyvale, Calif., Li-Qun Xia, Cupertino, Calif., and Derek R Witty, Fremont, Calif.

The patent application was filed on June 25, 2010 (12/824,095). The full-text of the patent can be found at *http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=8598020.PN.&OS=PN/8598020&RS=PN/8598020*

Written by Jude Bautista; edited by Marlyn Vitin.

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California Institute of Technology Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- California Institute of Technology, Pasadena, Calif., has been assigned a patent (8,598,137) developed by two co-inventors for the use of SAM68 "for modulating signaling through the TNF receptor." The co-inventors are Parameswaran Ramakrishnan, Pasadena, Calif., and David Baltimore, Pasadena, Calif.

The patent application was filed on Oct. 28, 2011 (13/284,792). The full-text of the patent can be found at *http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=8598137.PN.&OS=PN/8598137&RS=PN/8598137*

Written by Jude Bautista; edited by Marlyn Vitin.

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Applied Materials Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- Applied Materials, Santa Clara, Calif., has been assigned a patent (8,598,016) developed by six co-inventors for methods of "dicing substrates by both laser scribing and plasma etching." The co-inventors are Madhava Rao Yalamanchili, Morgan Hill, Calif., Wei-Sheng Lei, San Jose, Calif., Brad Eaton, Menlo Park, Calif., Saravjeet Singh, Santa Clara, Calif., Ajay Kumar, Cupertino, Calif., and Banqiu Wu, Sunnyvale, Calif.

The patent application was filed on June 15, 2011 (13/160,973). The full-text of the patent can be found at *http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=8598016.PN.&OS=PN/8598016&RS=PN/8598016*

Written by Jude Bautista; edited by Marlyn Vitin.

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Biomarin Pharmaceutical Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- Biomarin Pharmaceutical, Novato, Calif., has been assigned a patent (***8,598,121***) developed by seven co-inventors for "variants of C-type natriuretic peptide." The co-inventors are Daniel J. Wendt, Walnut Creek, Calif., Shinong Long, Milpitas, Calif., Sianna Castillo, San Francisco, Christopher P. Price, Kentfield, Calif., Mika Aoyagi-Scharber, Mill Valley, Calif., Michel Claude Vellard, San Rafael, Calif., and Augustus O. Okhamafe, Concord, Calif.

The patent application was filed on May 8, 2012 (13/466,672). The full-text of the patent can be found at http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=8598121.PN.&OS=PN/8598121&RS=PN/8598121

Written by Jude Bautista; edited by Marlyn Vitin.

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Microsoft Assigned Patent for System for the Efficient Indexing of Error Tolerant Set Containment

ALEXANDRIA, Va., Dec. 12 -- Microsoft, Redmond, Wash., has been assigned a patent (8,606,771) developed by Arvind Arasu, Bothell, Wash., Parag Agrawal, Stanford, Calif., and Kaushik Shriraghav, Bellevue, Wash., for "efficient indexing of error tolerant set containment."

The patent application was filed on Dec. 21, 2010 (12/973,909). The full-text of the patent can be found at http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=86.06.771.PN.&OS=PN/86.06.771&RS=PN/86.06.771

Written by Amal Ahmed; edited by Jaya Anand.

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Corcept Therapeutics Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- Corcept Therapeutics, Menlo Park, Calif., has been assigned a patent (8,598,149) developed by Joseph K. Belanoff, Woodside, Calif., for a method "for optimizing levels of mifepristone in a patient suffering from a mental disorder amenable to treatment by mifepristone."

The patent application was filed on Aug. 27, 2008 (12/199,114). The full-text of the patent can be found at http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=8598149.PN.&OS=PN/8598149&RS=PN/8598149

Written by Jude Bautista; edited by Marlyn Vitin.

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Fairchild Semiconductor Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- Fairchild Semiconductor, San Jose, Calif., has been assigned a patent (8,598,035) developed by seven co-inventors for "[s]emiconductor dice with backside trenches filled with elastic material for improved attachment, packages using the same, and methods of making the same." The co-inventors are Michael D. Gruenhagen, Salt Lake City, Suku Kim, South Jordan, Utah, James J. Murphy, South Jordan, Utah, Eddy Tjhia, Sunnyvale, Calif., Chung-Lin Wu, San Jose, Calif., Mark Larsen Sandy, Utah, and Douglas E. Dolan,York, Maine.

The patent application was filed on June 2, 2011 (13/151,495). The full-text of the patent can be found at http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=8598035.PN.&OS=PN/8598035&RS=PN/8598035

Written by Jude Bautista; edited by Marlyn Vitin.

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Lam Research Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- Lam Research, Fremont, Calif., has been assigned a patent (8,598,037) developed by two co-inventors for a method of "etching a silicon layer through a patterned mask." The co-inventors are Jaroslaw W. Winniczek, Daly City, Calif., and Robert P. Chebi, San Carlos, Calif.

The patent application was filed on Dec. 28, 2011 (13/339,301). The full-text of the patent can be found at *http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=8598037.PN.&OS=PN/8598037&RS=PN/8598037*

Written by Jude Bautista; edited by Marlyn Vitin.

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Micron Technology Assigned Patent for Non-volatile SRAM Cell that Incorporates Phase-change Memory

ALEXANDRIA, Va., Dec. 12 -- Micron Technology, Boise, Idaho, has been assigned a patent (8,605,490) developed by Richard Fackenthal, Carmichael, Calif., for a "non-volatile SRAM cell that incorporates phase-change memory into a CMOS process."

The patent application was filed on Oct. 12, 2009 (12/577,631). The full-text of the patent can be found at *http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO2&Sect2=HITOFF&p=1&u=%2Fnetahtml%2FPTO%2Fsearch-bool.html&r=10&f=G&l=50&co1=AND&d=PTXT&s1=20131210.PD.&s2=%28ID.ASST.%29&OS=ISD/12/10/2013+AND+AS/ID&RS=ISD/12/10/2013+AND+AS/ID*

Written by Kusum Sangma; edited by Jaya Anand.

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Illinois Tool Works Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- Illinois Tool Works, Glenview, Ill., has been assigned a patent (8,605,407) developed by four co-inventors for a "low maintenance AC gas flow driven static neutralizer and method." The co-inventors are Peter Gefter, South San Francisco, Calif., Lawrence Levit, Alamo, Calif., Leslie Partridge, San Jose, Calif., and Scott Gehlke, Saint-Satunin-les-Apt, France.

The patent application was filed on Aug. 15, 2011 (13/210,267). The full-text of the patent can be found at *http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO2&Sect2=HITOFF&p=1&u=%2Fnetahtml%2FPTO%2Fsearch-bool.html&r=43&f=G&l=50&co1=AND&d=PTXT&s1=20131210.PD.&s2=%28IL.ASST.%29&OS=ISD/12/10/2013+AND+AS/IL&RS=ISD/12/10/2013+AND+AS/IL*

Written by Kusum Sangma; edited by Jaya Anand.

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SunPower Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- SunPower, San Jose, Calif., has been assigned a patent (8,597,970) developed by three co-inventors for a method "for manufacturing high efficiency solar cells." The co-inventors are Peter J. Cousins, Menlo Park, Calif., David D. Smith, Campbell, Calif., and Seung B. Rim, Palo Alto, Calif.

The patent application was filed on Dec. 21, 2011 (13/333,904). The full-text of the patent can be found at *http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=8597970.PN.&OS=PN/8597970&RS=PN/8597970*

RENEE PRESTON

Written by Jude Bautista; edited by Marlyn Vitin.

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The Regents of the University of California Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- The Regents of the University of California, Oakland, Calif., has been assigned a patent (8,597,941) developed by four co-inventors for "[b]ioreactor for quantification of headspace VOC content from cultures." The co-inventors are Hye-Won Shin, Irvine, Calif., Brandon J. Umber, Corona Del Mar, Calif., Donald R. Blake, Irvine, Calif., and Dan M. Cooper, Irvine, Calif.

The patent application was filed on Sept. 3, 2010 (12/875,768). The full-text of the patent can be found at *http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=8597941.PN.&OS=PN/8597941&RS=PN/8597941*

Written by Jude Bautista; edited by Marlyn Vitin.

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LS9 Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- LS9, South San Francisco, Calif., has been assigned a patent 8,597,922) developed by three co-inventors for methods and compositions "for producing olefins." The co-inventors are Murtaza F. Alibhai, Austin, Texas, Mathew A. Rude, South San Francisco, Calif., and Andreas W. Schirmer, South San Francisco, Calif.

The patent application was filed on April 20, 2012 (13/452,449). The full-text of the patent can be found at *http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=8597922.PN.&OS=PN/8597922&RS=PN/8597922*

Written by Jude Bautista; edited by Marlyn Vitin.

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Northrop Grumman Systems Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- Northrop Grumman Systems, Falls Church, Va., has been assigned a patent (8,606,170) developed by Simon Ramo, Santa Monica, Calif., for a "method and apparatus for interactive, computer-based, automatically adaptable learning."

The patent application was filed on Jan. 20, 2012 (13/354,722). The full-text of the patent can be found at *http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO2&Sect2=HITOFF&p=1&u=%2Fnetahtml%2FPTO%2Fsearch-bool.html&r=12&f=G&l=50&co1=AND&d=PTXT&s1=20131210.PD.&s2=%28VA.ASST.%29&OS=ISD/12/10/2013+AND+AS/VA&RS=ISD/12/10/2013+AND+AS/VA*

Written by Kusum Sangma; edited by Jaya Anand.

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Dow AgroSciences Assigned Patent for Resistance to Auxinic Herbicides

ALEXANDRIA, Va., Dec. 12 -- Dow AgroSciences, Indianapolis, has been assigned a patent (8,603,755) developed by four co-inventors for "resistance to auxinic herbicides." The co-inventors are Terence A. Walsh, Zionsville, Ind., Glenn Hicks, Riverside, Calif., Mary Honma, San Francisco, and John P. Davies, Portland, Ore.

RENEE PRESTON

The patent application was filed on Nov. 23, 2011 (13/304,193). The full-text of the patent can be found at http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO2&Sect2=HITOFF&p=1&u=%2Fnetahtml%2FPTO%2Fsearch-bool.html&r=16&f=G&l=50&co1=AND&d=PTXT&s1=20131210.PD.&s2=%28IN.ASST.%29&OS=ISD/12/10/2013+AND+AS/IN&RS=ISD/12/10/2013+AND+AS/IN

Written by Kusum Sangma; edited by Jaya Anand.

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Battelle Memorial Institute, Advanced Image Resources Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- Battelle Memorial Institute, Columbus, Ohio, and Advanced Image Resources, Alpharetta, Ga., have been assigned a patent (8,603,714) developed by four co-inventors for a bio-based toner. The co-inventors are Bhima R. Vijayendran, Carlsbad, Calif., Jerry L. King II, Williamstown, W.Va., Jeffrey Clark, Milford, N.H., and Jan Bartus, Merrimack, N.H.

The patent application was filed on Feb. 14, 2011 (13/027,163). The full-text of the patent can be found at http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO2&Sect2=HITOFF&p=1&u=%2Fnetahtml%2FPTO%2Fsearch-bool.html&r=44&f=G&l=50&co1=AND&d=PTXT&s1=20131210.PD.&s2=%28OH.ASST.%29&OS=ISD/12/10/2013+AND+AS/OH&RS=ISD/12/10/2013+AND+AS/OH

Written by Kusum Sangma; edited by Jaya Anand.

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Danisco U.S. Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- Danisco U.S., Palo Alto, Calif., has been assigned a patent (8,597,916) developed by three co-inventors for "[m]ultimeric oxidoreductases." The co-inventors are Robert M. Caldwell, Belmont, Calif., M. Harunur Rashid, Sunnyvale, Calif., and Fernando Valle, Burlingame, Calif.

The patent application was filed on Dec. 6, 2012 (13/706,649). The full-text of the patent can be found at http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=8597916.PN.&OS=PN/8597916&RS=PN/8597916

Written by Niel Bautista; edited by Marlyn Vitin.

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Eco-Friendly Solutions Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- Eco-Friendly Solutions, San Juan Capistrano, Calif., has been assigned a patent (8,597,733) developed by Robert Scott Vonfelden, Concord, Calif., for methods "for manufacturing recyclable and repulpable packaging materials."

The patent application was filed on March 4, 2011 (13/041,310). The full-text of the patent can be found at http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=8597733.PN.&OS=PN/8597733&RS=PN/8597733

Written by Niel Bautista; edited by Marlyn Vitin.

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Spansion Assigned Patent

U.S. Patents Awarded to Inventors in California (Dec. 12)

ALEXANDRIA, Va., Dec. 12 -- Spansion, Sunnyvale, Calif., has been assigned a patent (8,598,005) developed by six co-inventors for method and manufacture "for embedded flash to achieve high quality spacers for core and high voltage devices and low temperature spacers for high performance logic devices." The co-inventors are Simon Siu-Sing Chan, Saratoga, Calif., Hidehiko Shiraiwa, San Jose, Calif., Chuan Lin, Cupertino, Calif., Lei Xue, Milpitas, Calif., Kenichi Ohtsuka, Sunnyvale, Calif., and Angela Tai Hui, Fremont, Calif.

The patent application was filed on July 18, 2011 (13/185,390). The full-text of the patent can be found at *http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=8598005.PN.&OS=PN/8598005&RS=PN/8598005*

Written by Jude Bautista; edited by Marlyn Vitin.

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Intematix Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- _Intematix, Fremont, Calif., has been assigned a patent (8,597,963) developed by Jonathan Melman, San Ramon, Calif., for a method of "manufacturing a light emitting devices."

The patent application was filed on May 19, 2010 (12/782,819). The full-text of the patent can be found at *http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=8597963.PN.&OS=PN/8597963&RS=PN/8597963*

Written by Jude Bautista; edited by Marlyn Vitin.

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Genomatica Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- Genomatica, San Diego, has been assigned a patent (8,597,918) developed by three co-inventors for a process of "separating components of a fermentation broth." The co-inventors are Warren Clark, Yale, Okla., Michael Japs, San Diego, and Mark J. Burk, San Diego.

The patent application was filed on June 3, 2010 (12/793,623). The full-text of the patent can be found at *http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=8597918.PN.&OS=PN/8597918&RS=PN/8597918*

Written by Niel Bautista; edited by Marlyn Vitin.

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Danisco U.S. Assigned Patent for Enzymatic Prevention

ALEXANDRIA, Va., Dec. 12 -- Danisco U.S., Palo Alto, Calif., has been assigned a patent (8,597,927) developed by three co-inventors for "[e]nzymatic prevention and control of biofilm." The co-inventors are Christopher C. Barnett, Granite Bay, Calif., Manoj Kumar, Fremont, Calif., and Gregory M. Whited, Belmont, Calif.

The patent application was filed on Dec. 16, 2008 (12/810,004). The full-text of the patent can be found at *http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=8597927.PN.&OS=PN/8597927&RS=PN/8597927*

Written by Jude Bautista; edited by Marlyn Vitin.

RENEE PRESTON

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Bridgelux Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- Bridgelux, Livermore, Calif., has been assigned a patent (8,597,988) developed by two co-inventors for a system "for flash-free overmolding of LED array substrates." The co-inventors are Alexander Shaikevitch, Livermore, Calif., and Vahid Moshtagh, Livermore, Calif.

The patent application was filed on Dec. 23, 2010 (12/977,596). The full-text of the patent can be found at *http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=8597988.PN.&OS=PN/8597988&RS=PN/8597988*

Written by Jude Bautista; edited by Marlyn Vitin.

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Sangamo BioSciences Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- Sangamo BioSciences, Richmond, Calif., has been assigned a patent (8,597,912) developed by two co-inventors for methods and compositions "for generation of Bak- and/or Bax-deficient cell lines using engineered nucleases." The co-inventors are Trevor Collingwood, Novato, Calif., and Philip D. Gregory, Orinda, Calif.

The patent application was filed on June 10, 2009 (12/456,043). The full-text of the patent can be found at *http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=8597912.PN.&OS=PN/8597912&RS=PN/8597912*

Written by Niel Bautista; edited by Marlyn Vitin.

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Videopression Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- Videopression, Wilmington, Del., has been assigned a patent (8,606,238) developed by Gregory T.H. Chew, Albany, Calif., Donald J. Pilcher, Half Moon Bay, Calif., and Kevin Thornton, Cupertino, Calif., for "user-initiated communications during multimedia content playback on a mobile communications device."

The patent application was filed on Feb. 8, 2012 (13/369,162). The full-text of the patent can be found at *http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO2&Sect2=HITOFF&p=1&u=%2Fnetahtml%2FPTO%2Fsearch-bool.html&r=13&f=G&l=50&co1=AND&d=PTXT&s1=20131210.PD.&s2=%28DE.ASST.%29&OS=ISD/12/10/2013+AND+AS/DE&RS=ISD/12/10/2013+AND+AS/DE*

Written by Deviprasad Jena; edited by Jaya Anand.

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Materia Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- Materia, Pasadena, Calif., has been assigned a patent (8,597,794) developed by two co-inventors for "[m]etathesis-active adhesion agents and methods for enhancing polymer adhesion to surfaces." The co-inventors are Michael A. Giardello, Pasadena, Calif., and Christopher M. Haar, Pasadena, Calif.

The patent application was filed on June 21, 2011 (13/165,515). The full-text of the patent can be found at *http://patft.uspto.gov/netacgi/nph-*

Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=8597794.PN.&OS=PN/8597794&RS=PN/8597794

Written by Niel Bautista; edited by Marlyn Vitin.

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Soraa Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- Soraa, Fremont, Calif., has been assigned a patent (8,597,967) developed by three co-inventors for a method and system "for dicing substrates containing gallium and nitrogen material ." The co-inventors are Michael R. Krames, Goleta, Calif., Tai Margalith, Pleasanton, Calif., and Rafael Aldaz, Pleasanton, Calif.

The patent application was filed on Nov. 17, 2011 (13/298,617). The full-text of the patent can be found at http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=8597967.PN.&OS=PN/8597967&RS=PN/8597967

Written by Jude Bautista; edited by Marlyn Vitin.

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Volterra Semiconductor Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- Volterra Semiconductor, Fremont, Calif., has been assigned a patent (8,598,000) developed by Marco A. Zuniga, Palo Alto, Calif., for a method of "making a transistor."

The patent application was filed on March 30, 2010 (12/750,580). The full-text of the patent can be found at http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=8598000.PN.&OS=PN/8598000&RS=PN/8598000

Written by Jude Bautista; edited by Marlyn Vitin.

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Amazon Technologies Assigned Patent for Adaptive Thresholding For Image Recognition

ALEXANDRIA, Va., Dec. 12 -- Amazon Technologies, Reno, Nev., has been assigned a patent (8,606,011) developed by four co-inventors for an "adaptive thresholding for image recognition." The co-inventors are Volodymyr V. Ivanchenko, Mountain View, Calif., Geoffrey Scott Heller, Seattle, Richard Howard Suplee, III, Bainbridge Island, Wash., and Daniel Bibireata, Seattle.

The patent application was filed on June 7, 2012 (13/491,398). The full-text of the patent can be found at http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO2&Sect2=HITOFF&p=1&u=%2Fnetahtml%2FPTO%2Fsearch-bool.html&r=17&f=G&l=50&co1=AND&d=PTXT&s1=20131210.PD.&s2=%28NV.ASST.%29&OS=ISD/12/10/2013+AND+AS/NV&RS=ISD/12/10/2013+AND+AS/NV

Written by Deviprasad Jena; edited by Jaya Anand.

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Holochip Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- Holochip, Albuquerque, N.M., has been assigned a patent (8,605,361) developed by Robert G. Batchko, Albuquerque, N.M., and Andrei Szilagyi, Danville, Calif., for "fluidic lens with reduced optical aberration."

The patent application was filed on Nov. 21, 2011 (13/301,492). The full-text of the patent can be found at *http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO2&Sect2=HITOFF&p=1&u=%2Fnetahtml%2FPTO%2Fsearch-bool.html&r=4&f=G&l=50&co1=AND&d=PTXT&s1=20131210.PD.&s2=%28NM.ASST.%29&OS=ISD/12/10/2013+AND+AS/NM&RS=ISD/12/10/2013+AND+AS/NM*

Written by Deviprasad Jena; edited by Jaya Anand.

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Blue Dolphin Solutions Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- Blue Dolphin Solutions, Las Vegas, has been assigned a patent (8,606,829) developed by four co-inventors for a "navigation of information via visual representations including nodes and contexts." The co-inventors are Eric Justin Gould, Austin, Texas, Janna Buckmaster, Austin, Texas, Todd Wilkens, San Francisco, and Paulus W. Trisnadi, Austin, Texas.

The patent application was filed on Feb. 28, 2012 (13/407,744). The full-text of the patent can be found at *http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO2&Sect2=HITOFF&p=1&u=%2Fnetahtml%2FPTO%2Fsearch-bool.html&r=12&f=G&l=50&co1=AND&d=PTXT&s1=20131210.PD.&s2=%28NV.ASST.%29&OS=ISD/12/10/2013+AND+AS/NV&RS=ISD/12/10/2013+AND+AS/NV*

Written by Deviprasad Jena; edited by Jaya Anand.

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Apple Assigned Patent for Electronic Devices Receiver Filters

ALEXANDRIA, Va., Dec. 12 -- Apple, Cupertino, Calif., has been assigned a patent (8,600,332) developed by John G. Dorsey, San Francisco, for "electronic devices having interferer aligned with receiver filters."

The patent application was filed on March 31, 2010 (12/752,058). The full text of the patent can be found at *http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=8600332.PN.&OS=PN/8600332&RS=PN/8600332*

Written by Eloisa Asedillo; edited by Vessie Ann Abalos.

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Pacific World Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- Pacific World, Aliso Viejo, Calif., has been assigned a patent (8,596,283) developed by two co-inventors for "paired artificial nails." The co-inventors are Jeanine Catherine Coppola, Irvine, Calif., and Joseph Michael Fracassi, Lake Forest, Calif.

The patent application was filed on March 9, 2012 (13/416,704). The full text of the patent can be found at *http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=8596283.PN.&OS=PN/8596283&RS=PN/8596283*

Written by Eloisa Asedillo; edited by Vessie Ann Abalos.

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Apple Assigned Patent for Over-the-Air Path Loss Calibration

ALEXANDRIA, Va., Dec. 12 -- Apple, Cupertino, Calif., has been assigned a patent (8,600,309) developed by two co-inventors for methods "for dynamic calibration of over-the-air path loss in over-the-air radio-frequency test systems." The co-inventors are Justin Gregg, San Francisco, and Thomas W. Chang, Sunnyvale, Calif.

The patent application was filed on Aug. 31, 2010 (12/872,711). The full text of the patent can be found at http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=8600309.PN.&OS=PN/8600309&RS=PN/8600309

Written by Eloisa Asedillo; edited by Vessie Ann Abalos.

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Lumenetix Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- Lumenetix, Scotts Valley, Calif., has been assigned a patent (8,596,337) developed by Matthew Weaver, Aptos, Calif., for a system and method "for active cooling utilizing a resonant shear technique."

The patent application was filed on Feb. 26, 2009 (12/393,988). The full text of the patent can be found at http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=8596337.PN.&OS=PN/8596337&RS=PN/8596337

Written by Eloisa Asedillo; edited by Vessie Ann Abalos.

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WD Media Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- WD Media, San Jose, Calif., has been assigned a patent (8,596,287) developed by two co-inventors for a "cross flow tank." The co-inventors are Chaoyuan C. Chen, San Jose, Calif., and Bing-Shiuan Chang, San Jose, Calif.

The patent application was filed on March 1, 2010 (12/715,276). The full text of the patent can be found at http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=8596287.PN.&OS=PN/8596287&RS=PN/8596287

Written by Eloisa Asedillo; edited by Vessie Ann Abalos.

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Donovan B. Yeates Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- Donovan B. Yeates, Escondido, Calif., has been assigned a patent (8,596,268) developed by Donovan B. Yeates, Escondido, Calif., for a method "for operating a compact, low flow resistance aerosol generator."

The patent application was filed on Sept. 24, 2010 (12/889,903). The full text of the patent can be found at http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=8596268.PN.&OS=PN/8596268&RS=PN/8596268

RENEE PRESTON

Written by Eloisa Asedillo; edited by Vessie Ann Abalos.

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Formula Plastics Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- Formula Plastics, Tecate, Calif., has been assigned a patent (8,596,260) developed by Elias Mora, Chula Vista, Calif., for a "solar water heater."

The patent application was filed on June 3, 2010 (12/793,562). The full text of the patent can be found at *http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=8596260.PN.&OS=PN/8596260&RS=PN/8596260*

Written by Eloisa Asedillo; edited by Vessie Ann Abalos.

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Broadcom Assigned Patent for Wireless Circuits

ALEXANDRIA, Va., Dec. 12 -- Broadcom, Irvine, Calif., has been assigned a patent (8,600,316) developed by three co-inventors for "wireless circuits with minimized port counts." The co-inventors are Louie J. Sanguinetti, Los Gatos, Calif., Ronald W. Dimpflmaier, Los Gatos, Calif., and Nicholas W. Lum, Santa Clara, Calif.

The patent application was filed on April 5, 2011 (13/080,588). The full text of the patent can be found at *http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=8600316.PN.&OS=PN/8600316&RS=PN/8600316*

Written by Eloisa Asedillo; edited by Vessie Ann Abalos.

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Hobbeezone Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- Hobbeezone, Manhattan Beach, Calif., has been assigned a patent (8,596,255) developed by Keith G. Meggs, Rancho Palos Verdes, Calif., for a "super absorbent polymer projectile launching device."

The patent application was filed on Dec. 20, 2010 (12/973,897). The full text of the patent can be found at *http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=8596255.PN.&OS=PN/8596255&RS=PN/8596255*

Written by Eloisa Asedillo; edited by Vessie Ann Abalos.

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West Corp. Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- West Corp., Omaha, Neb., has been assigned a patent (8,606,575) developed by Silke Witt-ehsani, Sunnyvale, Calif., for a "method and apparatus of providing semi-automated classifier adaptation for natural language processing."

The patent application was filed on Sept. 6, 2011 (13/225,852). The full-text of the patent can be found at *http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO2&Sect2=HITOFF&p=1&u=%2Fnetahtml%2FPTO%2Fsearch-*

bool.html&r=2&f=G&l=50&co1=AND&d=PTXT&s1=20131210.PD.&s2=%28NE.ASST.%29&OS=ISD/12/10/2013+AND+AS/NE&RS=ISD/12/10/2013+AND+AS/NE

Written by Deviprasad Jena; edited by Jaya Anand.

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Broadcom Assigned Patent for Linearization Signal Processing

ALEXANDRIA, Va., Dec. 12 -- Broadcom, Irvine, Calif., has been assigned a patent (8,600,317) developed by two co-inventors for the use of "linearization signal processing with context switching." The co-inventors are Timothy Ryan, San Francisco, and Ravichandran Ramachandran, San Jose, Calif.

The patent application was filed on March 15, 2012 (13/421,354). The full text of the patent can be found at http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=8600317.PN.&OS=PN/8600317&RS=PN/8600317

Written by Eloisa Asedillo; edited by Vessie Ann Abalos.

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Chevron USA Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- Chevron USA, San Ramon, Calif., has been assigned a patent (8,596,349) developed by two co-inventors for a "pulse fracturing device and method." The co-inventors are Joseph Henry Fram, Bakersfield, Calif., and Jean-Francis Leon, Le Bouscat, France.

The patent application was filed on July 13, 2012 (13/548,905). The full text of the patent can be found at http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=8596349.PN.&OS=PN/8596349&RS=PN/8596349

Written by Eloisa Asedillo; edited by Vessie Ann Abalos.

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Applied Materials Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- Applied Materials, Santa Clara, Calif., has been assigned a patent (8,596,336) developed by six co-inventors for an apparatus "for controlling the temperature of a substrate support." The co-inventors are Shane Nevil, Livermore, Calif., Paul Brillhart, Pleasanton, Calif., Richard Fovell, San Jose, Calif., Anisul H. Khan, Santa Clara, Calif., Jivko Dinev, Santa Clara, Calif., and Sang In Yi, Sunnyvale, Calif.

The patent application was filed on June 3, 2008 (12/132,101). The full text of the patent can be found at http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=8596336.PN.&OS=PN/8596336&RS=PN/8596336

Written by Eloisa Asedillo; edited by Vessie Ann Abalos.

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Advanced Cardiovascular Systems Assigned Patent

ALEXANDRIA, Va., Dec. 12 -- Advanced Cardiovascular Systems, Santa Clara, Calif., has been assigned a patent (8,596,215) developed by Jason Van Sciver, Los Gatos, Calif., for a "rotatable support elements for stents."

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The patent application was filed on July 18, 2011 (13/184,712). The full text of the patent can be found at http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=8596215.PN.&OS=PN/8596215&RS=PN/8596215

Written by Eloisa Asedillo; edited by Vessie Ann Abalos.

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Broadcom Assigned Patent for Configurable Front End Tranceiver

ALEXANDRIA, Va., Dec. 12 -- Broadcom, Irvine, Calif., has been assigned a patent (8,600,315) developed by two co-inventors for a method and system "for a configurable front end transceiver on a single chip that may comprise a power amplifier and a low noise amplifier." The co-inventors are Iqbal Bhatti, Rancho Palos Verdes, Calif., and Razieh Roufoogaran, Venice, Calif.

The patent application was filed on May 29, 2007 (11/754,467). The full text of the patent can be found at http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=8600315.PN.&OS=PN/8600315&RS=PN/8600315

Written by Eloisa Asedillo; edited by Vessie Ann Abalos.

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Broadcom Assigned Patent for Scanning Wireless Channels

ALEXANDRIA, Va., Dec. 12 -- Broadcom, Irvine, Calif., has been assigned a patent (8,600,314) developed by Rajesh Kumar Sinha, San Diego, for a system and method "for active scanning of wireless channels."

The patent application was filed on Oct. 12, 2011 (13/272,157). The full text of the patent can be found at http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=8600314.PN.&OS=PN/8600314&RS=PN/8600314

Written by Eloisa Asedillo; edited by Vessie Ann Abalos.

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Apple Assigned Patent for Simultaneous Sensitivity Testing

ALEXANDRIA, Va., Dec. 12 -- Apple, Cupertino, Calif., has been assigned a patent (8,600,311) developed by three co-inventors for a "simultaneous sensitivity testing for multiple devices in radio-frequency test systems." The co-inventors are Justin Gregg, San Francisco, Vishwanath Venkataraman, San Francisco, and Adil Syed, Santa Clara, Calif.

The patent application was filed on March 10, 2011 (13/044,857). The full text of the patent can be found at http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=8600311.PN.&OS=PN/8600311&RS=PN/8600311

Written by Eloisa Asedillo; edited by Vessie Ann Abalos.

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Qualcomm Assigned Patent for Spectral Sensing

U.S. Patents Awarded to Inventors in California (Dec. 12)

ALEXANDRIA, Va., Dec. 12 -- Qualcomm, San Diego, has been assigned a patent (8,600,312) developed by three co-inventors for a method and apparatus "for spe

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Load-Date: December 12, 2013

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US Patent Issued to Biomarin Pharmaceutical on Dec. 3 for "Variants of C-type natriuretic peptide" (California Inventors)

US Fed News

December 3, 2013 Tuesday 11:28 PM EST

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Length: 242 words

Dateline: ALEXANDRIA, Va.

Body

ALEXANDRIA, Va., Dec. 3 -- United States Patent no. ***8,598,121***, issued on Dec. 3, was assigned to Biomarin Pharmaceutical Inc. (Novato, Calif.).

"Variants of C-type natriuretic peptide" was invented by Daniel J. Wendt (Walnut Creek, Calif.), Shinong Long (Milpitas, Calif.), Sianna Castillo (San Francisco), Christopher P. Price (Kentfield, Calif.), Mika Aoyagi-Scharber (Mill Valley, Calif.), Michel Claude Vellard (San Rafael, Calif.) and Augustus O. Okhamafe (Concord, Calif.). According to the abstract* released by the U.S. Patent & Trademark Office: "The present disclosure provides variants of C-type natriuretic peptide (CNP), pharmaceutical compositions comprising CNP variants, and methods of making CNP variants. The CNP variants are useful as therapeutic agents for the treatment of diseases responsive to CNP, including but not limited to bone-related disorders, such as skeletal dysplasias (e.g., achondroplasia), and vascular smooth muscle disorders (e.g., restenosis and arteriosclerosis)." The patent was filed on May 8, 2012, under Application No. 13/466,672. *For further information, including images, charts and tables, please visit: *http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO2&Sect2=HITOFF&p=1&u=%2Fnetahtml%2FPTO%2Fsearch-bool.html&r=1&f=G&l=50&co1=AND&d=PTXT&s1=8598121&OS=8598121&RS=8598121* For any query with respect to this article or any other content requirement, please contact Editor at *htsyndication@hindustantimes.com*

Load-Date: December 3, 2013

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Biomarin Pharmaceutical Inc Granted United States Patent for Variants of C-Type Natriuretic Peptide

Global IP News. Pharmaceutical Patent News

December 4, 2013 Wednesday

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Length: 221 words

Body

Publication Name: Pharmaceutical Patent News Patent Application Number: 13/466,672 Patent Publication Number: ***8,598,121*** International Patent Classification Codes: A61K 38/22 (20060101), A61K 38/00 (20060101), A61K 38/16 (20060101), C12P 21/04 (20060101) Patent Status: Granted

Alexandria, Dec. 4 -- Biomarin Pharmaceutical Inc has been granted a patent (***8,598,121***) for variants of C-type natriuretic peptide. This invention was developed by Wendt Daniel J., Long Shinong, Castillo Sianna, Price Christopher P., Aoyagi-Scharber Mika, Vellard Michel Claude and Okhamafe Augustus O. The patent application number is 13/466,672. The International Patent Classification codes are A61K 38/22 (20060101), A61K 38/00 (20060101), A61K 38/16 (20060101) and C12P 21/04 (20060101).

According to the abstract released by the U.S. Patent and Trademark Office: "The present disclosure provides variants of C-type natriuretic peptide (CNP), pharmaceutical compositions comprising CNP variants, and methods of making CNP variants. The CNP variants are useful as therapeutic agents for the treatment of diseases responsive to CNP, including but not limited to bone-related disorders, such as skeletal dysplasias (e.g., achondroplasia), and vascular smooth muscle disorders (e.g., restenosis and arteriosclerosis)."

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Load-Date: January 26, 2015

End of Document

RENEE PRESTON

US Patent granted to Biomarin Pharmaceutical Inc (California) on December 03 titled as "Variants of C-type natriuretic peptide"

US Official News

December 3, 2013 Tuesday

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Length: 213 words

Dateline: ALEXANDRIA, Va

Body

United States Patent and Trademark Office has granted patent no. ***8,598,121***, on December 03, 2013, to Biomarin Pharmaceutical Inc (California), titled as "Variants of C-type natriuretic peptide"

Inventors: Wendt; Daniel J. (Walnut Creek, CA), Long; Shinong (Milpitas, CA), Castillo; Sianna (San Francisco, CA), Price; Christopher P. (Kentfield, CA), Aoyagi-Scharber; Mika (Mill Valley, CA), Vellard; Michel Claude (San Rafael, CA), Okhamafe; Augustus O. (Concord, CA)

Assignee: Biomarin Pharmaceutical Inc. (Novato, CA)

According to the abstract released by the U.S. Patent & Trademark Office: "The present disclosure provides variants of C-type natriuretic peptide (CNP), pharmaceutical compositions comprising CNP variants, and methods of making CNP variants. The CNP variants are useful as therapeutic agents for the treatment of diseases responsive to CNP, including but not limited to bone-related disorders, such as skeletal dysplasias (e.g., achondroplasia), and vascular smooth muscle disorders (e.g., restenosis and arteriosclerosis)."

The patent was filed on May 8, 2012 Application no. 13/466,672

For more information please visit: http://www.uspto.gov/. In case of any query regarding this article or other content needs please contact: editorial@plusmediasolutions.com

Load-Date: December 4, 2013

End of Document

RENEE PRESTON

US Patent granted to Biomarin Pharmaceutical Inc (California) on December 03 titled as "Variants of C-type natriuretic peptide"

Plus Patent News

December 3, 2013 Tuesday

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Length: 213 words

Dateline: ALEXANDRIA, Va

Body

United States Patent and Trademark Office has granted patent no. ***8,598,121***, on December 03, 2013, to Biomarin Pharmaceutical Inc (California), titled as "Variants of C-type natriuretic peptide"

Inventors: Wendt; Daniel J. (Walnut Creek, CA), Long; Shinong (Milpitas, CA), Castillo; Sianna (San Francisco, CA), Price; Christopher P. (Kentfield, CA), Aoyagi-Scharber; Mika (Mill Valley, CA), Vellard; Michel Claude (San Rafael, CA), Okhamafe; Augustus O. (Concord, CA)

Assignee: Biomarin Pharmaceutical Inc. (Novato, CA)

According to the abstract released by the U.S. Patent & Trademark Office: "The present disclosure provides variants of C-type natriuretic peptide (CNP), pharmaceutical compositions comprising CNP variants, and methods of making CNP variants. The CNP variants are useful as therapeutic agents for the treatment of diseases responsive to CNP, including but not limited to bone-related disorders, such as skeletal dysplasias (e.g., achondroplasia), and vascular smooth muscle disorders (e.g., restenosis and arteriosclerosis)."

The patent was filed on May 8, 2012 Application no. 13/466,672

For more information please visit: http://www.uspto.gov/. In case of any query regarding this article or other content needs please contact: editorial@plusmediasolutions.com

Load-Date: December 4, 2013

End of Document

RENEE PRESTON

Tabla / Mercados Financieros

El Norte (Mexico)

March 4, 2010 Thursday

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Section: NEGOCIOS; Pág. 2; Edic. 72; Nº26018

Length: 1079 words

Body

DÓLAR 48 HORAS

(Pesos por dólar / venta)

3 Mar 12.7070

MONEDAS

EN MÉXICO (Cotización de venta)

	Cierre	Var. %
Dólar menudeo peso/dólar	12.8000	-0.030
dólar/peso	0.0781	0.030
Dólar Fix peso/dólar	12.6900	-0.036
dólar/peso	0.0788	0.036
Dólar 48 hrs. peso/dólar	12.7070	-0.036
dólar/peso	0.0786	0.036
EURO peso/euro	17.5528	0.076
euro/peso	0.0570	-0.076

EN EL MUNDO (Divisas por dólar, cotización de venta)

	Cierre	Var. %
Euro/dólar	0.729	-0.005
Dólar/euro	1.371	0.005
Libra esterlina	0.662	-0.006
Yen japonés	88.370	-0.400
Peso argentino	3.848	-0.002
Real brasileño	1.780	-0.008
Peso chileno	516.450	-3.680

EL PESO EN CHICAGO $/DLS

(Cotización del futuro al cierre)

	Cierre	Anterior
Abril 10	12.7551	12.8000
Junio 10	12.8493	12.8949
Julio 10	12.8908	12.9366

ENERGÉTICOS

	Cierre	Anterior
BRENT (Dls. por barril)	78.83	77.38
WTI (Dls. por barril)	80.87	79.68
MEZCLA (Dls. por barril)	72.68	71.67

RENEE PRESTON

Tabla / Mercados Financieros

HENRY HUB 4.77 4.78

METALES

	Cierre Anterior
Centenario (pesos)	17,800 17,600
Oro Onza Troy (dlls)	1,137 1,135
Plata Oz Libertad (pesos)	245 230
Aluminio Londres (dlls)	2,210 2,168
Cobre NY (dlls/lb)	3.42 3.40
Zinc Alto Grado (dls/ton)	2,197 2,197

FONDOS DE DEUDA

Nombre	Administradora	Último Hecho	Variación (%) Diaria	Variación (%) Mensual	Variación (%) Anual
HZPZONCC0-A	IMFBANAMEX	33.73	0.03	2.21	17.60
HZESC0-B	IMFBANAMEX	2.73	0.00	1.69	15.11
ING-30EBOE1	IIMMEXICO	2.35	0.00	1.10	10.80
BMERLPGB	BMERGEST	13.83	0.00	0.91	10.79
IXELPBS	IXEFONDOS	1.48	0.00	1.56	9.85
VALMX17B0FI	OVALMEX	1.74	-0.00	0.28	8.98
IXELPBF3	IXEFONDOS	1.45	0.00	1.47	8.70
VALMX16B0NC	OVALMEX	35.06	0.01	0.64	8.29
VALMX17B2FI	OVALMEX	1.46	-0.00	0.21	8.04
SCOTIACT	SCOTIAF	20.69	0.00	0.47	7.75
APOLO10E	APOLO	1.69	0.00	0.59	7.67
ING-GOBBOE1	IIMMEXICO	1.62	0.00	0.43	7.61
VALMX16B1NC	OVALMEX	33.89	0.01	0.57	7.47
APOLO10B-6	APOLO	1.68	0.00	0.59	7.47
SCOTIA2C1	SCOTIAF	61.97	0.01	0.46	6.45
MONEXM+BEM-1	MONEX-OPER	25.96	0.00	0.35	5.41
ICAGUBB-E2	INVERCAP	1.12	0.00	0.35	5.12
VALMX14B1CF	OVALMEX	16.45	0.00	0.00	4.94
ACTIEURE	ACTINVER	131.74	0.45	-4.39	-5.97
ACTIEURM-2	ACTINVER	127.78	0.43	-4.43	-6.96
IXEEUROBI	IXEFONDOS	1.04	0.00	-4.45	-10.89
ACTIPLUE	ACTINVER	1.04	0.00	-1.13	-10.98
HZEUROB0-D	IMFBANAMEX	12.32	0.05	-4.53	-11.18

Los de mayor rendimiento en los últimos 12 meses con activos superiores a 300 millones de pesos

FONDOS DE RENTA VARIABLE

Nombre	Administradora	Último Hecho	Variación (%) Diaria	Variación (%) Mensual	Variación (%) Anual
VALUEV5B	VALUEOP	14.36	-0.07	-1.53	158.54
NTE+SELC	OPBANORTE	1.85	0.02	3.17	118.29
NTE+SELB	OPBANORTE	1.61	0.02	2.83	109.66
ACTICREFF	ACTINVER	19.63	0.17	3.30	95.80
ING-PATB1	IIMMEXICO	8.48	0.07	3.72	95.46
SCOTIA7C1	SCOTIAF	26.67	0.24	3.67	92.25
ACTIPATFF	ACTINVER	8.13	0.10	3.27	91.74
HZPATC0-C	IMFBANAMEX	3.46	0.04	2.41	91.35
APOLOIPB7	APOLO	1.23	0.01	3.60	90.79
GBMAAAB	GBMOPE	2.32	0.02	1.83	90.21
VALMX20B0	OVALMEX	7.80	0.07	3.63	89.97
ST&ER-IB1	GESANTAMEX	32.01	0.30	3.61	89.81
ING-IPCB	IIMMEXICO	5.53	0.05	3.55	89.72
NTE+INE	OPBANORTE	23.10	0.21	3.66	88.81
ACTIVARB	ACTINVER	61.16	0.55	3.47	88.56
IXECONBS	IXEFONDOS	0.15	0.00	3.97	87.75
VECTCRF	VECTOR	1.43	0.01	2.41	85.91
ACTOTALB	ACTINVER	165.93	1.78	1.61	85.46
GBMBRAB	GBMOPE	1.47	0.02	4.30	85.26

NTE+INC OPBANORTE 22.22 0.00 0.00 75.34
MIURAB SINTER 1.13 0.01 -0.05 73.52
HSBCBOLBFF OPEHSBC 28.70 0.26 3.61 63.20
S-ASIAVB OPSCHRODER 1.06 0.01 -1.84 50.91

Los de mayor rendimiento en los últimos 12 meses con activos superiores a 100 millones de pesos

LA BOLSA EN MÉXICO (Puntos del IPC)

3 Mar 32,353.54
Variación día 0.93% 2
Variación mensual 3.41% 2
Importe (millones de pesos) 11,634
Volumen (millones de acciones) 423

ACCIONES DEL IPC (*) LAS REGIAS

Instrumento Valor Var. Unitaria Var. % Volumen Total Var. Anual Máx. 12 meses Mín 12 meses
ALFAA (*) 86.47 -0.59 -0.68 1,511 380.66 89.80 15.68
AMXL 29.19 0.59 2.06 45,993 56.10 32.28 15.00
ARA* 8.54 0.00 0.00 3,259 140.56 9.76 3.30
AUTLANB (*) 28.42 -0.10 -0.35 248 43.53 33.80 17.55
AXTELCPO (*) 10.37 0.22 2.17 5,143 111.20 13.60 4.40
BIMBOA 97.79 1.05 1.09 568 127.10 99.00 45.06
BOLSAA 19.50 0.92 4.95 2,920 111.96 19.75 7.52
CEMEXCPO (*) 12.85 0.44 3.55 46,536 68.95 20.00 3.85
COMERCIUBC 11.05 -0.01 -0.09 453 323.37 12.00 2.60
COMPARTO 69.60 -1.17 -1.65 1,002 189.76 73.50 24.01
ELEKTRA* 672.53 -4.82 -0.71 68 121.65 749.90 293.00
FEMSAUBD (*) 56.96 0.32 0.56 4,805 78.22 64.48 30.27
GAPB 45.18 1.56 3.58 2,876 87.78 45.40 21.57
GCARSOA1 45.53 0.25 0.55 712 67.08 49.90 27.00
GEOB 38.69 0.48 1.26 4,851 210.26 41.59 12.13
GFINBURO 44.82 0.97 2.21 859 59.45 51.68 28.25
GFNORTEO (*) 49.34 0.83 1.71 7,394 249.19 51.00 13.04
GMEXICOB 31.92 0.57 1.82 19,393 278.42 32.80 8.22
GMODELOC 69.09 1.29 1.90 1,883 76.93 74.30 38.20
HOMEX* 58.37 0.25 0.43 1,011 88.84 95.50 25.90
ICA* 30.77 0.62 2.06 7,923 53.16 35.50 18.70
ICHB 42.58 0.40 0.95 464 40.95 49.99 26.00
KIMBERA 68.39 0.86 1.27 447 50.70 68.50 44.25
MEXCHEM* 31.18 -0.08 -0.26 2,153 252.76 31.90 7.95
OMAB (*) 20.99 1.27 6.44 1,388 59.74 23.25 12.20
PEÑOLES* 264.82 5.01 1.93 185 100.80 316.01 133.00
SIMECB 32.34 -0.11 -0.34 820 68.17 38.64 19.18
SORIANAB (*) 35.65 -0.37 -1.03 1,868 82.82 36.90 19.00
TELECOMA1 59.34 1.55 2.68 845 52.70 64.50 34.30

TELINTL 11.66 0.07 0.60 *8,598 121*.25 12.15 4.98
TELMEXL 9.90 -0.01 -0.10 9,400 -2.46 12.90 9.50
TLEVISACPO 49.19 0.18 0.37 6,580 38.72 57.00 33.60
TVAZTCACPO 6.92 0.03 0.44 1,503 52.42 7.44 4.17
URBI* 29.53 -0.05 -0.17 1,700 152.39 32.50 10.61
WALMEXV 64.16 -0.83 -1.28 9,896 123.71 65.27 26.00

MÁS REGIAS

ARCA* 42.17 0.09 0.21 3,811 73.90 44.20 24.32
BEVIDESB 10.50 0.00 0.00 0 -2.78 10.80 7.49
CYDSASAA 14.00 -0.01 -0.07 40 91.78 15.00 7.30
GFAMSAA 24.98 0.51 2.08 1,029 482.42 31.75 4.24
GRUMAB 25.60 -0.40 -1.54 1,273 479.19 29.30 3.65
LAMOSA* 13.00 0.00 0.00 0 33.06 13.50 6.80
PASAB 14.30 0.00 0.00 0 98.61 15.79 6.02

VALUEGFO 15.00 0.00 0.00 0 55.76 15.00 9.63
VITROA 7.92 1.02 14.78 299 95.07 8.34 3.80

MERCADOS EN EL MUNDO

Indicador	Cierre	Var. %
Dow Jones	10,396.76	-0.09
FTSE 100	5,533.21	0.90
Merval	2,291.32	0.24
Nikkei	10,253.14	0.31
Dax 30	5,817.88	0.72
CAC 40	3,842.52	0.80
S&P 500	1,118.79	0.04
Nasdaq	2,280.68	0.00

TASAS

EN MÉXICO

	Último	Anterior
Cetes 1 día	4.52	4.55
Cetes 28 Días*	4.49	4.49
Cetes 91 Días*	4.66	4.66
Papel Bancario 1 día	4.52	4.53
Papel Bancario 28 días	4.58	4.63
Papel Bancario 91 días	4.69	4.70
TIIE 28 días	4.94	4.92
Bono a 10 años	7.55	7.56
UDI	4.413429	4.412810

EN EL MUNDO

	Último	Anterior
T-bill 1 mes EU	0.09	0.08
T-bill 3 meses EU	0.14	0.13
Bono 10 años EU	3.61	3.60
Tasa de descuento (FED)	0.75	0.50
Prime rate	3.25	4.00
Libor 1 mes	0.23	0.23
Libor 3 meses	0.25	0.25
Euribor 1 mes	0.41	0.42
Euribor 3 meses	0.66	0.66

Fuente: Eficiencia Informativa

Load-Date: March 4, 2010

End of Document

Tabla / Mercados Financieros

Reforma (Mexico)

March 4, 2010 Thursday

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Section: NEGOCIOS; Pág. 2; Edic. 17; Nº5916

Length: 1045 words

Body

DÓLAR 48 HORAS

(Pesos por dólar / venta)

3 Mar 12.7070

MONEDAS

EN MÉXICO (Cotización de venta)

	Cierre	Var. %
Dólar menudeo peso/dólar	12.8000	-0.030
dólar/peso	0.0781	0.030
Dólar Fix peso/dólar	12.6900	-0.036
dólar/peso	0.0788	0.036
Dólar 48 hrs. peso/dólar	12.7070	-0.036
dólar/peso	0.0786	0.036
EURO peso/euro	17.5528	0.076
euro/peso	0.0570	-0.076

EN EL MUNDO (Divisas por dólar, cotización de venta)

	Cierre	Var. %
Euro/dólar	0.729	-0.005
Dólar/euro	1.371	0.005
Libra esterlina	0.662	-0.006
Yen japonés	88.370	-0.400
Peso argentino	3.848	-0.002
Real brasileño	1.780	-0.008
Peso chileno	516.450	-3.680

EL PESO EN CHICAGO $/DLS

(Cotización del futuro al cierre)

	Cierre	Anterior
Abril 10	12.7551	12.8000
Junio 10	12.8493	12.8949
Julio 10	12.8908	12.9366

ENERGÉTICOS

	Cierre	Anterior
BRENT (Dls. por barril)	78.83	77.38
WTI (Dls. por barril)	80.87	79.68
MEZCLA (Dls. por barril)	72.68	71.67

RENEE PRESTON

HENRY HUB 4.77 4.78

METALES

	Cierre Anterior
Centenario (pesos)	17,800 17,600
Oro Onza Troy (dlls)	1,137 1,135
Plata Oz Libertad (pesos)	245 230
Aluminio Londres (dlls)	2,210 2,168
Cobre NY (dlls/lb)	3.42 3.40
Zinc Alto Grado (dls/ton)	2,197 2,197

FONDOS DE DEUDA

Nombre	Administradora	Último Hecho	Variación (%) Diaria	Variación (%) Mensual	Variación (%) Anual
HZPZONCC0-A	IMFBANAMEX	33.73	0.03	2.21	17.60
HZESC0-B	IMFBANAMEX	2.73	0.00	1.69	15.11
ING-30EBOE1	IIMMEXICO	2.35	0.00	1.10	10.80
BMERLPGB	BMERGEST	13.83	0.00	0.91	10.79
IXELPBS	IXEFONDOS	1.48	0.00	1.56	9.85
VALMX17B0FI	OVALMEX	1.74	-0.00	0.28	8.98
IXELPBF3	IXEFONDOS	1.45	0.00	1.47	8.70
VALMX16B0NC	OVALMEX	35.06	0.01	0.64	8.29
VALMX17B2FI	OVALMEX	1.46	-0.00	0.21	8.04
SCOTIACT	SCOTIAF	20.69	0.00	0.47	7.75
APOLO10E	APOLO	1.69	0.00	0.59	7.67
ING-GOBBOE1	IIMMEXICO	1.62	0.00	0.43	7.61
VALMX16B1NC	OVALMEX	33.89	0.01	0.57	7.47
APOLO10B-6	APOLO	1.68	0.00	0.59	7.47
SCOTIA2C1	SCOTIAF	61.97	0.01	0.46	6.45
MONEXM+BEM-1	MONEX-OPER	25.96	0.00	0.35	5.41
ICAGUBB-E2	INVERCAP	1.12	0.00	0.35	5.12
VALMX14B1CF	OVALMEX	16.45	0.00	0.00	4.94
ACTIEURE	ACTINVER	131.74	0.45	-4.39	-5.97
ACTIEURM-2	ACTINVER	127.78	0.43	-4.43	-6.96
IXEEUROBI	IXEFONDOS	1.04	0.00	-4.45	-10.89
ACTIPLUE	ACTINVER	1.04	0.00	-1.13	-10.98
HZEUROB0-D	IMFBANAMEX	12.32	0.05	-4.53	-11.18

Los de mayor rendimiento en los últimos 12 meses con activos superiores a 300 millones de pesos

FONDOS DE RENTA VARIABLE

Nombre	Administradora	Último Hecho	Variación (%) Diaria	Variación (%) Mensual	Variación (%) Anual
VALUEV5B	VALUEOP	14.36	-0.07	-1.53	158.54
NTE+SELC	OPBANORTE	1.85	0.02	3.17	118.29
NTE+SELB	OPBANORTE	1.61	0.02	2.83	109.66
ACTICREFF	ACTINVER	19.63	0.17	3.30	95.80
ING-PATB1	IIMMEXICO	8.48	0.07	3.72	95.46
SCOTIA7C1	SCOTIAF	26.67	0.24	3.67	92.25
ACTIPATFF	ACTINVER	8.13	0.10	3.27	91.74
HZPATC0-C	IMFBANAMEX	3.46	0.04	2.41	91.35
APOLOIPB7	APOLO	1.23	0.01	3.60	90.79
GBMAAAB	GBMOPE	2.32	0.02	1.83	90.21
VALMX20B0	OVALMEX	7.80	0.07	3.63	89.97
ST&ER-IB1	GESANTAMEX	32.01	0.30	3.61	89.81
ING-IPCB	IIMMEXICO	5.53	0.05	3.55	89.72
NTE+INE	OPBANORTE	23.10	0.21	3.66	88.81
ACTIVARB	ACTINVER	61.16	0.55	3.47	88.56
IXECONBS	IXEFONDOS	0.15	0.00	3.97	87.75
VECTCRF	VECTOR	1.43	0.01	2.41	85.91
ACTOTALB	ACTINVER	165.93	1.78	1.61	85.46
GBMBRAB	GBMOPE	1.47	0.02	4.30	85.26

NTE+INC OPBANORTE 22.22 0.00 0.00 75.34
MIURAB SINTER 1.13 0.01 -0.05 73.52
HSBCBOLBFF OPEHSBC 28.70 0.26 3.61 63.20
S-ASIAVB OPSCHRODER 1.06 0.01 -1.84 50.91

Los de mayor rendimiento en los últimos 12 meses con activos superiores a 100 millones de pesos

LA BOLSA EN MÉXICO (Puntos del IPC)

3 Mar 32,353.54
Variación día 0.93% 2
Variación mensual 3.41% 2
Importe (millones de pesos) 11,634
Volumen (millones de acciones) 423

LAS MÁS NEGOCIADAS

Acción Cierre Var.%
CEMEXCPO 12.85 3.55
AMXL 29.19 2.06
ICA* 30.77 2.06
GMEXICOB 31.92 1.82
GFNORTEO 49.34 1.71
GEOB 38.69 1.26
FEMSAUBD 56.96 0.56
TLEVISACPO 49.19 0.37
HERDEZ* 17.50 -0.06
WALMEXV 64.16 -1.28

ACCIONES DEL IPC

Instrumento Valor Var. Unitaria Var. % Volumen Total Var. Anual P. Máx. (últ. 12 meses) P. Mín (últ. 12 meses)
ALFAA 86.47 -0.59 -0.68 1,511 380.66 89.80 15.68
AMXL 29.19 0.59 2.06 45,993 56.10 32.28 15.00
ARA* 8.54 0.00 0.00 3,259 140.56 9.76 3.30
ASURB 66.61 -1.49 -2.19 226 59.74 75.11 36.11
AUTLANB 28.42 -0.10 -0.35 248 43.53 33.80 17.55
AXTELCPO 10.37 0.22 2.17 5,143 111.20 13.60 4.40
BIMBOA 97.79 1.05 1.09 568 127.10 99.00 45.06
BOLSAA 19.50 0.92 4.95 2,920 111.96 19.75 7.52
CEMEXCPO 12.85 0.44 3.55 46,536 68.95 20.00 3.85
COMERCIUBC 11.05 -0.01 -0.09 453 323.37 12.00 2.60
COMPARTO 69.60 -1.17 -1.65 1,002 189.76 73.50 24.01
ELEKTRA* 672.53 -4.82 -0.71 68 121.65 749.90 293.00
FEMSAUBD 56.96 0.32 0.56 4,805 78.22 64.48 30.27
GAPB 45.18 1.56 3.58 2,876 87.78 45.40 21.57
GCARSOA1 45.53 0.25 0.55 712 67.08 49.90 27.00
GEOB 38.69 0.48 1.26 4,851 210.26 41.59 12.13
GFAMSAA 24.98 0.51 2.08 1,029 482.42 31.75 4.24
GFINBURO 44.82 0.97 2.21 859 59.45 51.68 28.25
GFNORTEO 49.34 0.83 1.71 7,394 249.19 51.00 13.04
GMEXICOB 31.92 0.57 1.82 19,393 278.42 32.80 8.22
GMODELOC 69.09 1.29 1.90 1,883 76.93 74.30 38.20
GRUMAB 25.60 -0.40 -1.54 1,273 479.19 29.30 3.65
HOMEX* 58.37 0.25 0.43 1,011 88.84 95.50 25.90
ICA* 30.77 0.62 2.06 7,923 53.16 35.50 18.70
KIMBERA 68.39 0.86 1.27 447 50.70 68.50 44.25
MEXCHEM* 31.18 -0.08 -0.26 2,153 252.76 31.90 7.95
PEÑOLES* 264.82 5.01 1.93 185 100.80 316.01 133.00
SORIANAB 35.65 -0.37 -1.03 1,868 82.82 36.90 19.00
TELECOMA1 59.34 1.55 2.68 845 52.70 64.50 34.30

TELINTL 11.66 0.07 0.60 ***8,598 121***.25 12.15 4.98

Tabla / Mercados Financieros

TELMEXL 9.90 -0.01 -0.10 9,400 -2.46 12.90 9.50
TLEVISACPO 49.19 0.18 0.37 6,580 38.72 57.00 33.60
TVAZTCACPO 6.92 0.03 0.44 1,503 52.42 7.44 4.17
URBI* 29.53 -0.05 -0.17 1,700 152.39 32.50 10.61
WALMEXV 64.16 -0.83 -1.28 9,896 123.71 65.27 26.00

MERCADOS EN EL MUNDO

Indicador	Cierre	Var. %
Dow Jones	10,396.76	-0.09
FTSE 100	5,533.21	0.90
Merval	2,291.32	0.24
Nikkei	10,253.14	0.31
Dax 30	5,817.88	0.72
CAC 40	3,842.52	0.80
S&P 500	1,118.79	0.04
Nasdaq	2,280.68	0.00

TASAS

EN MÉXICO

	Último	Anterior
Cetes 1 día	4.52	4.55
Cetes 28 Días*	4.49	4.49
Cetes 91 Días*	4.66	4.66
Papel Bancario 1 día	4.52	4.53
Papel Bancario 28 días	4.58	4.63
Papel Bancario 91 días	4.69	4.70
TIIE 28 días	4.94	4.92
Bono a 10 años	7.55	7.56
UDI	4.413429	4.412810

EN EL MUNDO

	Último	Anterior
T-bill 1 mes EU	0.09	0.08
T-bill 3 meses EU	0.14	0.13
Bono 10 años EU	3.61	3.60
Tasa de descuento (FED)	0.75	0.50
Prime rate	3.25	4.00
Libor 1 mes	0.23	0.23
Libor 3 meses	0.25	0.25
Euribor 1 mes	0.41	0.42
Euribor 3 meses	0.66	0.66

Fuente: Eficiencia Informativa

Load-Date: March 4, 2010

End of Document

Tabla / Mercados Financieros

Mural (Mexico)

March 4, 2010 Thursday

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Section: NEGOCIOS; Pág. 2; Edic. 12; Nº4113

Length: 1040 words

Body

DÓLAR 48 HORAS

(Pesos por dólar / venta)

Mar. 3 12.7070

MONEDAS

EN MÉXICO

(Cotización de venta)

	Cierre	Var %
Dólar menudeo peso/dólar	12.8000	-0.030
dólar/peso	0.0781	0.030
Dólar Fix peso/dólar	12.6900	-0.036
dólar/peso	0.0788	0.036
Dólar 48 hrs. peso/dólar	12.7070	-0.036
dólar/peso	0.0786	0.036
EURO peso/euro	17.5528	0.076
euro/peso	0.0570	-0.076

EN EL MUNDO

(Divisas por dólar, cotización de venta)

	Cierre	Var %
Euro/dólar	0.729	-0.005
Dólar/euro	1.371	0.005
Libra esterlina	0.662	-0.006
Yen japonés	88.370	-0.400
Peso argentino	3.848	-0.002
Real brasileño	1.780	-0.008
Peso chileno	516.450	-3.680

EL PESO EN CHICAGO $/DLS

(Cotización del futuro al cierre)

	Cierre	Anterior
Abril 10	12.7551	12.8000
Junio 10	12.8493	12.8949
Julio 10	12.8908	12.9366

ENERGÉTICOS

RENEE PRESTON

Tabla / Mercados Financieros

	Cierre	Anterior
BRENT (Dls. por barril)	78.83	77.38
WTI (Dls. por barril)	80.87	79.68
MEZCLA (Dls. por barril)	72.68	71.67
HENRY HUB	4.77	4.78

METALES

	Cierre	Anterior
Centenario (pesos)	17,800	17,600
Oro Onza Troy (dlls)	1,137	1,135
Plata Oz Libertad (pesos)	245	230
Aluminio Londres (dlls)	2,210	2,168
Cobre NY (dlls/lb)	3.42	3.40
Zinc Alto Grado (dls/ton)	2,197	2,197

FONDOS DE DEUDA

Nombre	Administradora	Último Hecho	Variación (%) Diaria	Variación (%) Mensual	Variación (%) Anual
HZPZONCC0-A	IMFBANAMEX	33.73	0.03	2.21	17.60
HZESC0-B	IMFBANAMEX	2.73	0.00	1.69	15.11
ING-30EBOE1	IIMMEXICO	2.35	0.00	1.10	10.80
BMERLPGB	BMERGEST	13.83	0.00	0.91	10.79
IXELPBS	IXEFONDOS	1.48	0.00	1.56	9.85
VALMX17B0FI	OVALMEX	1.74	-0.00	0.28	8.98
IXELPBF3	IXEFONDOS	1.45	0.00	1.47	8.70
VALMX16B0NC	OVALMEX	35.06	0.01	0.64	8.29
VALMX17B2FI	OVALMEX	1.46	-0.00	0.21	8.04
SCOTIACT	SCOTIAF	20.69	0.00	0.47	7.75
APOLO10E	APOLO	1.69	0.00	0.59	7.67
ING-GOBBOE1	IIMMEXICO	1.62	0.00	0.43	7.61
VALMX16B1NC	OVALMEX	33.89	0.01	0.57	7.47
APOLO10B-6	APOLO	1.68	0.00	0.59	7.47
SCOTIA2C1	SCOTIAF	61.97	0.01	0.46	6.45
MONEXM+BEM-1	MONEX-OPER	25.96	0.00	0.35	5.41
ICAGUBB-E2	INVERCAP	1.12	0.00	0.35	5.12
VALMX14B1CF	OVALMEX	16.45	0.00	0.00	4.94
ACTIEURE	ACTINVER	131.74	0.45	-4.39	-5.97
ACTIEURM-2	ACTINVER	127.78	0.43	-4.43	-6.96
IXEEUROBI	IXEFONDOS	1.04	0.00	-4.45	-10.89
ACTIPLUE	ACTINVER	1.04	0.00	-1.13	-10.98
HZEUROB0-D	IMFBANAMEX	12.32	0.05	-4.53	-11.18

Los de mayor rendimiento en los últimos 12 meses con activos superiores a 300 millones de pesos

FONDOS DE RENTA VARIABLE

Nombre	Administradora	Último Hecho	Variación (%) Diaria	Variación (%) Mensual	Variación (%) Anual
VALUEV5B	VALUEOP	14.36	-0.07	-1.53	158.54
NTE+SELC	OPBANORTE	1.85	0.02	3.17	118.29
NTE+SELB	OPBANORTE	1.61	0.02	2.83	109.66
ACTICREFF	ACTINVER	19.63	0.17	3.30	95.80
ING-PATB1	IIMMEXICO	8.48	0.07	3.72	95.46
SCOTIA7C1	SCOTIAF	26.67	0.24	3.67	92.25
ACTIPATFF	ACTINVER	8.13	0.10	3.27	91.74
HZPATC0-C	IMFBANAMEX	3.46	0.04	2.41	91.35
APOLOIPB7	APOLO	1.23	0.01	3.60	90.79
GBMAAAB	GBMOPE	2.32	0.02	1.83	90.21
VALMX20B0	OVALMEX	7.80	0.07	3.63	89.97
ST&ER-IB1	GESANTAMEX	32.01	0.30	3.61	89.81
ING-IPCB	IIMMEXICO	5.53	0.05	3.55	89.72
NTE+INE	OPBANORTE	23.10	0.21	3.66	88.81
ACTIVARB	ACTINVER	61.16	0.55	3.47	88.56

IXECONBS IXEFONDOS 0.15 0.00 3.97 87.75
VECTCRF VECTOR 1.43 0.01 2.41 85.91
ACTOTALB ACTINVER 165.93 1.78 1.61 85.46
GBMBRAB GBMOPE 1.47 0.02 4.30 85.26
NTE+INC OPBANORTE 22.22 0.00 0.00 75.34
MIURAB SINTER 1.13 0.01 -0.05 73.52
HSBCBOLBFF OPHSBC 28.70 0.26 3.61 63.20
S-ASIAVB OPSCHRODER 1.06 0.01 -1.84 50.91

Los de mayor rendimiento en los últimos 12 meses con activos superiores a 100 millones de pesos

LA BOLSA EN MÉXICO

(Puntos del IPC)

Mar. 3 32,353.54
Variación día 0.93%
Variación mensual 3.41%
Importe (millones de pesos) 11,634
Volumen (millones de acciones) 423

LAS MÁS NEGOCIADAS

Acción Cierre Var.%
CEMEXCPO 12.85 3.55
AMXL 29.19 2.06
ICA* 30.77 2.06
GMEXICOB 31.92 1.82
GFNORTEO 49.34 1.71
GEOB 38.69 1.26
FEMSAUBD 56.96 0.56
TLEVISACPO 49.19 0.37
HERDEZ* 17.50 -0.06
WALMEXV 64.16 -1.28

ACCIONES DEL IPC

Instrumento Valor Var. Unitaria Var. % Volumen Total Var. Anual Máx. 12 meses Mín. 12 meses
ALFAA 86.47 -0.59 -0.68 1,511 380.66 89.80 15.68
AMXL 29.19 0.59 2.06 45,993 56.10 32.28 15.00
ARA* 8.54 0.00 0.00 3,259 140.56 9.76 3.30
ASURB 66.61 -1.49 -2.19 226 59.74 75.11 36.11
AUTLANB 28.42 -0.10 -0.35 248 43.53 33.80 17.55
AXTELCPO 10.37 0.22 2.17 5,143 111.20 13.60 4.40
BIMBOA 97.79 1.05 1.09 568 127.10 99.00 45.06
BOLSAA 19.50 0.92 4.95 2,920 111.96 19.75 7.52
CEMEXCPO 12.85 0.44 3.55 46,536 68.95 20.00 3.85
COMERCIUBC 11.05 -0.01 -0.09 453 323.37 12.00 2.60
COMPARTO 69.60 -1.17 -1.65 1,002 189.76 73.50 24.01
ELEKTRA* 672.53 -4.82 -0.71 68 121.65 749.90 293.00
FEMSAUBD 56.96 0.32 0.56 4,805 78.22 64.48 30.27
GAPB 45.18 1.56 3.58 2,876 87.78 45.40 21.57
GCARSOA1 45.53 0.25 0.55 712 67.08 49.90 27.00
GEOB 38.69 0.48 1.26 4,851 210.26 41.59 12.13
GFAMSAA 24.98 0.51 2.08 1,029 482.42 31.75 4.24
GFINBURO 44.82 0.97 2.21 859 59.45 51.68 28.25
GFNORTEO 49.34 0.83 1.71 7,394 249.19 51.00 13.04
GMEXICOB 31.92 0.57 1.82 19,393 278.42 32.80 8.22
GMODELOC 69.09 1.29 1.90 1,883 76.93 74.30 38.20
GRUMAB 25.60 -0.40 -1.54 1,273 479.19 29.30 3.65
HOMEX* 58.37 0.25 0.43 1,011 88.84 95.50 25.90
ICA* 30.77 0.62 2.06 7,923 53.16 35.50 18.70
KIMBERA 68.39 0.86 1.27 447 50.70 68.50 44.25

Tabla / Mercados Financieros

MEXCHEM* 31.18 -0.08 -0.26 2,153 252.76 31.90 7.95
PEÑOLES* 264.82 5.01 1.93 185 100.80 316.01 133.00
SORIANAB 35.65 -0.37 -1.03 1,868 82.82 36.90 19.00
TELECOMA1 59.34 1.55 2.68 845 52.70 64.50 34.30

TELINTL 11.66 0.07 0.60 ***8,598 121***.25 12.15 4.98
TELMEXL 9.90 -0.01 -0.10 9,400 -2.46 12.90 9.50
TLEVISACPO 49.19 0.18 0.37 6,580 38.72 57.00 33.60
TVAZTCACPO 6.92 0.03 0.44 1,503 52.42 7.44 4.17
URBI* 29.53 -0.05 -0.17 1,700 152.39 32.50 10.61
WALMEXV 64.16 -0.83 -1.28 9,896 123.71 65.27 26.00

MERCADOS EN EL MUNDO

Indicador	Cierre	Var.%
Dow Jones	10,396.76	-0.09
FTSE 100	5,533.21	0.90
Merval	2,291.32	0.24
Nikkei	10,253.14	0.31
Dax 30	5,817.88	0.72
CAC 40	3,842.52	0.80
S&P 500	1,118.79	0.04
Nasdaq	2,280.68	0.00

TASAS

EN MÉXICO

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TIIE 28 días	4.94	4.92
Bono a 10 años	7.55	7.56
UDI	4.413429	4.412810

EN EL MUNDO

	Último	Anterior
T-bill 1 mes EU	0.09	0.08
T-bill 3 meses EU	0.14	0.13
Bono 10 años EU	3.61	3.60
Tasa de descuento (FED)	0.75	0.50
Prime rate	3.25	4.00
Libor 1 mes	0.23	0.23
Libor 3 meses	0.25	0.25
Euribor 1 mes	0.41	0.42
Euribor 3 meses	0.66	0.66

Fuente: Eficiencia Informativa

Load-Date: March 4, 2010

End of Document

InVision Technologies Reports Strong Second-quarter Results; Company Earns $0.52 Per Share, Revenues Double From First Quarter

Business Wire

July 23, 2002, Tuesday

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Length: 2163 words

Dateline: NEWARK, Calif., July 23, 2002

Body

InVision Technologies, Inc. (Nasdaq:INVN) today reported results for the second quarter ended June 30, 2002. For the quarter, InVision reported total revenues of $68.4 million compared to revenues of $17.4 million a year ago and $33.2 million in the first quarter of 2002. Net income was $9.6 million, or $0.52 cents per diluted share, compared to$144,000, or $0.01 per diluted share, for the second quarter of 2001, and compared to $2.8 million, or $0.17 per diluted share, for the first quarter of 2002.

Gross margin in the second quarter was 42% of revenues compared to 31% in the same period last year, and 40% in the first quarter of 2002.

For the second quarter, operating expenses were $11.4 million, or 17% of revenues, compared with $5.0 million, or 29% of revenues, in the second quarter of 2001. In the first quarter of 2002, InVision's operating expenses were $8.7 million, or 26% of revenues.

For the first six months of fiscal 2002, InVision reported revenues of $101.6 million compared with revenues of $34.9 million in the first six months of fiscal 2001. The company reported net income of $12.4 million, or $0.73 per diluted share, for the first six months of fiscal 2002, versus $233,000, or $0.02 per diluted share, in the first six months of fiscal 2001.

"For the quarter, our product revenues were approximately one-third greater than product revenues for the entire year 2001. At the same time, effective leveraging of existing infrastructure produced historically strong gross margin and operating expense performance," said Sergio Magistri, Ph.D., InVision's president and chief executive officer. "We are very pleased with our performance for the second quarter, and believe that it demonstrates our ability to rapidly grow our business, while at the same time delivering very strong financial performance."

As of June 30, 2002, total company backlog was $375.1 million, of which $366.6 million was for explosives detection systems (EDS) products and services. Of the EDS backlog, 77% was from the U.S. Transportation Security Administration (TSA), with the remainder primarily from international customers. Since then, InVision announced additional orders from the TSA for $106.8 million in completed systems and parts.

"This most recent order includes the TSA's first sizeable order for CTX 9000 DSi units. This is significant for InVision because it reflects the position of most of the larger airports in the U.S. to move to faster, automated EDS solutions integrated into the baggage handling systems of airports," said Dr. Magistri. "These integrated solutions provide superior throughput and customer convenience. However, it can take more than a year to plan, design and construct integrated baggage handling systems. As a result, we expect to see demand in the U.S. for the CTX 9000 DSi to continue well beyond this year."

Current highlights

RENEE PRESTON

-- Since March, the TSA has ordered 458 complete systems across InVision's full product line, and has funded the company to purchase parts kits for an additional 142 systems;

-- InVision continues to see solid indications of robust international demand. During the second quarter, $43.9 million in orders were booked from customers in France, Italy, Canada, and Israel, as well as the first order from a U.S. airline for installation abroad;

-- During the second quarter, InVision's manufacturing subcontractor CoorsTek began production of CTX 2500 EDS in its own facility, and has prepared for volume production of both 2500 CTX and 5500 CTX models beginning in the third quarter;

-- Total headcount related to EDS products and service went to 425 from 328 during the quarter, an increase of 30%;

-- InVision opened a field service office in France to serve European customers. This effort is part of a comprehensive plan to strengthen the service organization worldwide. During the second quarter, InVision added 18% to its service organization headcount;

-- In May, InVision received a $1 million grant from the TSA to develop an upgrade to the CTX(TM) family of explosives detection systems utilizing quadrupole resonance technology. The company's Quantum Magnetics subsidiary will do the research on this effort to reduce operator alert rates and improve overall performance on existing systems; and

-- Andrew Siegel joined the management team as the Vice President of Business Development, a new position.

Looking forward

Dr. Magistri said, "We expect third-quarter revenues of at least$110 million and EPS of at least $1.00 per share. For the fourth quarter, InVision will continue to deliver on existing orders, and will build to new orders, as our customers require.

"In June, the TSA hired a team led by Boeing Co. and Siemens Corp. to oversee the installation of the systems. InVision has begun a close working relationship with the Boeing/Siemens team. The current national and international focus on aviation security highlights the contribution InVision can make to safer airline travel, and we look forward to continuing our work," said Dr. Magistri.

InVision Technologies, Inc. will webcast a presentation regarding these results and InVision's outlook for the future over the Internet at 1:45 pm (PDT) today. To listen, please log on to www.inVision-tech.com, and follow the link that will be posted on the front page. A replay of the webcast will be available shortly after the presentation and will remain available through midnight (EDT) on July 30, 2002.

InVision Technologies develops, manufactures, markets and supports explosives detection systems based on advanced Computed Tomography (CT) technology for civil aviation security. InVision's wholly owned subsidiary Quantum Magnetics develops weapons, narcotics, explosives and other detection systems based on quadrupole resonance and other proprietary magnetic sensing technologies. InVision's wholly owned subsidiary Inovec develops, manufactures, markets and supports scanning, optimization and control systems for the forest products industry. Additional information about InVision can be obtained on the company's web site at http://www.invision-tech.com.

This news release contains forward-looking statements regarding InVision's projected third-quarter financial results and InVision's expectations that U.S. demand for the CTX 9000 DSi EDS will continue well beyond this year. Investors are cautioned to consider the important risk factors that could cause actual results to differ materially from those in the forward-looking statements in this news release. These risk factors include: the risk that InVision may not be able to obtain in a timely manner components necessary to build the number of EDS units it expects to build

in the third quarter; the risk that the TSA may cancel or decrease the orders that it has already placed; the risk that InVision may not be able to sustain or increase its manufacturing capacity due to unanticipated staffing or other problems; the risk that InVision's manufacturing subcontractors cannot perform as anticipated; the risk that U.S. airports will not implement plans to build automated baggage handling systems incorporating InVision's CTX 9000 DSi EDS; and other risks set forth under the caption "Business Risks" in InVision's most recent Form 10-Q filed with the Securities and Exchange Commission.

Note to Editors: CTX, CTX 2500, CTX 5500 DS and CTX 9000 DSi are trademarks of InVision Technologies, Inc. QScan and LiquiScan are registered trademarks of Quantum Magnetics, Inc. and i-Portal is a trademark of Quantum Magnetics, Inc.

InVision Technologies, Inc.
Consolidated Statements of Operations
(In thousands, except per share data)
(Unaudited)

	Three Months Ended		Six Months Ended	
	June 30, 2002	July 1, 2001	June 30, 2002	July 1, 2001
Revenues:				
Product revenue	$ 62,001	$ 10,587	$ 88,096	$ 21,808
Service revenue	3,423	2,847	6,889	5,404
Government contract revenue	3,002	3,966	6,639	7,685
Total revenues	68,426	17,400	101,624	34,897
Cost of revenues:				
Product costs	35,007	6,941	49,924	13,356
Service costs	2,994	1,866	5,308	3,699
Government contract costs	1,989	3,256	4,532	6,077
Total cost of revenues	39,990	12,063	59,764	23,132
Gross profit	28,436	5,337	41,860	11,765
Operating expenses:				
Research and development	4,718	1,907	8,030	4,757
Selling, general and administrative	6,655	3,141	12,034	6,611
Total operating expenses	11,373	5,048	20,064	11,368
Income from operations	17,063	289	21,796	397
Interest expense	(116)	(83)	(210)	(148)
Interest and other (expenses)/income, net	(694)	--	(564)	105

Income before provision for income taxes	16,253	206	21,022	354
Provision for income taxes	6,647	62	8,598	121
Net income	$ 9,606	$ 144	$ 12,424	$ 233
Net income per share:				
Basic	$ 0.58	$ 0.01	$ 0.82	$ 0.02
Diluted	$ 0.52	$ 0.01	$ 0.73	$ 0.02
Weighted average shares outstanding:				
Basic	16,579	12,932	15,132	12,784
Diluted	18,387	13,835	17,124	13,789

InVision Technologies, Inc.
Consolidated Balance Sheets
(In thousands, except share data)

	June 30, 2002 (Unaudited)	December 31, 2001
Assets		
Current assets:		
Cash and cash equivalents	$ 187,010	$ 11,386
Short-term investments	--	1,992
Accounts receivable, net	54,200	27,239
Inventories	65,472	27,104
Deferred income taxes	4,975	4,082
Other current assets	15,484	5,464
Total current assets	327,141	77,267
Property and equipment, net	6,236	5,713
Deferred income taxes	1,289	2,237
Intangible assets, net	3,859	4,011
Other assets	154	505
Total assets	$ 338,679	$ 89,733
Liabilities and stockholders' equity		
Current liabilities:		
Accounts payable	$ 31,512	$ 8,375
Accrued liabilities	23,965	12,822
Deferred revenue	116,037	4,377
Short term debt	1,364	1,880
Current maturities of long-term obligations	184	179

Total current liabilities	173,062	27,633
Long-term obligations	670	680
Commitments and contingencies		
Stockholders' equity:		
Preferred stock, no par value, 5,000,000 shares authorized; no shares issued and outstanding	--	--
Common stock, $0.001 par value, 60,000,000 and 20,000,000 shares authorized; 16,876,000 and 13,730,000 shares issued; 16,675,000 and 13,539,000 shares outstanding	17	14
Additional paid-in capital	159,548	66,011
Deferred stock compensation expense	(474)	--
Accumulated other comprehensive loss	(1,911)	--
Retained earnings (accumulated deficit)	8,966	(3,458)
Treasury stock, at cost (201,000 and 191,000 shares)	(1,199)	(1,147)
Total stockholders' equity	164,947	61,420
Total liabilities and stockholders' equity	$ 338,679	$ 89,733

CONTACT: InVision Technologies, Inc.

Jordan Goldstein, 510/739-2448

URL: http://www.businesswire.com

Load-Date: July 24, 2002

End of Document

OPAP AKIMA-18
MAR 0 3 2018
PATENT & TRADEMARK OFFICE

3-5-18

OK

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No.: 15/646,822

Filed: 07-11-2017

CON REI of 14/604,262 filed 1-23-2015 REI of 13/466,672 filed 5-8-2012, now US 8,598,121, CON of 12/784,117 filed 5-20-2010, which claims benefit of 61/254,563 filed 10-23-2009 and 61/180,112, filed 5-20-2009)

Title: Variants of C-Type Natriuretic Peptide

Applicant: BioMarin Pharmaceutical Inc.

Confirmation No.: 7727

Art Unit: 1629

Examiner: Not yet assigned

CON REISSUE APPLICATION UNDERGOING PREEXAM PROCESSING

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450
Attention Director Art Unit 3991

Protest under 37 CFR 1.291

Ascendis Pharma A/S, of Tuborg Boulevard 5, 2900 Hellerup, Denmark, as real party in interest, submits the following protest against the above-captioned application under 37 CFR 1.291. This is the first protest submitted by Ascendis Pharma in this application. A protest can be submitted any time up to allowance in a reissue case so the protest is timely filed.

All claims of the present reissue application should be rejected under 35 USC 251 for attempting to correct an uncorrectable error, that is, a failure to file a divisional application on nonelected subject matter following a restriction requirement in the underlying patent being reissued (US 8,598,121). Claims 21 and 22 should also be rejected for lack of written description under 35 USC 112, first paragraph.

Listing of documents and brief statement of relevance

This protest relies on the following documents (Exhibits A-I), for which copies are provided.

A. Claims pending in 13/466,672, filed May 8, 2012, now US 8,598,121 at the date of issuance of a restriction requirement: This document is relevant to establish the claims and sequences subject to a restriction requirement in the original prosecution.

B. Restriction requirement dated February 13, 2013 from 13/466,672: This document defines the groups and species of the restriction requirement.

C. Response to restriction requirement dated April 12, 2013 from 13/466,672: This document identifies which group and sequence were elected (Group I and SEQ ID NO:185).

D. Interview summary dated July 30, 2013 from 13/466,672: This document discloses a modification of the restriction requirement in which certain groups and sequences (SEQ ID NOS. 179, 180 and 190) were rejoined, and all other non-elected sequences were cancelled.

E. Granted claims from 13/466,672: This document shows that the granted claims were consistent with the interview summary.

F. Claims now pending in the present application: This document shows that claims directed in part to non-elected SEQ ID NOS. from 13/466,672 are now being pursued.

G. *Ex part Joachim Werres,* Appeal 2006-2966 (BPAI 2007), *Ex parte Morrison et al.,* Appeal 2012-12266 (PTAB 2013), affirmed (2014-1050 Fed. Cir. 2014), Ex *Parte Solomon,* Appeal 2012-2100 (BPAI 2012), affirmed (2012-1689 Fed. Cir. 2013) and *Ex parte Wu et al.,* Appeal 2016-0348 (PTAB 2017) are exemplary Board decisions sustaining rejections of claims presented in a reissue application under 35 USC 251 due to failure to file a divisional application on non-elected subject matter.

H. PTO Briefs from Federal Circuit appeals in *Ex parte Morrison* and *Ex parte Solomon* from Exhibit G. These briefs provide a useful summary of the *Orita, Weiler, Doyle* line

of cases limiting pursuit of non-elected claims in a divisional application.

I. Tsuberry et al., J. Biol. Chem. 279, 38118-38124 (2004)) is an exemplary reference showing that linkage of peptide drugs to PEG via a hydrolysable linkage was an art-known technique for improving half-life without compromising efficacy.

Analysis

MPEP 1412.01 provides as follows:

> **I. FAILURE TO TIMELY FILE A DIVISIONAL APPLICATION PRIOR TO ISSUANCE OF ORIGINAL PATENT**
>
> Where a restriction (or an election of species) requirement was made in an application and applicant permitted the elected invention to issue as a patent without filing a divisional application on the non-elected invention(s), the non-elected invention(s) cannot be recovered by filing a reissue application. A reissue applicant's failure to timely file a divisional application covering the non-elected invention(s) in response to a restriction (or an election of species) requirement is not considered to be error causing a patent granted on the elected claims to be partially inoperative by reason of claiming less than the applicant had a right to claim. Accordingly, this is not correctable by reissue of the original patent under 35 U.S.C. 251. *In re Watkinson,* 900 F.2d 230, 14 USPQ2d 1407 (Fed. Cir. 1990); *In re Orita,* 550 F.2d 1277, 1280, 193 USPQ 145, 148 (CCPA 1977); See also *In re Mead,* 581 F.2d 251, 198 USPQ 412 (CCPA 1978). In this situation, the reissue claims should be rejected under 35 U.S.C. 251 for lack of defect in the original patent and lack of error in obtaining the original patent. Compare with *In re Doyle,* 293 F.3d 1355, 63 USPQ2d 1161 (Fed. Cir. 2002) where the court permitted the patentee to file a reissue application to present a so-called linking claim, a claim broad enough to read on or link the invention elected (and patented) together with the invention not elected. The non-elected invention(s) were inadvertently not filed as a divisional application.

The rationale for this rule is that the error does not lie in the patent sought to be reissued in which the divisional claims could never have been pursued but in the failure to file a divisional application.

Patentees claimed exactly what they had a right to claim in the

> patent, no more nor less, and appellants' failure to timely re-file does not change this fact.
>
> *In re Doyle*, 293 F3d. 1355 (Fed. Cir. 2002)

Here, a restriction requirement in the underlying patent being reissued US 8,598,121 required the following elections of groups and species.

I. Claims 1-4, 30, 45 and 46, drawn to a variant of C-type natriuretic peptide (CNP), classified in class 530, subclass 300.

II. Claim 7, drawn to a method of treating a bone-related disorder or skeletal dysplasia, classified in class 514, subclass 12.4.

III. Claim 9, drawn to a method of treating a vascular smooth muscle disorder, classified in class 514, subclass 12.4.

IV. Claims 10, 16, 18, 22-24, 31, 35, drawn to a method for recombinant production of a CNP variant and a host cell used in said method, classified in class 435, subclass 69.1/325.

V. Claims 39 and 41, drawn to a method of treating a CNP-responsive condition or disorder, classified in class 514, subclass 12.4.

VI. Claim 44, drawn to a method of increasing long bone growth, classified in class 514, subclass 12.4.

Species Election #1

This application contains claims directed to the following patentably distinct species: sequences as disclosed in claims 1, 9, 10, 30, 31, 39, and 46. The species are independent or distinct because each sequence is a distinct separate polypeptide. In addition, these species are not obvious variants of each other based on the current record.

Applicant is required under 35 U.S.C. 121 to elect a single disclosed species, or a single grouping of patentably indistinct species, for prosecution on the merits to which the claims shall be restricted if no generic claim is finally held to be allowable. Currently, no claim is generic.

The applicant elected group I and SEQ ID NO:185, a C-type natriuretic peptide of 53 amino acids (CNP53) for continued prosecution.

Subsequently, the Examiner indicated that the case would be allowed subject to rejoinder of certain sequences based on CNP53 (i.e., SEQ ID NO:179, 190 and 180), and rejoinder of method claims recited in independent claims 7, 10 and 44, corresponding to Groups II, IV and VI (likewise limited to SEQ ID NOS. 185, 179, 190 and 180) with cancellation of non-elected sequences (see interview summary of July 30, 2013 reproduced below).

> *Examiner indicated SEQ ID NO:185, the elected species is free of the prior art. Examiner indicated will search other sequences based on CNP53, SEQ ID NOs:179,190 and 180. These have been found free of the art. Examiner will rejoin and examine appropriate method claims:7, 10 and dependents and 44. Atty Neville authorized cancellation of non-elected sequences and claims to non-elected sequences. .*

Consistent with this interview summary, US 8,598,121 issued with claims to a CNP variant peptide, a pharmaceutical composition comprising the variant, a method of treating a bone related disorder or skeletal dysplasia with such a variant and a method of increasing long bone growth with such a variant, with in all claims the CNP variant being limited to a peptide having the sequence of SEQ ID NO:185, 179, 190 or 180. Analogous claims in which the peptide included the sequence of numerous other non-elected SEQ ID NOS. were cancelled and not pursued by way of a divisional application.

Instead of filing claims to non-elected sequences in divisional applications of US 8,598,121, such claims have been reintroduced in the present reissue application. The independent claims currently pending are directed to a large Markush group of sequences, which includes both the elected SEQ ID NOS:185, 179, 190 and 180 from the underlying patent as well as numerous other SEQ ID NOS., many of which were present at the time of the original restriction requirement in the parent patent and were subsequently cancelled. For example, at least SEQ ID NOS. 36, 75, 144, 146-179, 183-185, 188-190 and 193 of the present Markush group were present at the time of the restriction requirement in the original prosecution.

The inclusion of both elected and non-elected sequences in the claims does not

circumvent the prohibition on pursuing non-elected subject matter under the *In re Doyle* exception referred to in MPEP 1412.01. In *In re Doyle*, a patent issued on an elected species claim and a reissue was filed with a linking claim generic to both the elected species and a non-elected species. Such a claim was never presented in the original prosecution, but the court found that it could and should have been and failure to present it was an error correctable by reissue.

But the present situation is distinct from *In re Doyle*. In contrast to the situation in *Doyle* when a linking claim was never presented in the original prosecution, claims of the type now presented (i.e., listing both elected and nonelected sequences) were presented in the original prosecution, and amended to cancel nonelected species as a condition of allowability. Thus, in contrast to the situation in *Doyle*, the applicant here acquiesced in the restriction requirement by pursuing claims in the original prosecution of the same type as are now being presented in a reissue and amending the claims to remove nonelected subject matter.

A similar issue was presented in *Ex parte Joachim Werres,* Appeal 2006-2966 (BPAI 2007). The applicant in that case limited the claims to an elected species in the original prosecution and then tried to pursue broader claim including both the elected species and nonelected species recited in the alternative in a reissue. The applicant argued that error arose in allowing the original patent to issue without a generic claim. But the Board found that the applicant "deliberately agreed to an Examiner's amendment that limited or narrowed generic claim 17 to the elected species only" (Decision at p. 8). Such is precisely what occurred in the present case. The Board in *Werres* explained its decision as follows:

> A correctable 251 error does not include these deliberate decisions by Appellant whereby the '177 patent was allowed to issue with selected species claims only (i.e., without a generic claim). *Orita,* 550 F.2d at 1280, 193 USPQ at 148. These deliberate choices do not comprise error correctable by reissue regardless of the propriety of the election requirement or claim amendment made by the Examiner. *Watkinson,* 900 F.2d at 231-232, 14 USPQ2d at 1408-09. There is a distinction between a 251 error and a deliberate choice subsequently found to be disadvantageous. *Serenkin,* 479 F.3d at 1364, 81 USPQ2d at 2015.

Werres at p. 8.

The present case is further distinguished from *Doyle* in that the purportedly linking claim presented merely lists elected and many unelected species in the alternative rather than presenting a true generic claim. It is inconceivable that the applicant will be allowed to continue to pursue the claims in their current form directed to a vast number of distinct sequences. Either as a result of a further restriction requirement or rejection due to an improper Markush group, the present claims will almost certainly have to be amended to be directed to a single sequence or group of patentably indistinct sequences (as they were in the original prosecution). In such case, the claims will presumably be directed to entirely unelected subject matter, leaving no similarity in the present situation to that in *In re Doyle*.

That a few of the CNP sequences recited in the present claims were not claimed at the time of the restriction requirement in the original prosecution should not change the analysis. The applicant was required to elect a single *disclosed* sequence of CNP variant, so the election applied to all disclosed sequences of CNP variants in the application. If the applicant had included any additional sequences to the many already present in the claims of the parent case, they would have been treated in exactly the same way. That is, the applicant would have been required to cancel them to obtain allowance of the elected sequences (SEQ ID NOS. 179, 180, 185 and 190). Applicants could no more have obtained allowance of unelected sequences never present in the claims than unelected sequences that were present in the claims and cancelled in the original prosecution. Attempting to pursue such claims does not therefore represent correcting an error in claim scope of the original patent but an error in failure to file a divisional application to obtain a further patent on a distinct invention as delineated in a restriction requirement.

That there are differences in the form of the claims now pending from those present in the original prosecution also does not avoid the prohibition on pursuing non-elected subject matter of MPEP 1412.01. Specifically whereas original claim 1 was directed to a variant of a CNP peptide of specified sequence, present claim 15 and dependents are directed to a macromolecule in which such a peptide is linked through a hydrolysable linker to a polymer.

Likewise whereas original claim 2 was directed to a pharmaceutical composition comprising a CNP variant of claim 1 and a pharmaceutical acceptable, excipient, carrier or diluent, present claim 18 and dependents are directed to a sustained release composition in which a polymer is coupled to a CNP variant by a hydrolysable linker. Similar distinctions are featured in present method claims (independent claims 23 and 27 and their dependents) compared with corresponding claim 7 from the original prosecution.

The additional recitation that CNP peptides are linked to a polymer via a hydrolysable linker does not confer any patentable distinction over corresponding claims present in the original prosecution. It has been known for many years that linkage of peptides to PEG via a hydrolysable linkage provides a means to extend half-life without compromising activity (see, e.g., Tsuberry et al., J. Biol. Chem. 279, 38118-38124 (2004)). Characterizing a CNP peptide linked to a polymer as a macromolecule is merely a difference in form without any limiting effect. The recitation of "sustained release" in the preamble of claim 18 does not appear to be of limiting effect beyond the linkage of a CNP peptide to a polymer by a hydrolysable linkage recited in the body of the claim, which itself provides for sustained release. But even if the preamble is considered further limiting of the claim, a sustained release formulation is a well-known type of pharmaceutical composition and not patentably distinct from the pharmaceutical composition of claim 2 in the original prosecution. Claims of corresponding scope to those now present could have been presented in dependent form within the claim groupings of the original restriction requirement (e.g., The CNP variant of claim 1 linked to a polymer by a hydrolysable linkage forming a macromolecule, or the pharmaceutical composition of claim 2, which is a sustained-release composition). Thus, the additional recitation of linkage via a hydrolysable linker to a polymer or of a sustained release formulation do not change the analysis or escape the prohibition against pursuing non-elected subject matter.

The issue of claims differing in form but without patentable distinction between the original prosecution and reissue was considered by *Ex parte Morrison et al.,* Appeal 2012-12266 (BPAI 2013). An original non-elected group of claims was directed to a method of providing a

tow assembly, and the newly presented claim was directed to a tow assembly per se. The Board found there were no substantial differences between the claims even though directed to distinct statutory categories. Because the claims were "simply not patentably distinct" the Board sustained the rejection under 35 USC 251.

The applicant may allege that present claims 21 and 22 have no corresponding claims in the original prosecution. Claim 21 is directed to a method of stimulating cGMP production in a cell expressing natriuretic peptide receptor B (NPR-B) comprising contacting the cell expressing NPR-B with a CNP variant. Claim 22 is directed to a method of overcoming cell growth arrest induces by a constitutively active mutant fibroblast growth factor receptor 3 (FGFR3) comprising contacting a cell expressing the constitutively active FGFR-3 with a CNP variant. Although claims 21 and 22 may appear to be directed to methods performed on isolated cells patentably distinct from the treatment methods pursued in the original prosecution, under a broadest reasonable interpretation, the cells contacted in claims 21 and 22 can be construed as being in a patient with the contacting occurring in the method of treatment. Claims 21 and 22 can thus be construed as methods of treatment encompassing either claim 7 or claim 9 from the original prosecution with the functional results of stimulating cGMP production in a cell expressing natriuretic peptide receptor B (NPR-B) or overcoming cell growth arrest induced by a constitutively active mutant fibroblast growth factor receptor 3 (FGFR3) occurring as an inherent result of treatment and of no further limiting effect. Thus, claims 21 and 22 are not necessarily patentably distinct from claims 7 and 9 in the original prosecution.

But even if claims 21 and 22 were considered patentably distinct from claims pending in the original prosecution, they would have been treated in the same way as other method claims that were present in the original prosecution. That is, they would have been placed in a restriction group separate from the elected group of CNP variant peptides, subject to rejoinder on allowance of the elected group if amended to define the CNP variant peptide in the same manner as the elected group. In other words, if such claims were present in the original prosecution, they would have either have to have been amended to be limited to the elected CNP variants or cancelled (the choice presented for the method claims that were present).

These claims could not have been pursued to allowance with non-elected sequence in the original prosecution. Thus, insofar as claims 21 and 22 are directed to non-elected species, their absence from the original patent does not represent any error in the original prosecution, but rather an uncorrectable failure to file a divisional application on nonelected subject matter.

Several cases have applied the prohibition against pursuing non-elected claims in a reissue to claims that were not pursued in the original prosecution but would have been subject to restriction had they been pursued. In *in re Weiler,* 790 F2d 1576 (Fed. Cir. 1986), the applicant accepted issuance of an original patent with claims to a single elected invention a method of quantitative analysis of limonin, which was one of three inventions delineated in a restriction requirement. Weiler then filed a reissue application claiming inter alia a method of developing citrus fruit strains low in limonin or antibodies against limonin. The court considered these claims patentably distinct from any of the groups in the original prosecution. The court sustained the rejection of these claims as follows:

> By acquiescing in the examiner's restriction requirement and failing to file divisional applications on the subject matter of non-elected claims, Weiler foreclosed (because that was not error) his right to claim that subject matter. If it was not error to forego divisional applications on subject matter to which claims had been made in the original prosecution, it cannot on the present record have been error to forego divisional applications on subject matter which claims have never been made.

The Board has also addressed several cases in which it was arguable whether newly presented claims in a reissue case would have been placed in a non-elected group from the original prosecution or in a new non-elected group and found this of no consequence to the lack of correctable error under 35 USC 251. For example, *Ex Parte Solomon,* Appeal 2012-2100 (BPAI 2012) considered a claim directed to a method of treating a beta-amyloid disease when the original prosecution contained a non-elected group III of claims directed to treating a protein aggregation disease. The Board found that the claims presented in the reissue if presented in the original prosecution would have fallen either in non-elected group III or a new group, and sustained the rejection. The Board explained:

> Consonant with *Orita*, should Appellant "prevail, the copendency requirement would become meaningless, for should an applicant fail to file a divisional application while maintain copendency as required by section 120, he could simply revert to section 251 in order to cure his mistake." *Orita,* 550 F.2d at 1281. Here, permitting appellant to correct the failure to file divisional applications by reissue would defeat the copendency requirement of 35 U.S.C. 120, the result found undesirable by the CCPA in *Orita.*
>
> *Ex parte Solomon* at pp. 10-11.

Likewise in *Ex parte Wu et al.,* Appeal 2016-0348 (PTAB 2017), the Board considered a reissue case including method claims that if presented in the original prosecution would have been grouped with either unelected Group II also directed to methods or a new group distinct from the elected Group I. The Board found that "Appellants are not entitled to obtain method claims per se, regardless of scope, because they conceded by their acquiescence in the restriction requirement that they were not entitled to claim method claims in the original application." Here likewise, the applicant conceded by its acquiescence in the restriction requirement in the original prosecution that it was not entitled to claim non-elected sequences as CNP peptides, compositions or methods of use and should not be allowed to pursue such in a reissue either.

A useful summary of case law discussed in this protest is provided by the PTO's briefs in the Federal Circuit appeals of *Ex parte Morrison* and *Ex parte Solomon*. In short:

Orita, *Weiler*, and *Doyle* hold that when an applicant makes a deliberate choice in response to a restriction requirement to prosecute a single invention and fails to file divisional application(s) to the remaining, nonelected inventions—whether originally claimed or not—error under § 251 does not exist. These cases are based on the rationale that once an applicant acquiesces to a restriction requirement, any invention distinct from the one elected and prosecuted to allowance can only be pursued in a timely-filed divisional application.

PTO Brief in *Ex parte Solomon* (2012-1689 Fed. Cir. 2012) at p. 18

This reissue case is in its essence an attempt to pursue non-elected subject matter not fitting the narrow exception of *in re Doyle*. As indicated in MPEP 1412.01, a reissue applicant's failure to timely file a divisional application covering the non-elected invention(s) in response to a restriction (or an election of species) requirement is not considered to be an error correctable by reissue of the original patent under 35 U.S.C. 251. In this situation, the reissue claims should be rejected under 35 U.S.C. 251 for lack of defect in the original patent and lack of error in obtaining the original patent.

Claims 21 and 22 should also be rejected under 35 USC 112, first paragraph for lack of written description in the original patent specification. While there is no *in haec verba* requirement, newly added claim limitations must be supported in the specification through express, implicit, or inherent disclosure. If a representative number of adequately described species are not disclosed for a genus, the claim to that genus must be rejected as lacking adequate written description (MPEP 2163). Here, the original patent does not describe claims 21 and 22 *in haec verba* but, if at all, only by exemplification. The exemplification is in the

context of methods of screening CNP peptide in cell cultures in vitro to check the CNP peptides have the desired activity (see col. 51, lines 45-66, Example 5 and Example 7). There is no exemplification or other description of stimulating cGMP production in a cell expressing natriuretic peptide receptor B (NPR-B) comprising contacting the cell expressing NPR-B or overcoming cell growth arrest induced by a constitutively active mutant fibroblast growth factor receptor 3 (FGFR3) in the context of a treatment method. Because of the lack of *in haec verba* description and exemplification of only one species, the specification fails to provide written description throughout the genus encompassed by these claims under a broadest reasonable interpretation. The claims should therefore be rejected for lack of written description.

Please charge any fee occasioned by this protest to deposit account 16-0605.

Respectfully submitted

/Joe Liebeschuetz/

Joe Liebeschuetz
Reg. No. 37, 505
On behalf of Ascendis Pharma A/S.

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I certify this correspondence is being mailed by Express Mail (Express Mail Label No. EF 141591764 US) to the Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 on December 7, 2017.
By: /Joe Liebeschuetz/
Joe Liebeschuetz
Reg. 37, 505.

Certificate of Service

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OPAP AKIMA-18
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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No.: 15/646,822 Filed: 07-11-2017
CON REI of 14/604,262 filed 1-23-2015 REI of 13/466,672 filed 5-8-2012, now US 8,598,121, CON of 12/784,117 filed 5-20-2010, which claims benefit of 61/254,563 filed 10-23-2009 and 61/180,112, filed 5-20-2009)
Title: Variants of C-Type Natriuretic Peptide Confirmation No.: 7727
Applicant: BioMarin Pharmaceutical Inc.
Art Unit: 3991 Examiner: Raley, Johnny F.
CON REISSUE APPLICATION UNDERGOING PREEXAM PROCESSING

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450
Attention Director Art Unit 3991

COMMUNICATION

We are resubmitting a copy of a protest originally filed December 7, 2017 with proof of receipt by the PTO because we understand that, although received by the PTO, the protest appears to be have been misdirected and has not appeared on PAIR.

This transmittal is being served on the patent owner at its current attorneys of record. The actual protest was already served on the patent owner's attorneys when originally filed, and is not being served again.

Respectfully submitted

/Joe Liebeschuetz/

Joe Liebeschuetz
Reg. No. 37, 505
On behalf of Ascendis Pharma A/S

Certificate of Express Mailing
I certify this correspondence is being mailed by Express Mail (Express Mail Label No. EF 141591795 US) to the Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 on March 2, 2018.
By: /Joe Liebeschuetz/ Joe Liebeschuetz Reg. 37, 505.

Certificate of Service
I certify under 37 CFR 1.248 a copy of the Resubmission of Protest (transmittal only) has been sent by Express Mail (Express Mail Label No. EF 141591818 US) to the attorneys of record GREENBERG TRAURIG (BOS) ONE INTERNATIONAL PLACE, 20th FL ATTN: PATENT ADMINISTRATOR, BOSTON MA 02110.
By: /Joe Liebeschuetz/ Joe Liebeschuetz, Reg. 37, 505.

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MAR 0 3 2018
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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Atty. Dkt. No. **065588-495115**

Appl. No.: 15/646,822 Filed: 07-11-2017
Title: Variants of C-Type Natriuretic Peptide
Applicant: BioMarin Pharmaceutical Inc.

OPAP
DEC 08 2017
PATENT & TRADEMARK OFFICE

Documents Enclosed:

1) Protest under 37 CFR 1.291;
2) Processing Fee Transmittal (in duplicate);
3) PTO SB08A and SB08B;
4) Exhibits A-I

Express Mail No.EF 141591764 US on 12/07/2017

Kindly acknowledge receipt by placing office stamp hereon and returning postcard to:
Attn.: Joe Liebeschuetz
ALSTON & BIRD LLP
1950 University Avenue, 5th Floor
East Palo Alto, CA 94303-2282

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PTO/SB/17i (05-15)
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PROCESSING FEE Under 37 CFR 1.17(i) TRANSMITTAL

(Fees are subject to annual revision)

Send completed form to: **Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450**

Application Number	15/646,822
Filing Date	07-11-2017
First Named Inventor	Daniel J. Wendt
Art Unit	1629
Examiner Name	Unassigned
Practitioner Docket No.	065588-495115

Enclosed is a paper filed under 37 CFR 1.291 that requires a processing fee (37 CFR 1.17(i)(1) or (2)).

Payment of $ 140.00 is enclosed.

This form should be included with the above-mentioned paper and faxed or mailed to the Office using the appropriate Mail Stop, if applicable.
For transmittal of petition fees under 37 CFR 1.17(f), (g) or (h), see form PTO/SB/17p or PTO/AIA/17p, as applicable.

☐ Applicant asserts small entity status. See 37 CFR 1.27.

☐ Applicant certifies micro entity status. See 37 CFR 1.29.
Form PTO/SB/15A or B or equivalent must either be enclosed or have been submitted previously.

Payment of Fees

☑ The Commissioner is hereby authorized to charge the following fees to Deposit Account No. 160605:

☑ processing fee under 37 CFR 1.17(i)(1) ☐ processing fee under 37 CFR 1.17(i)(2) ☐ any deficiency of fees and credit of any overpayments

☐ Check in the amount of $ _____ is enclosed. ☐ Payment via EFS-Web.

☐ Payment by credit card (Form PTO-2038 or equivalent is enclosed). Do not provide credit card information on this form.

Processing Fees Under 37 CFR 1.17(i)(1):

Undiscounted Fee $140 (Fee Code 1053) Small Entity Fee $70 (Fee Code 2053) Micro Entity Fee $35 (Fee Code 3053)

For papers filed under:
§ 1.28(c)(3) - for processing a non-itemized fee deficiency based on an error in small entity status.
§ 1.29(k)(3) - for processing a non-itemized fee deficiency based on an error in micro entity status.
§ 1.41(b) - for supplying the name or names of the inventor or joint inventors in an application without either an application data sheet or the inventor's oath or declaration, except in provisional applications.
§ 1.48 - for correcting inventorship, except in provisional applications.
§ 1.52(d) - for processing a nonprovisional application filed with a specification in a language other than English.
§ 1.53(c)(3) - to convert a provisional application filed under § 1.53(c) into a nonprovisional application under § 1.53(b).
§ 1.71(g)(2) - for processing a belated amendment under § 1.71(g).
§ 1.102(e) - for requesting prioritized examination of an application.
§ 1.103(b) - for requesting limited suspension of action, continued prosecution application for a design patent (§ 1.53(d)).
§ 1.103(c) - for requesting limited suspension of action, request for continued examination (§ 1.114).
§ 1.103(d) - for requesting deferred examination of an application.
§ 1.291(c)(5) - for processing a second or subsequent protest by the same real party in interest.
§ 3.81 - for a patent to issue to assignee, assignment submitted after payment of the issue fee.

Processing Fees Under 37 CFR 1.17(i)(2):

Fee $130 (small and micro entity discounts are not available for the 37 CFR 1.17(i)(2) processing fee)

For papers filed under:
§ 1.217 - for processing a redacted copy of a paper submitted in the file of an application in which a redacted copy was submitted for the patent application publication. **FEE CODE 1808**
§ 1.221 - for requesting voluntary publication or republication of an application. **FEE CODE 1803**

SUBMITTED BY

Signature	/Joe Liebeschuetz/	Registration No. (Attorney/Agent) 37505	Telephone 650-838-2038
Name (Print/Type)	Joe Liebeschuetz		Date 12/07/2017

This collection of information is required by 37 CFR 1.17. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 5 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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Modified PTO/SB/08A (01-08)

Substitute for form 1449A/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 2

Complete if Known	
Application Number	15/646,822
Filing Date	July 11, 2017
First Named Inventor	Daniel J. Wendt
Art Unit	1629
Examiner Name	Not applicable
Attorney Docket Number	065588-495115

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3] Number[4] Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T[6]
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Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. [1] Applicant's unique citation designation number (optional). [2] Kind Codes of U.S. Patent Documents at www.uspto.gov or MPEP 901.04. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST. 16 if possible. [6] Applicant is to place a check mark here if English language Translation is attached.

Modified PTO/SB/08B (01-08)

Substitute for form 1449B/PTO				Complete if Known	
INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	15/646,822
				Filing Date	July 11, 2017
				First Named Inventor	Daniel J. Wendt
				Art Unit	1629
(Use as many sheets as necessary)				Examiner Name	Not applicable
Sheet	2	of	2	Attorney Docket Number	065588-495115

NON PATENT LITERATURE DOCUMENTS			
Examiner Initials *	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T [2]
	A	US Application 13/466,672 claims pending as of the mailing of the Restriction Requirement on February 13, 2013.	
	B	US Application 13/466,672 Restriction Requirement mailed February 13, 2013.	
	C	US Application 13/466,672 Response to Restriction Requirement submitted April 12, 2013.	
	D	US Application 13/466,672 Examiner-Initiated Interview Summary mailed July 30, 2013.	
	E	US Patent 8,598,121 B2 claims as granted on December 3, 2013.	
	F	US Application 15/332,793 claims as amended in a Preliminary Amendment submitted October 24, 2016.	
	G1	*Ex parte Joachim Werres,* Appeal 2006-2966 (BPAI 2007).	☐
	G2	*Ex parte Morrison et al.,* Appeal 2012-012266 (PTAB 2013) affirmed (2014-1050 Fed. Cir. 2014).	
	G3	Ex *Parte Solomon*, Appeal 2012-002100 (BPAI 2012), affirmed (2012-1689 Fed. Cir. 2013)	
	G4	*Ex parte Wu et al.*, Appeal 2016-0348 (PTAB 2017).	
	H1	PTO Brief from Federal Circuit appeals in *Ex parte Morrison (2012-1050 Fed. Cir. 2014).*	☐
	H2	PTO Brief from Federal Circuit appeals in *Ex parte Solomon (2012-1689 Fed. Cir. 2013).*	
	I	TSUBERRY et al., "Prolonging the Action of Protein and Peptide Drugs by a Novel Approach of Reversible Polyethylene Glycol Modification", J. Biol. Chem., Vol. 279, No. 37, pp 38118-3824 (2004).	☐

Exhibit A

Application No.: 13/466,672 Docket No.: 30610/44556C1

AMENDMENT TO THE CLAIMS

1. (Currently amended) A variant of C-type natriuretic peptide (CNP) selected from the group consisting of:

GDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP53) (SEQ ID NO: 179);

PDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP53) (SEQ ID NO: 185);

MDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP53) (SEQ ID NO: 190)

DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP-53(M48N)] (SEQ ID NO: 180);

LRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-52) (SEQ ID NO: 146);

RVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-51) (SEQ ID NO: 147);

VDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-50) (SEQ ID NO: 148);

DTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-49) (SEQ ID NO: 149);

TKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-48) (SEQ ID NO: 150);

KSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-47) (SEQ ID NO: 151);

SRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-46) (SEQ ID NO: 152);

RAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-45) (SEQ ID NO: 153);

AAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-44) (SEQ ID NO: 154);

AWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-43) (SEQ ID NO: 155);

WARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-42) (SEQ ID NO: 156);

ARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-41) (SEQ ID NO: 157);

RLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-40) (SEQ ID NO: 158);

LLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-39) (SEQ ID NO: 159);

LQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-38) (SEQ ID NO: 160);

QEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-37) (SEQ ID NO: 60);

EHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-36) (SEQ ID NO: 161);

HPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-35) (SEQ ID NO: 162);

PNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-34) (SEQ ID NO: 163);

NARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-33) (SEQ ID NO: 164);

ARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-32) (SEQ ID NO: 165);

RKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-31) (SEQ ID NO: 166);

KYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-30) (SEQ ID NO: 167);

YKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-29) (SEQ ID NO: 168);

KGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-28) (SEQ ID NO: 169);

GANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-27) (SEQ ID NO: 170);

ANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-26) (SEQ ID NO: 171);

NKKGLSKGCFGLKLDRIGSMSGLGC (CNP-25) (SEQ ID NO: 172);

KKGLSKGCFGLKLDRIGSMSGLGC (CNP-24) (SEQ ID NO: 173);

KGLSKGCFGLKLDRIGSMSGLGC (CNP-23) (SEQ ID NO: 174);

LSKGCFGLKLDRIGSMSGLGC (CNP-21) (SEQ ID NO: 175);

SKGCFGLKLDRIGSMSGLGC (CNP-20) (SEQ ID NO: 176);

KGCFGLKLDRIGSMSGLGC (CNP-19) (SEQ ID NO: 177);

GCFGLKLDRIGSMSGLGC (CNP-18) (SEQ ID NO: 178);

~~QEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP37(M32N)] (SEQ ID NO: 182);~~

~~PQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP37) (SEQ ID NO: 186);~~

~~MQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP37) (SEQ ID NO: 192);~~

GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP37) (SEQ ID NO: 75);

~~GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [Gly-CNP37(M32N)]) (SEQ ID NO: 181);~~

~~PGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-Gly-CNP37)) (SEQ ID NO: 145);~~

~~MGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-Gly-CNP37)) (SEQ ID NO: 191);~~

GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (HSA-CNP27) (SEQ ID NO: 144);

GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSNSGLGC [HSA-CNP27(M22N)) (SEQ ID NO: 183)];

PGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-HSA-CNP27)) (SEQ ID NO: 188);

MGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-HSA-CNP27)) (SEQ ID NO: 193);

GANRRGLSRGCFGLKLDRIGSMSGLGC [CNP27(K4,5,9R)] (SEQ ID NO: 36);

GANRRGLSRGCFGLKLDRIGSNSGLGC [CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);

PGANRRGLSRGCFGLKLDRIGSMSGLGC [Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189);

MGANRRGLSRGCFGLKLDRIGSMSGLGC [Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194);

PEG1K-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-CNP27(K4,5,9R)] (SEQ ID NO: 36);

PEG1K-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEG1K-CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);

PEG1K-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189);

PEG1K-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194);

PEO12-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-CNP27(K4,5,9R)] (SEQ ID NO: 36);

PEO12-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEO12-CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);

PEO12-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189);

PEO12-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194);

PEO24-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-CNP27(K4,5,9R)] (SEQ ID NO: 36);

PEO24-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEO24-CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);

PEO24-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189); and

PEO24-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194), wherein the CNP variant can be conjugated to a moiety selected from a hydrophobic acid and an amino acid sequence from 1 to 20 amino acids useful in bone targeting.

2. (Currently amended) A pharmaceutical composition comprising a CNP variant of claim 1, and a pharmaceutically acceptable excipient, carrier or diluent.

3. (Original) The composition of claim 2, which is a lyophilized formulation prepared from a formulation that comprises a citric acid/citrate buffer or an acetic acid/acetate buffer having a pH from about 4 to about 6.

4. (Currently amended) The composition of claim 3, wherein the lyophilized formulation is prepared from a formulation that further comprises (a) an isotonicity-adjusting agent or a bulking agent or (b) an antioxidant ~~selected from the group consisting of mannitol, sucrose, sorbitol, and combinations thereof~~.

5-6. (Cancel)

7. (Currently amended) A method of treating a bone-related disorder or skeletal dysplasia, comprising administering a CNP variant to a subject in need thereof, wherein the CNP variant is a CNP variant according to claim 1, and wherein the bone-related disorder or skeletal dysplasia is selected from the group consisting of ~~osteoarthritis, hypophosphatemic rickets,~~ achondroplasia, hypochondroplasia, short stature, dwarfism~~, osteochondrodysplasias, thanatophoric dysplasia, osteogenesis imperfecta, achondrogenesis, chondrodysplasia punctata,~~ and homozygous achondroplasia~~, chondrodysplasia punctata, camptomelic dysplasia, congenital lethal hypophosphatasia, perinatal lethal type of osteogenesis imperfecta, short rib polydactyly syndromes, hypochondroplasia, rhizomelic type of chondrodysplasia punctata, Jansen type metaphyseal dysplasia, spondyloepiphyseal dysplasia congenita, atelosteogenesis, diastrophic dysplasia, congenital short femur, Langer type mesomelic dysplasia, Nievergelt type mesomelic dysplasia, Robinow syndrome, Reinhardt syndrome, acrodysostosis, peripheral dysostosis, Kniest dysplasia, fibrochondrogenesis, Roberts syndrome, acromesomelic dysplasia, micromelia, Morquio syndrome, Kniest syndrome, metatrophic dysplasia, and spondyloepimetaphyseal dysplasia~~.

8. (Cancel)

9. (Currently amended) A method of treating a vascular smooth muscle disorder, comprising administering a CNP variant to a subject, wherein the CNP variant is a CNP variant according to claim 1, and wherein the vascular smooth muscle disorder is selected from the group consisting of hypertension, restenosis, arteriosclerosis, acute decompensated heart failure, congestive heart failure, cardiac edema, nephredema, hepatic edema, acute renal insufficiency, and chronic renal insufficiency, wherein the CNP variant is selected from the group consisting of

GDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP53) (SEQ ID NO: 179);

PDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP53) (SEQ ID NO: 185);

MDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP53) (SEQ ID NO: 190)

DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP-53(M48N)] (SEQ ID NO: 180);

LRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-52) (SEQ ID NO: 146);

RVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-51) (SEQ ID NO: 147);

VDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-50) (SEQ ID NO: 148);

DTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-49) (SEQ ID NO: 149);

TKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-48) (SEQ ID NO: 150);

KSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-47) (SEQ ID NO: 151);

SRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-46) (SEQ ID NO: 152);

RAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-45) (SEQ ID NO: 153);

AAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-44) (SEQ ID NO: 154);

AWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-43) (SEQ ID NO: 155);

WARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-42) (SEQ ID NO: 156);

ARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-41) (SEQ ID NO: 157);

RLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-40) (SEQ ID NO: 158);

LLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-39) (SEQ ID NO: 159);

LQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-38) (SEQ ID NO: 160);

QEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-37) (SEQ ID NO: 60);
EHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-36) (SEQ ID NO: 161);
HPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-35) (SEQ ID NO: 162);
PNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-34) (SEQ ID NO: 163);
NARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-33) (SEQ ID NO: 164);
ARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-32) (SEQ ID NO: 165);
RKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-31) (SEQ ID NO: 166);
KYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-30) (SEQ ID NO: 167);
YKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-29) (SEQ ID NO: 168);
KGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-28) (SEQ ID NO: 169);
GANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-27) (SEQ ID NO: 170);
ANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-26) (SEQ ID NO: 171);
NKKGLSKGCFGLKLDRIGSMSGLGC (CNP-25) (SEQ ID NO: 172);
KKGLSKGCFGLKLDRIGSMSGLGC (CNP-24) (SEQ ID NO: 173);
KGLSKGCFGLKLDRIGSMSGLGC (CNP-23) (SEQ ID NO: 174);
LSKGCFGLKLDRIGSMSGLGC (CNP-21) (SEQ ID NO: 175);
SKGCFGLKLDRIGSMSGLGC (CNP-20) (SEQ ID NO: 176);
KGCFGLKLDRIGSMSGLGC (CNP-19) (SEQ ID NO: 177);
GCFGLKLDRIGSMSGLGC (CNP-18) (SEQ ID NO: 178);
QEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP37(M32N)] (SEQ ID NO: 182);
PQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP37) (SEQ ID NO: 186);
MQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP37) (SEQ ID NO: 192);
GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP37) (SEQ ID NO: 75);
GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [Gly-CNP37(M32N)]) (SEQ ID NO: 181);
PGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-Gly-CNP37)) (SEQ ID NO: 145);

MGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-Gly-CNP37)) (SEQ ID NO: 191);

GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (HSA-CNP27) (SEQ ID NO: 144);

GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSNSGLGC [HSA-CNP27(M22N)) (SEQ ID NO: 183)];

PGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-HSA-CNP27)) (SEQ ID NO: 188);

MGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-HSA-CNP27)) (SEQ ID NO: 193);

GANRRGLSRGCFGLKLDRIGSMSGLGC [CNP27(K4,5,9R)] (SEQ ID NO: 36);

GANRRGLSRGCFGLKLDRIGSNSGLGC [CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);

PGANRRGLSRGCFGLKLDRIGSMSGLGC [Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189);

MGANRRGLSRGCFGLKLDRIGSMSGLGC [Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194);

PEG1K-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-CNP27(K4,5,9R)] (SEQ ID NO: 36);

PEG1K-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEG1K-CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);

PEG1K-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189);

PEG1K-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194);

PEO12-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-CNP27(K4,5,9R)] (SEQ ID NO: 36);

PEO12-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEO12-CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);

PEO12-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189);

PEO12-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194);

PEO24-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-CNP27(K4,5,9R)] (SEQ ID NO: 36);

PEO24-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEO24-CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);
PEO24-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189); and
PEO24-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194), wherein the CNP variant can be conjugated to a moiety selected from a hydrophobic acid and an amino acid sequence from 1 to 20 amino acids useful in bone targeting.

10. (Currently amended) A method for recombinant production of a CNP variant, comprising culturing in a medium a host cell comprising a first polynucleotide encoding a CNP variant polypeptide linked to a second polynucleotide encoding a cleavable peptide or protein under conditions that result in expression of a fusion polypeptide encoded by the polynucleotides, wherein the fusion polypeptide comprises the CNP variant polypeptide directly linked to the cleavable peptide or protein or indirectly linked thereto via a linker, wherein the CNP variant is selected from the group consisting of:

R-CNP22(K4R) (Analog AZ) (SEQ ID NO: 41);
ER-CNP22(K4R) (Analog BA) (SEQ ID NO: 39);
GANPR-CNP22(K4R) (Analog CI) (SEQ ID NO: 37);
GANQQ-CNP22(K4R) (Analog CH) (SEQ ID NO: 69);
AAWARLLQEHPNA-CNP22 (Analog CA) (SEQ ID NO: 61);
AAWARLLQEHPNAR-CNP22 (Analog CB)(SEQ ID NO: 62);
DLRVDTKSRAAWAR-CNP22 (Analog CC) (SEQ ID NO: 63);
GQPREPQVYTLPPS-CNP22 (Analog CF) (SEQ ID NO: 79);
GERAFKAWAVARLSQ-CNP22 (Analog CE) (SEQ ID NO: 81);
GHHSHEQHPHGANQQ-CNP22 (Analog CQ) (SEQ ID NO: 76);
GQEHPNARKYKGANPK-CNP22 (Analog CS) (SEQ ID NO: 71);
GQEHPNARKYKGANQK-CNP22 (Analog CT) (SEQ ID NO: 130);
GAHHPHEHDTHGANQQ-CNP22 (Analog CR) (SEQ ID NO: 77);
GQTHSSGTQSGANQQ-CNP22(K4R) (Analog CN) (SEQ ID NO: 87);
SPKMVQGSG-CNP17-KVLRRH (Analog CD) (SEQ ID NO: 68);
PNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-34);

GANRRGLSRGCFGLKLDRIGSMSGLGC [CNP27(K4,5,9R)] (SEQ ID NO: 36);
GANRRGLSRGCFGLKLDRIGSNSGLGC [CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);
PGANRRGLSRGCFGLKLDRIGSMSGLGC [Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189);
MGANRRGLSRGCFGLKLDRIGSMSGLGC [Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194);
GDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP53)) (SEQ ID NO: 179);
PDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP53)) (SEQ ID NO: 185);
MDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP53)) (SEQ ID NO: 190);
DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP-53(M48N)]) (SEQ ID NO: 180);
QEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-37) (SEQ ID NO: 60);
GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP37) (SEQ ID NO: 75);
GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (HSA-CNP27) (SEQ ID NO: 144);
GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSNSGLGC [HSA-CNP27(M22N)]) (SEQ ID NO: 183);
PGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-HSA-CNP27)) (SEQ ID NO: 188);
MGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-HSA-CNP27)) (SEQ ID NO: 193);

LRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-52) (SEQ ID NO: 146);

RVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-51) (SEQ ID NO: 147);
VDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-50) (SEQ ID NO: 148);
DTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-49) (SEQ ID NO: 149);

TKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-48) (SEQ ID NO: 150);
KSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-47) (SEQ ID NO: 151);
SRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-46) (SEQ ID NO: 152);
RAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-45) (SEQ ID NO: 153);
AAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-44) (SEQ ID NO: 154);
AWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-43) (SEQ ID NO: 155);
WARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-42) (SEQ ID NO: 156);
ARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-41) (SEQ ID NO: 157);
RLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-40) (SEQ ID NO: 158);
LLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-39) (SEQ ID NO: 159);
LQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-38) (SEQ ID NO: 160);
EHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-36) (SEQ ID NO: 161);
HPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-35) (SEQ ID NO: 162);
PNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-34) (SEQ ID NO: 163);
NARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-33) (SEQ ID NO: 164);
ARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-32) (SEQ ID NO: 165);
RKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-31) (SEQ ID NO: 166);
KYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-30) (SEQ ID NO: 167);
YKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-29) (SEQ ID NO: 168);
KGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-28) (SEQ ID NO: 169);
GANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-27) (SEQ ID NO: 170);

ANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-26) (SEQ ID NO: 171);
NKKGLSKGCFGLKLDRIGSMSGLGC (CNP-25) (SEQ ID NO: 172);
KKGLSKGCFGLKLDRIGSMSGLGC (CNP-24) (SEQ ID NO: 173);
KGLSKGCFGLKLDRIGSMSGLGC (CNP-23) (SEQ ID NO: 174);
LSKGCFGLKLDRIGSMSGLGC (CNP-21) (SEQ ID NO: 175);
SKGCFGLKLDRIGSMSGLGC (CNP-20) (SEQ ID NO: 176);
KGCFGLKLDRIGSMSGLGC (CNP-19) (SEQ ID NO: 177); and
GCFGLKLDRIGSMSGLGC (CNP-18) (SEQ ID NO: 178).

11-15. (Cancel)

16. (Original) The method of claim 10, wherein the cleavable peptide or protein is selected from the group consisting of histidine tags, human transcription factor TAF12, TAF12 fragments, TAF12 histone fold domain, mutants of TAF12 and fragments thereof, TAF12(C/A), TAF12(D/E), TAF12(4D/4E), TAF12(6D/6E), TAF12(10D/10E), TAF12(C/A & D/E), TAF12(C/A & 4D/4E), TAF12(C/A & 6D/6E), TAF12(C/A & 10D/10E), ketosteroid isomerase, maltose-binding protein, ß-galactosidase, glutathione-S-transferase, thioredoxin, chitin-binding domain, BMP-2, BMP-2 mutants, BMP-2(C/A), and mutants and fragments thereof.

17. (Cancel)

18. (Original) The method of claim 10, wherein the host cell is bacterial.

19-21. (Cancel)

22. (Original) The method of claim 10, wherein the fusion polypeptide is expressed as a soluble protein or as an inclusion body.

23. (Original) The method of claim10, further comprising isolating the expressed fusion polypeptide from the host cell or culture medium.

24. (Original) The method of claim 23, further comprising contacting the isolated fusion polypeptide with a cleaving agent selected from the group consisting of formic acid,

cyanogen bromide (CNBr), hydroxylamine, protein self cleavage, Factor Xa, enterokinase, ProTEV, and SUMO protease.

25-29. (Cancel)

30. (Currently amended) A CNP variant produced according to the method of claim 10, wherein the CNP variant is selected from the group consisting of:
R-CNP22(K4R) (Analog AZ) (SEQ ID NO: 41);
ER-CNP22(K4R) (Analog BA) (SEQ ID NO: 39);
GANPR-CNP22(K4R) (Analog CI) (SEQ ID NO: 37);
GANQQ-CNP22(K4R) (Analog CH) (SEQ ID NO: 69);
AAWARLLQEHPNA-CNP22 (Analog CA) (SEQ ID NO: 61);
AAWARLLQEHPNAR-CNP22 (Analog CB)(SEQ ID NO: 62);
DLRVDTKSRAAWAR-CNP22 (Analog CC) (SEQ ID NO: 63);
GQPREPQVYTLPPS-CNP22 (Analog CF) (SEQ ID NO: 79);
GERAFKAWAVARLSQ-CNP22 (Analog CE) (SEQ ID NO: 81);
GHHSHEQHPHGANQQ-CNP22 (Analog CQ) (SEQ ID NO: 76);
GQEHPNARKYKGANPK-CNP22 (Analog CS) (SEQ ID NO: 71);
GQEHPNARKYKGANQK-CNP22 (Analog CT) (SEQ ID NO: 130);
GAHHPHEHDTHGANQQ-CNP22 (Analog CR) (SEQ ID NO: 77);
GQTHSSGTQSGANQQ-CNP22(K4R) (Analog CN) (SEQ ID NO: 87);
SPKMVQGSG-CNP17-KVLRRH (Analog CD) (SEQ ID NO: 68);
PNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-34);
GANRRGLSRGCFGLKLDRIGSMSGLGC [CNP27(K4,5,9R)] (SEQ ID NO: 36);
GANRRGLSRGCFGLKLDRIGSNSGLGC [CNP27(K4,5,9R, M22N)];
PGANRRGLSRGCFGLKLDRIGSMSGLGC [Pro-CNP27(K4,5,9R)];
MGANRRGLSRGCFGLKLDRIGSMSGLGC [Met-CNP27(K4,5,9R)];
GDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP53)) (SEQ ID NO: 179);
PDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP53)) (SEQ ID NO: 185);
MDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP53) (SEQ ID NO: 190);

DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP-53(M48N)] (SEQ ID NO: 180);

QEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-37) (SEQ ID NO: 60);

QEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP37(M32N)] (SEQ ID NO: 182);

PQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP37) (SEQ ID NO: 186);

MQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP37) (SEQ ID NO: 192);

GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP37) (SEQ ID NO: 75);

GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [Gly-CNP37(M32N)] (SEQ ID NO: 181);

~~PGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-Gly-CNP37) (SEQ ID NO: 145);~~

MGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-Gly-CNP37) (SEQ ID NO: 191);

GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (HSA-CNP27) (SEQ ID NO: 144);

GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSNSGLGC [HSA-CNP27(M22N)] (SEQ ID NO: 183);

PGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-HSA-CNP27) (SEQ ID NO: 188); and

MGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-HSA-CNP27) (SEQ ID NO: 193)[[;]].

31. (Currently amended) A host cell comprising an expression vector, the vector comprising a first polynucleotide encoding a CNP variant polypeptide linked to a second polynucleotide encoding a cleavable peptide or protein, wherein the CNP variant is selected from the group consisting of

GDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP53) (SEQ ID NO: 179);

PDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP53) (SEQ ID NO: 185);

MDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP53) (SEQ ID NO: 190)

DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP-53(M48N)] (SEQ ID NO: 180);

LRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-52) (SEQ ID NO: 146);

RVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-51) (SEQ ID NO: 147);

VDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-50) (SEQ ID NO: 148);

DTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-49) (SEQ ID NO: 149);

TKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-48) (SEQ ID NO: 150);

KSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-47) (SEQ ID NO: 151);

SRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-46) (SEQ ID NO: 152);

RAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-45) (SEQ ID NO: 153);

AAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-44) (SEQ ID NO: 154);

AWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-43) (SEQ ID NO: 155);

WARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-42) (SEQ ID NO: 156);

ARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-41) (SEQ ID NO: 157);

RLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-40) (SEQ ID NO: 158);

LLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-39) (SEQ ID NO: 159);
LQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-38) (SEQ ID NO: 160);
QEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-37) (SEQ ID NO: 60);
EHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-36) (SEQ ID NO: 161);
HPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-35) (SEQ ID NO: 162);
PNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-34) (SEQ ID NO: 163);
NARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-33) (SEQ ID NO: 164);
ARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-32) (SEQ ID NO: 165);
RKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-31) (SEQ ID NO: 166);
KYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-30) (SEQ ID NO: 167);
YKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-29) (SEQ ID NO: 168);
KGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-28) (SEQ ID NO: 169);
GANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-27) (SEQ ID NO: 170);
ANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-26) (SEQ ID NO: 171);
NKKGLSKGCFGLKLDRIGSMSGLGC (CNP-25) (SEQ ID NO: 172);
KKGLSKGCFGLKLDRIGSMSGLGC (CNP-24) (SEQ ID NO: 173);
KGLSKGCFGLKLDRIGSMSGLGC (CNP-23) (SEQ ID NO: 174);
LSKGCFGLKLDRIGSMSGLGC (CNP-21) (SEQ ID NO: 175);
SKGCFGLKLDRIGSMSGLGC (CNP-20) (SEQ ID NO: 176);
KGCFGLKLDRIGSMSGLGC (CNP-19) (SEQ ID NO: 177);
GCFGLKLDRIGSMSGLGC (CNP-18) (SEQ ID NO: 178);
QEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP37(M32N)] (SEQ ID NO: 182);
PQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP37) (SEQ ID NO: 186);
MQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP37) (SEQ ID NO: 192);
GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP37) (SEQ ID NO: 75);

GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [Gly-CNP37(M32N)]) (SEQ ID NO: 181);

PGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-Gly-CNP37)) (SEQ ID NO: 145);

MGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-Gly-CNP37)) (SEQ ID NO: 191);

GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (HSA-CNP27) (SEQ ID NO: 144);

GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSNSGLGC [HSA-CNP27(M22N)) (SEQ ID NO: 183)];

PGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-HSA-CNP27)) (SEQ ID NO: 188);

MGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-HSA-CNP27)) (SEQ ID NO: 193);

GANRRGLSRGCFGLKLDRIGSMSGLGC [CNP27(K4,5,9R)] (SEQ ID NO: 36);

GANRRGLSRGCFGLKLDRIGSNSGLGC [CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);

PGANRRGLSRGCFGLKLDRIGSMSGLGC [Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189);

MGANRRGLSRGCFGLKLDRIGSMSGLGC [Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194);

PEG1K-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-CNP27(K4,5,9R)] (SEQ ID NO: 36);

PEG1K-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEG1K-CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);

PEG1K-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189);

PEG1K-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194);

PEO12-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-CNP27(K4,5,9R)] (SEQ ID NO: 36);

PEO12-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEO12-CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);

PEO12-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189);

PEO12-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194);
PEO24-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-CNP27(K4,5,9R)] (SEQ ID NO: 36);
PEO24-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEO24-CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);
PEO24-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189); and
PEO24-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194).

32-34. (Cancel)

35. (Original) The host cell of claim 31, wherein the host cell is cultured in a medium under conditions suitable for expression of a fusion polypeptide encoded by the polynucleotides, wherein the fusion polypeptide comprises the CNP variant polypeptide directly linked to the cleavable peptide or protein or indirectly linked thereto via a linker.

36-38. (Cancel)

39. (Currently amended) A method of treating a CNP-responsive condition or disorder, comprising

administering a CNP peptide or variant to a subject, and

monitoring the level of at least one bone- or cartilage-associated biomarker in the subject,

wherein an increase in the level of the at least one bone- or cartilage-associated biomarker indicates a therapeutic effect of the CNP peptide or variant on the subject or the condition or disorder, wherein the CNP variant is selected from the group consisting of

GDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP53) (SEQ ID NO: 179);
PDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP53) (SEQ ID NO: 185);

MDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP53) (SEQ ID NO: 190)

DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP-53(M48N)] (SEQ ID NO: 180);

LRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-52) (SEQ ID NO: 146);

RVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-51) (SEQ ID NO: 147);

VDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-50) (SEQ ID NO: 148);

DTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-49) (SEQ ID NO: 149);

TKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-48) (SEQ ID NO: 150);

KSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-47) (SEQ ID NO: 151);

SRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-46) (SEQ ID NO: 152);

RAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-45) (SEQ ID NO: 153);

AAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-44) (SEQ ID NO: 154);

AWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-43) (SEQ ID NO: 155);

WARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-42) (SEQ ID NO: 156);

ARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-41) (SEQ ID NO: 157);

RLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-40) (SEQ ID NO: 158);

LLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-39) (SEQ ID NO: 159);

LQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-38) (SEQ ID NO: 160);

QEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-37) (SEQ ID NO: 60);

EHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-36) (SEQ ID NO: 161);

HPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-35) (SEQ ID NO: 162);

PNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-34) (SEQ ID NO: 163);

NARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-33) (SEQ ID NO: 164);

ARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-32) (SEQ ID NO: 165);

RKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-31) (SEQ ID NO: 166);

KYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-30) (SEQ ID NO: 167);

YKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-29) (SEQ ID NO: 168);

KGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-28) (SEQ ID NO: 169);

GANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-27) (SEQ ID NO: 170);

ANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-26) (SEQ ID NO: 171);

NKKGLSKGCFGLKLDRIGSMSGLGC (CNP-25) (SEQ ID NO: 172);

KKGLSKGCFGLKLDRIGSMSGLGC (CNP-24) (SEQ ID NO: 173);

KGLSKGCFGLKLDRIGSMSGLGC (CNP-23) (SEQ ID NO: 174);

LSKGCFGLKLDRIGSMSGLGC (CNP-21) (SEQ ID NO: 175);

SKGCFGLKLDRIGSMSGLGC (CNP-20) (SEQ ID NO: 176);

KGCFGLKLDRIGSMSGLGC (CNP-19) (SEQ ID NO: 177);

GCFGLKLDRIGSMSGLGC (CNP-18) (SEQ ID NO: 178);

QEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP37(M32N)] (SEQ ID NO: 182);

PQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP37) (SEQ ID NO: 186);

MQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP37) (SEQ ID NO: 192);

GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP37) (SEQ ID NO: 75);

GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [Gly-CNP37(M32N)]) (SEQ ID NO: 181);

PGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-Gly-CNP37)) (SEQ ID NO: 145);

MGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-Gly-CNP37)) (SEQ ID NO: 191);

GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (HSA-CNP27) (SEQ ID NO: 144);

GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSNSGLGC [HSA-CNP27(M22N)) (SEQ ID NO: 183)];

PGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-HSA-CNP27)) (SEQ ID NO: 188);

MGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-HSA-CNP27)) (SEQ ID NO: 193);

GANRRGLSRGCFGLKLDRIGSMSGLGC [CNP27(K4,5,9R)] (SEQ ID NO: 36);

GANRRGLSRGCFGLKLDRIGSNSGLGC [CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);

PGANRRGLSRGCFGLKLDRIGSMSGLGC [Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189);

MGANRRGLSRGCFGLKLDRIGSMSGLGC [Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194);

PEG1K-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-CNP27(K4,5,9R)] (SEQ ID NO: 36);

PEG1K-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEG1K-CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);

PEG1K-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189);

PEG1K-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194);

PEO12-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-CNP27(K4,5,9R)] (SEQ ID NO: 36);

PEO12-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEO12-CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);

PEO12-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189);

PEO12-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194);

PEO24-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-CNP27(K4,5,9R)] (SEQ ID NO: 36);
PEO24-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEO24-CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);
PEO24-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189); and
PEO24-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194), wherein the CNP variant can be conjugated to a moiety selected from a hydrophobic acid and an amino acid sequence from 1 to 20 amino acids useful in bone targeting.

40. (Cancel)

41. (Currently amended) The method of claim ~~37 or~~ 39, wherein the at least one bone- or cartilage-associated biomarker is selected from the group consisting of CNP, cGMP, propeptides of collagen type II and fragments thereof, collagen type II and fragments thereof, osteocalcin, proliferating cell nuclear antigen (PCNA), propeptides of type I procollagen (PINP) and fragments thereof, collagen type I and fragments thereof, aggrecan chondroitin sulfate, and alkaline phosphatase.

42-43. (Cancel)

44. (New) A method of increasing long bone growth, comprising administering a CNP variant according to Claim 1 to a subject in need thereof, where the administration increases long bone growth.

45. (New) A CNP variant according to Claim 1 useful for increasing long bone growth or treating achondroplasia, hypochondroplasia, short stature, dwarfism, or homozygous achondroplasia in a subject in need thereof.

46. (New) A CNP variant where the CNP variant is selected from QEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP37(M32N)] (SEQ ID NO: 182);

PQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP37) (SEQ ID NO: 186);

MQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP37) (SEQ ID NO: 192);

GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [Gly-CNP37(M32N)]) (SEQ ID NO: 181);

PGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-Gly-CNP37)) (SEQ ID NO: 145); and

MGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-Gly-CNP37)) (SEQ ID NO: 191),

wherein the variant is conjugated to a moiety selected from a hydrophobic acid and an amino acid sequence from 1 to 20 amino acids useful in bone targeting.

UNITED STATES PATENT AND TRADEMARK OFFICE

Exhibit B

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/466,672	05/08/2012	Daniel J. Wendt	30610/44556C1	1368

90849 7590 02/13/2013
Marshall, Gerstein & Borun LLP (Biomarin)
233 South Wacker Drive
6300 Willis Tower
Chicago, IL 60606

EXAMINER
SHAFER, SHULAMITH H

ART UNIT	PAPER NUMBER
1647	

NOTIFICATION DATE	DELIVERY MODE
02/13/2013	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

lbigornia@bmrn.com
mgbdocket@marshallip.com
mflores@bmrn.com

PTOL-90A (Rev. 04/07)

Office Action Summary	Application No.	Applicant(s)	
	13/466,672	WENDT ET AL.	
	Examiner	Art Unit	
	SHULAMITH H. SHAFER	1647	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 1 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

5)☒ Claim(s) *1-4,7,9,10,16,18,22-24,30,31,35,39,41 and 44-46* is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☐ Claim(s) _____ is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☒ Claim(s) *See Continuation Sheet* are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)

2) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.

4) ☐ Other: _____.

Continuation of Disposition of Claims: Claims subject to restriction and/or election requirement are 1-4,7,9,10,16,18,22-24,30,31,35,39,41 and 44-46.

DETAILED ACTION

Applicants' amendment of 20 August 2012 is acknowledged. Claims 1-4, 7, 9, 10, 16, 18, 22-24, 30, 31, 35, 39, 41, and 44-46 are pending in the instant application.

Election/Restrictions

Restriction to one of the following inventions is required under 35 U.S.C. 121:

I. Claims 1-4, 30, 45 and 46, drawn to a variant of C-type natriuretic peptide (CNP), classified in class 530, subclass 300.

II. Claim 7, drawn to a method of treating a bone-related disorder or skeletal dysplasia, classified in class 514, subclass 12.4.

III. Claim 9, drawn to a method of treating a vascular smooth muscle disorder, classified in class 514, subclass 12.4.

IV. Claims 10, 16, 18, 22-24, 31, 35, drawn to a method for recombinant production of a CNP variant and a host cell used in said method, classified in class 435, subclass 69.1/325.

V. Claims 39 and 41, drawn to a method of treating a CNP-responsive condition or disorder, classified in class 514, subclass 12.4.

VI. Claim 44, drawn to a method of increasing long bone growth, classified in class 514, subclass 12.4.

The inventions are distinct, each from the other because of the following reasons:

Inventions II-VI are directed to related processes. The related inventions are distinct if: (1) the inventions as claimed are either not capable of use together or can have a materially different design, mode of operation, function, or effect; (2) the inventions do not overlap in scope, i.e., are mutually exclusive; and (3) the inventions as claimed are not obvious variants. See MPEP § 806.05(j). In the instant case, the inventions as claimed are directed to methods that are distinct both physically and functionally, and are not required one for the other. Inventions II, III, V and VI are all drawn to methods of treatment, while Invention IV is drawn to a method of producing a CNP variant recombinantly. While Inventions II, III, V and VI are all directed to methods

of treatment, Invention II is directed to a method of treating a bone-related disorder or skeletal dysplasia, Invention III is drawn to a method of treating a vascular smooth muscle disorder, Invention V is drawn to a method of treating a CNP-responsive condition or disorder and monitoring the level of at least one bone- or cartilage-associated biomarker, while Invention VI is directed to a method of increasing long bone growth; thus each Invention requires a search for a distinct patient or subject population. All of these methods recite different goals, method steps and conclusion. Thus, a search of all these methods would result in an undue burden, as the searches would not be co-extensive. A search and examination of all these methods in one patent application would result in an undue burden, since the searches for the methods are not co-extensive. Furthermore, the inventions as claimed do not encompass overlapping subject matter and there is nothing of record to show them to be obvious variants.

Inventions I and II/III/V and VI are related as product and process of use. The inventions can be shown to be distinct if either or both of the following can be shown: (1) the process for using the product as claimed can be practiced with another materially different product or (2) the product as claimed can be used in a materially different process of using that product. See MPEP § 806.05(h). In the instant case the CNP variant of Invention I can be used to generate antibodies.

Inventions IV and I are related as process of making and product made. The inventions are distinct if either or both of the following can be shown: (1) that the process as claimed can be used to make another and materially different product or (2) that the product as claimed can be made by another and materially different process (MPEP § 806.05(f)). In the instant case the CNP variant of Invention I can be made by chemical synthesis.

Restriction for examination purposes as indicated is proper because all these inventions listed in this action are independent or distinct for the reasons given above

and there would be a serious search and examination burden if restriction were not required because one or more of the following reasons apply:

(a) the inventions have acquired a separate status in the art in view of their different classification;

(b) the inventions have acquired a separate status in the art due to their recognized divergent subject matter;

(c) the inventions require a different field of search (for example, searching different classes/subclasses or electronic resources, or employing different search queries);

(d) the prior art applicable to one invention would not likely be applicable to another invention;

(e) the inventions are likely to raise different non-prior art issues under 35 U.S.C. 101 and/or 35 U.S.C. 112, first paragraph.

Applicant is advised that the reply to this requirement to be complete must include (i) an election of a invention to be examined even though the requirement may be traversed (37 CFR 1.143) **and (ii) identification of the claims encompassing the elected invention**.

The election of an invention may be made with or without traverse. To reserve a right to petition, the election must be made with traverse. If the reply does not distinctly and specifically point out supposed errors in the restriction requirement, the election shall be treated as an election without traverse. Traversal must be presented at the time of election in order to be considered timely. Failure to timely traverse the requirement will result in the loss of right to petition under 37 CFR 1.144. If claims are added after the election, applicant must indicate which of these claims are readable on the elected invention.

If claims are added after the election, applicant must indicate which of these claims are readable upon the elected invention.

Should applicant traverse on the ground that the inventions are not patentably distinct, applicant should submit evidence or identify such evidence now of record showing the inventions to be obvious variants or clearly admit on the record that this is

the case. In either instance, if the examiner finds one of the inventions unpatentable over the prior art, the evidence or admission may be used in a rejection under 35 U.S.C. 103(a) of the other invention.

Species Election #1

This application contains claims directed to the following patentably distinct species: sequences as disclosed in claims 1, 9, 10, 30, 31, 39, and 46. The species are independent or distinct because each sequence is a distinct separate polypeptide. In addition, these species are not obvious variants of each other based on the current record.

Applicant is required under 35 U.S.C. 121 to elect a single disclosed species, or a single grouping of patentably indistinct species, for prosecution on the merits to which the claims shall be restricted if no generic claim is finally held to be allowable. Currently, no claim is generic.

There is a search and/or examination burden for the patentably distinct species as set forth above because at least the following reason(s) apply:

The species are independent or distinct because each sequence is a distinct separate polypeptide; a search for the recited sequences would not be coextensive.

Species Election #2

This application contains claims directed to the following patentably distinct species bone-related disorders. The species are independent or distinct because each disorder comprises a condition of unique etiology, progression and prognosis. In addition, these species are not obvious variants of each other based on the current record.

1. achondroplasia,
2. hypochondroplasia,
3. short stature,
4. dwarfism,
5. homozygous achondroplasia,

If Group II is elected, Applicant is required under 35 U.S.C. 121 to elect a single disclosed species, or a single grouping of patentably indistinct species, for prosecution on the merits to which the claims shall be restricted if no generic claim is finally held to be allowable. Currently, no claim is generic.

There is a search and/or examination burden for the patentably distinct species as set forth above because at least the following reason(s) apply:

The species are independent or distinct because each disorder comprises a condition of unique etiology, progression and prognosis; a search for prior art directed at achondroplasia, for example, would not necessarily reveal art relevant to an unspecified dwarfism.

Species Election #3

This application contains claims directed to the following patentably distinct species: vascular smooth muscle disorder. The species are independent or distinct because each species comprises a condition of unique etiology, progression and prognosis. In addition, these species are not obvious variants of each other based on the current record.

6. hypertension,
7. restenosis,
8. arteriosclerosis,
9. acute decompensated heart failure,
10. congestive heart failure,
11. cardiac edema,
12. nephredema,
13. hepatic edema,
14. acute renal insufficiency, and
15. chronic renal insufficiency.

If Group III is elected, Applicant is required under 35 U.S.C. 121 to elect a single disclosed species, or a single grouping of patentably indistinct species, for prosecution on the merits to which the claims shall be restricted if no generic claim is finally held to be allowable. Currently, no claim is generic.

There is a search and/or examination burden for the patentably distinct species as set forth above because at least the following reason(s) apply:

The species are independent or distinct because each disorder comprises a condition of unique etiology, progression and prognosis; a search for prior art directed at hypertension, for example, would not necessarily reveal art relevant to chronic renal insufficiency.

Species Election #4

This application contains claims directed to the following patentably distinct species: bone or cartilage associated biomarker. The species are independent or distinct because each biomarker is a compound of distinct structure and function. In addition, these species are not obvious variants of each other based on the current record.

16. CNP,
17. cGMP,
18. propeptides of collagen type II and fragments thereof,
19. collagen type II and fragments thereof,
20. osteocalcin,
21. proliferating cell nuclear antigen (PCNA),
22. propeptides of type I procollagen (PINP) and fragments thereof,
23. collagen type I and fragments thereof,
24. aggrecan chondroitin sulfate, and
25. alkaline phosphatase.

If Group V is elected, Applicant is required under 35 U.S.C. 121 to elect a single disclosed species, or a single grouping of patentably indistinct species, for prosecution on the merits to which the claims shall be restricted if no generic claim is finally held to be allowable. Currently, claim 39 is generic.

There is a search and/or examination burden for the patentably distinct species as set forth above because at least the following reason(s) apply:

The species are independent or distinct because each each compound has a distinct structure and function; a search for the recited compounds would not be coextensive.

Applicant is advised that the reply to this requirement to be complete must include (i) an election of a species or a grouping of patentably indistinct species to be examined even though the requirement may be traversed (37 CFR 1.143) **and (ii) identification of the claims encompassing the elected species or grouping of patentably indistinct species**, including any claims subsequently added. An argument that a claim is allowable or that all claims are generic is considered nonresponsive unless accompanied by an election.

The election may be made with or without traverse. To preserve a right to petition, the election must be made with traverse. If the reply does not distinctly and specifically point out supposed errors in the election of species requirement, the election shall be treated as an election without traverse. Traversal must be presented at the time of election in order to be considered timely. Failure to timely traverse the requirement will result in the loss of right to petition under 37 CFR 1.144. If claims are added after the election, applicant must indicate which of these claims are readable on the elected species or grouping of patentably indistinct species.

Should applicant traverse on the ground that the species, or groupings of patentably indistinct species from which election is required, are not patentably distinct, applicant should submit evidence or identify such evidence now of record showing them to be obvious variants or clearly admit on the record that this is the case. In either instance, if the examiner finds one of the species unpatentable over the prior art, the evidence or admission may be used in a rejection under 35 U.S.C. 103(a) of the other species.

Upon the allowance of a generic claim, applicant will be entitled to consideration of claims to additional species which depend from or otherwise require all the limitations of an allowable generic claim as provided by 37 CFR 1.141.

The examiner has required restriction between product and process claims. Where applicant elects claims directed to the product, and the product claims are

subsequently found allowable, withdrawn process claims that depend from or otherwise require all the limitations of the allowable product claim will be considered for rejoinder. All claims directed to a nonelected process invention must require all the limitations of an allowable product claim for that process invention to be rejoined.

In the event of rejoinder, the requirement for restriction between the product claims and the rejoined process claims will be withdrawn, and the rejoined process claims will be fully examined for patentability in accordance with 37 CFR 1.104. Thus, to be allowable, the rejoined claims must meet all criteria for patentability including the requirements of 35 U.S.C. 101, 102, 103 and 112. Until all claims to the elected product are found allowable, an otherwise proper restriction requirement between product claims and process claims may be maintained. Withdrawn process claims that are not commensurate in scope with an allowable product claim will not be rejoined. See MPEP § 821.04(b). Additionally, in order to retain the right to rejoinder in accordance with the above policy, applicant is advised that the process claims should be amended during prosecution to require the limitations of the product claims. **Failure to do so may result in a loss of the right to rejoinder**. Further, note that the prohibition against double patenting rejections of 35 U.S.C. 121 does not apply where the restriction requirement is withdrawn by the examiner before the patent issues. See MPEP § 804.01.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to SHULAMITH H. SHAFER whose telephone number is (571)272-3332. The examiner can normally be reached on Monday through Friday, 8 AM to 5 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Joanne Hama can be reached on 571-272-2911. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

SHULAMITH H. SHAFER
Primary Examiner
Art Unit 1647

/SHULAMITH H. SHAFER/
Primary Examiner, Art Unit 1647

Exhibit C

Docket No.: 30610/44556C1
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Daniel J. Wendt et al.

Application No.: 13/466,672

Filed: May 8, 2012

For: Variants of C-type Natriuretic Peptide

Confirmation No.: 1368

Art Unit: 1646

Examiner: S. Shafer

RESPONSE TO RESTRICTION REQUIREMENT

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Madam:

This paper is filed in response to an Office Action (hereinafter "the Action") mailed February 13, 2013 and setting out a Restriction Requirement in the above-mentioned application.

Amendments to the Claims begin on page 2 of this paper.

Remarks begin on page 24 of this paper.

AMENDMENT TO THE CLAIMS

1. (Currently amended) A variant of C-type natriuretic peptide (CNP) selected from the group consisting of:

GDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP53) (SEQ ID NO: 179);

PDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP53) (SEQ ID NO: 185);

MDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP53) (SEQ ID NO: 190)

DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP-53(M48N)] (SEQ ID NO: 180);

LRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-52) (SEQ ID NO: 146);

RVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-51) (SEQ ID NO: 147);

VDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-50) (SEQ ID NO: 148);

DTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-49) (SEQ ID NO: 149);

TKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-48) (SEQ ID NO: 150);

KSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-47) (SEQ ID NO: 151);

SRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-46) (SEQ ID NO: 152);

RAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-45) (SEQ ID NO: 153);

AAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-44) (SEQ ID NO: 154);

AWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-43) (SEQ ID NO: 155);

WARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-42) (SEQ ID NO: 156);

ARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-41) (SEQ ID NO: 157);

RLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-40) (SEQ ID NO: 158);

LLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-39) (SEQ ID NO: 159);

LQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-38) (SEQ ID NO: 160);

QEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-37) (SEQ ID NO: 60);

EHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-36) (SEQ ID NO: 161);

HPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-35) (SEQ ID NO: 162);

PNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-34) (SEQ ID NO: 163);

NARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-33) (SEQ ID NO: 164);

ARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-32) (SEQ ID NO: 165);

RKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-31) (SEQ ID NO: 166);

KYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-30) (SEQ ID NO: 167);

YKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-29) (SEQ ID NO: 168);

KGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-28) (SEQ ID NO: 169);

GANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-27) (SEQ ID NO: 170);

ANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-26) (SEQ ID NO: 171);

NKKGLSKGCFGLKLDRIGSMSGLGC (CNP-25) (SEQ ID NO: 172);

KKGLSKGCFGLKLDRIGSMSGLGC (CNP-24) (SEQ ID NO: 173);

KGLSKGCFGLKLDRIGSMSGLGC (CNP-23) (SEQ ID NO: 174);

LSKGCFGLKLDRIGSMSGLGC (CNP-21) (SEQ ID NO: 175);

SKGCFGLKLDRIGSMSGLGC (CNP-20) (SEQ ID NO: 176);

KGCFGLKLDRIGSMSGLGC (CNP-19) (SEQ ID NO: 177);

GCFGLKLDRIGSMSGLGC (CNP-18) (SEQ ID NO: 178);

GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP37) (SEQ ID NO: 75);

GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (HSA-CNP27) (SEQ ID NO: 144);

GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSNSGLGC [HSA-CNP27(M22N)) (SEQ ID NO: 183)];

PGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-HSA-CNP27)) (SEQ ID NO: 188);

MGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-HSA-CNP27)) (SEQ ID NO: 193);

~~GANRRGLSRGCFGLKLDRIGSMSGLGC [CNP27(K4,5,9R)] (SEQ ID NO: 36);~~

GANRRGLSRGCFGLKLDRIGSNSGLGC [CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);

PGANRRGLSRGCFGLKLDRIGSMSGLGC [Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189);

MGANRRGLSRGCFGLKLDRIGSMSGLGC [Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194);

~~PEG1K-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-CNP27(K4,5,9R)] (SEQ ID NO: 36);~~

PEG1K-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEG1K-CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);

PEG1K-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189);

PEG1K-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194);

~~PEO12-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-CNP27(K4,5,9R)] (SEQ ID NO: 36);~~

PEO12-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEO12-CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);

PEO12-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189);

PEO12-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194);

~~PEO24-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-CNP27(K4,5,9R)] (SEQ ID NO: 36);~~

PEO24-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEO24-CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);

PEO24-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189); and

PEO24-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194), wherein the CNP variant can be conjugated to a moiety selected from a hydrophobic acid and an amino acid sequence from 1 to 20 amino acids useful in bone targeting.

2. (Previously presented) A pharmaceutical composition comprising a CNP variant of claim 1, and a pharmaceutically acceptable excipient, carrier or diluent.

3. (Original) The composition of claim 2, which is a lyophilized formulation prepared from a formulation that comprises a citric acid/citrate buffer or an acetic acid/acetate buffer having a pH from about 4 to about 6.

4. (Previously presented) The composition of claim 3, wherein the lyophilized formulation is prepared from a formulation that further comprises (a) an isotonicity-adjusting agent or a bulking agent or (b) an antioxidant.

5-6. (Cancel)

7. (Previously presented) A method of treating a bone-related disorder or skeletal dysplasia, comprising administering a CNP variant to a subject in need thereof, wherein the CNP variant is a CNP variant according to claim 1, and wherein the bone-related disorder or skeletal dysplasia is selected from the group consisting of achondroplasia, hypochondroplasia, short stature, dwarfism and homozygous achondroplasia.

8. (Cancel)

9. (Currently amended) A method of treating a vascular smooth muscle disorder, comprising administering a CNP variant to a subject, wherein the CNP variant is a CNP variant according to claim 1, and wherein the vascular smooth muscle disorder is selected from the group consisting of hypertension, restenosis, arteriosclerosis, acute decompensated heart failure, congestive heart failure, cardiac edema, nephredema, hepatic edema, acute renal insufficiency, and chronic renal insufficiency, wherein the CNP variant is selected from the group consisting of

GDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP53) (SEQ ID NO: 179);

PDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP53) (SEQ ID NO: 185);

MDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP53) (SEQ ID NO: 190)

DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP-53(M48N)] (SEQ ID NO: 180);

LRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-52) (SEQ ID NO: 146);

RVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-51) (SEQ ID NO: 147);

VDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-50) (SEQ ID NO: 148);

DTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-49) (SEQ ID NO: 149);

TKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-48) (SEQ ID NO: 150);

KSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-47) (SEQ ID NO: 151);

SRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-46) (SEQ ID NO: 152);

RAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-45) (SEQ ID NO: 153);

AAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-44) (SEQ ID NO: 154);

AWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-43) (SEQ ID NO: 155);

WARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-42) (SEQ ID NO: 156);

ARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-41) (SEQ ID NO: 157);

RLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-40) (SEQ ID NO: 158);

LLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-39) (SEQ ID NO: 159);

LQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-38) (SEQ ID NO: 160);

~~QEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-37) (SEQ ID NO: 60);~~

EHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-36) (SEQ ID NO: 161);

HPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-35) (SEQ ID NO: 162);

PNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-34) (SEQ ID NO: 163);

NARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-33) (SEQ ID NO: 164);

ARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-32) (SEQ ID NO: 165);

RKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-31) (SEQ ID NO: 166);

KYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-30) (SEQ ID NO: 167);

YKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-29) (SEQ ID NO: 168);

KGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-28) (SEQ ID NO: 169);

GANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-27) (SEQ ID NO: 170);

ANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-26) (SEQ ID NO: 171);

NKKGLSKGCFGLKLDRIGSMSGLGC (CNP-25) (SEQ ID NO: 172);

KKGLSKGCFGLKLDRIGSMSGLGC (CNP-24) (SEQ ID NO: 173);

KGLSKGCFGLKLDRIGSMSGLGC (CNP-23) (SEQ ID NO: 174);

LSKGCFGLKLDRIGSMSGLGC (CNP-21) (SEQ ID NO: 175);

SKGCFGLKLDRIGSMSGLGC (CNP-20) (SEQ ID NO: 176);

KGCFGLKLDRIGSMSGLGC (CNP-19) (SEQ ID NO: 177);

GCFGLKLDRIGSMSGLGC (CNP-18) (SEQ ID NO: 178);

QEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP37(M32N)] (SEQ ID NO: 182);

PQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP37) (SEQ ID NO: 186);

MQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP37) (SEQ ID NO: 192);

GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP37) (SEQ ID NO: 75);

GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [Gly-CNP37(M32N)]) (SEQ ID NO: 181);

PGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-Gly-CNP37)) (SEQ ID NO: 145);

MGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-Gly-CNP37)) (SEQ ID NO: 191);

GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (HSA-CNP27) (SEQ ID NO: 144);

GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSNSGLGC [HSA-CNP27(M22N)) (SEQ ID NO: 183)];

PGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-HSA-CNP27)) (SEQ ID NO: 188);

MGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-HSA-CNP27)) (SEQ ID NO: 193);

~~GANRRGLSRGCFGLKLDRIGSMSGLGC [CNP27(K4,5,9R)] (SEQ ID NO: 36);~~

GANRRGLSRGCFGLKLDRIGSNSGLGC [CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);

PGANRRGLSRGCFGLKLDRIGSMSGLGC [Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189);

MGANRRGLSRGCFGLKLDRIGSMSGLGC [Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194);

~~PEG1K-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-CNP27(K4,5,9R)] (SEQ ID NO: 36);~~

PEG1K-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEG1K-CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);

PEG1K-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189);

PEG1K-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194);

~~PEO12-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-CNP27(K4,5,9R)] (SEQ ID NO: 36);~~

PEO12-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEO12-CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);

PEO12-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189);

PEO12-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194);

~~PEO24-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-CNP27(K4,5,9R)] (SEQ ID NO: 36);~~

PEO24-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEO24-CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);

PEO24-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189); and

PEO24-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194), wherein the CNP variant can be conjugated to a moiety selected from a hydrophobic acid and an amino acid sequence from 1 to 20 amino acids useful in bone targeting.

10. (Previously presented) A method for recombinant production of a CNP variant, comprising culturing in a medium a host cell comprising a first polynucleotide encoding a CNP variant polypeptide linked to a second polynucleotide encoding a cleavable peptide or protein under conditions that result in expression of a fusion polypeptide encoded by the polynucleotides, wherein the fusion polypeptide comprises the CNP variant polypeptide directly linked to the cleavable peptide or protein or indirectly linked thereto via a linker, wherein the CNP variant is selected from the group consisting of:

R-CNP22(K4R) (Analog AZ) (SEQ ID NO: 41);

ER-CNP22(K4R) (Analog BA) (SEQ ID NO: 39);

GANPR-CNP22(K4R) (Analog CI) (SEQ ID NO: 37);

GANQQ-CNP22(K4R) (Analog CH) (SEQ ID NO: 69);

AAWARLLQEHPNA-CNP22 (Analog CA) (SEQ ID NO: 61);

AAWARLLQEHPNAR-CNP22 (Analog CB)(SEQ ID NO: 62);

DLRVDTKSRAAWAR-CNP22 (Analog CC) (SEQ ID NO: 63);

GQPREPQVYTLPPS-CNP22 (Analog CF) (SEQ ID NO: 79);

GERAFKAWAVARLSQ-CNP22 (Analog CE) (SEQ ID NO: 81);

GHHSHEQHPHGANQQ-CNP22 (Analog CQ) (SEQ ID NO: 76);

GQEHPNARKYKGANPK-CNP22 (Analog CS) (SEQ ID NO: 71);
GQEHPNARKYKGANQK-CNP22 (Analog CT) (SEQ ID NO: 130);
GAHHPHEHDTHGANQQ-CNP22 (Analog CR) (SEQ ID NO: 77);
GQTHSSGTQSGANQQ-CNP22(K4R) (Analog CN) (SEQ ID NO: 87);
SPKMVQGSG-CNP17-KVLRRH (Analog CD) (SEQ ID NO: 68);
PNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-34);
GANRRGLSRGCFGLKLDRIGSMSGLGC [CNP27(K4,5,9R)] (SEQ ID NO: 36);
GANRRGLSRGCFGLKLDRIGSNSGLGC [CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);
PGANRRGLSRGCFGLKLDRIGSMSGLGC [Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189);
MGANRRGLSRGCFGLKLDRIGSMSGLGC [Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194);
GDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP53)) (SEQ ID NO: 179);
PDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP53)) (SEQ ID NO: 185);
MDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP53)) (SEQ ID NO: 190);
DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP-53(M48N)]) (SEQ ID NO: 180);
QEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-37) (SEQ ID NO: 60);
GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP37) (SEQ ID NO: 75);
GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (HSA-CNP27) (SEQ ID NO: 144);
GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSNSGLGC [HSA-CNP27(M22N)]) (SEQ ID NO: 183);
PGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-HSA-CNP27)) (SEQ ID NO: 188);
MGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-HSA-CNP27)) (SEQ ID NO: 193);

LRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-52) (SEQ ID NO: 146);

RVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-51) (SEQ ID NO: 147);

VDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-50) (SEQ ID NO: 148);

DTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-49) (SEQ ID NO: 149);

TKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-48) (SEQ ID NO: 150);

KSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-47) (SEQ ID NO: 151);

SRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-46) (SEQ ID NO: 152);

RAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-45) (SEQ ID NO: 153);

AAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-44) (SEQ ID NO: 154);

AWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-43) (SEQ ID NO: 155);

WARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-42) (SEQ ID NO: 156);

ARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-41) (SEQ ID NO: 157);

RLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-40) (SEQ ID NO: 158);

LLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-39) (SEQ ID NO: 159);

LQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-38) (SEQ ID NO: 160);

EHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-36) (SEQ ID NO: 161);

HPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-35) (SEQ ID NO: 162);

PNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-34) (SEQ ID NO: 163);

NARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-33) (SEQ ID NO: 164);

ARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-32) (SEQ ID NO: 165);
RKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-31) (SEQ ID NO: 166);
KYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-30) (SEQ ID NO: 167);
YKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-29) (SEQ ID NO: 168);
KGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-28) (SEQ ID NO: 169);
GANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-27) (SEQ ID NO: 170);
ANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-26) (SEQ ID NO: 171);
NKKGLSKGCFGLKLDRIGSMSGLGC (CNP-25) (SEQ ID NO: 172);
KKGLSKGCFGLKLDRIGSMSGLGC (CNP-24) (SEQ ID NO: 173);
KGLSKGCFGLKLDRIGSMSGLGC (CNP-23) (SEQ ID NO: 174);
LSKGCFGLKLDRIGSMSGLGC (CNP-21) (SEQ ID NO: 175);
SKGCFGLKLDRIGSMSGLGC (CNP-20) (SEQ ID NO: 176);
KGCFGLKLDRIGSMSGLGC (CNP-19) (SEQ ID NO: 177); and
GCFGLKLDRIGSMSGLGC (CNP-18) (SEQ ID NO: 178).

11-15. (Cancel)

16. (Original) The method of claim 10, wherein the cleavable peptide or protein is selected from the group consisting of histidine tags, human transcription factor TAF12, TAF12 fragments, TAF12 histone fold domain, mutants of TAF12 and fragments thereof, TAF12(C/A), TAF12(D/E), TAF12(4D/4E), TAF12(6D/6E), TAF12(10D/10E), TAF12(C/A & D/E), TAF12(C/A & 4D/4E), TAF12(C/A & 6D/6E), TAF12(C/A & 10D/10E), ketosteroid isomerase, maltose-binding protein, ß-galactosidase, glutathione-S-transferase, thioredoxin, chitin-binding domain, BMP-2, BMP-2 mutants, BMP-2(C/A), and mutants and fragments thereof.

17. (Cancel)

18. (Original) The method of claim 10, wherein the host cell is bacterial.

19-21. (Cancel)

22. (Original) The method of claim 10, wherein the fusion polypeptide is expressed as a soluble protein or as an inclusion body.

23. (Original) The method of claim10, further comprising isolating the expressed fusion polypeptide from the host cell or culture medium.

24. (Original) The method of claim 23, further comprising contacting the isolated fusion polypeptide with a cleaving agent selected from the group consisting of formic acid, cyanogen bromide (CNBr), hydroxylamine, protein self cleavage, Factor Xa, enterokinase, ProTEV, and SUMO protease.

25-29. (Cancel)

30. (Currently amended) A CNP variant produced according to the method of claim 10, wherein the CNP variant is selected from the group consisting of:
~~R-CNP22(K4R) (Analog AZ) (SEQ ID NO: 41);~~
~~ER-CNP22(K4R) (Analog BA) (SEQ ID NO: 39);~~
~~GANPR-CNP22(K4R) (Analog CI) (SEQ ID NO: 37);~~
~~GANQQ-CNP22(K4R) (Analog CH) (SEQ ID NO: 69);~~
~~AAWARLLQEHPNA-CNP22 (Analog CA) (SEQ ID NO: 61);~~
~~AAWARLLQEHPNAR-CNP22 (Analog CB)(SEQ ID NO: 62);~~
~~DLRVDTKSRAAWAR-CNP22 (Analog CC) (SEQ ID NO: 63);~~
~~GQPREPQVYTLPPS-CNP22 (Analog CF) (SEQ ID NO: 79);~~
~~GERAFKAWAVARLSQ-CNP22 (Analog CE) (SEQ ID NO: 81);~~
~~GHHSHEQHPHGANQQ-CNP22 (Analog CQ) (SEQ ID NO: 76);~~
~~GQEHPNARKYKGANPK-CNP22 (Analog CS) (SEQ ID NO: 71);~~
~~GQEHPNARKYKGANQK-CNP22 (Analog CT) (SEQ ID NO: 130);~~
~~GAHHPHEHDTHGANQQ-CNP22 (Analog CR) (SEQ ID NO: 77);~~
~~GQTHSSGTQSGANQQ-CNP22(K4R) (Analog CN) (SEQ ID NO: 87);~~
~~SPKMVQGSG-CNP17-KVLRRH (Analog CD) (SEQ ID NO: 68);~~
PNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-34);
~~GANRRGLSRGCFGLKLDRIGSMSGLGC [CNP27(K4,5,9R)] (SEQ ID NO: 36);~~
GANRRGLSRGCFGLKLDRIGSNSGLGC [CNP27(K4,5,9R, M22N)];
PGANRRGLSRGCFGLKLDRIGSMSGLGC [Pro-CNP27(K4,5,9R)];
MGANRRGLSRGCFGLKLDRIGSMSGLGC [Met-CNP27(K4,5,9R)];

GDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP53)) (SEQ ID NO: 179);

PDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP53)) (SEQ ID NO: 185);

MDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP53) (SEQ ID NO: 190);

DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP-53(M48N)] (SEQ ID NO: 180);

~~QEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-37) (SEQ ID NO: 60);~~

QEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP37(M32N)] (SEQ ID NO: 182);

PQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP37) (SEQ ID NO: 186);

MQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP37) (SEQ ID NO: 192);

~~GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP37) (SEQ ID NO: 75);~~

GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [Gly-CNP37(M32N)] (SEQ ID NO: 181);

MGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-Gly-CNP37) (SEQ ID NO: 191);

GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (HSA-CNP27) (SEQ ID NO: 144);

GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSNSGLGC [HSA-CNP27(M22N)] (SEQ ID NO: 183);

PGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-HSA-CNP27) (SEQ ID NO: 188); and

MGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-HSA-CNP27) (SEQ ID NO: 193).

31. (Currently amended) A host cell comprising an expression vector, the vector comprising a first polynucleotide encoding a CNP variant polypeptide linked to a second

polynucleotide encoding a cleavable peptide or protein, wherein the CNP variant is selected from the group consisting of

GDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP53) (SEQ ID NO: 179);

PDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP53) (SEQ ID NO: 185);

MDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP53) (SEQ ID NO: 190)

DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP-53(M48N)] (SEQ ID NO: 180);

LRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-52) (SEQ ID NO: 146);

RVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-51) (SEQ ID NO: 147);

VDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-50) (SEQ ID NO: 148);

DTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-49) (SEQ ID NO: 149);

TKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-48) (SEQ ID NO: 150);

KSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-47) (SEQ ID NO: 151);

SRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-46) (SEQ ID NO: 152);

RAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-45) (SEQ ID NO: 153);

AAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-44) (SEQ ID NO: 154);

AWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-43) (SEQ ID NO: 155);

WARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-42) (SEQ ID NO: 156);

ARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-41) (SEQ ID NO: 157);

RLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-40) (SEQ ID NO: 158);

LLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-39) (SEQ ID NO: 159);

LQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-38) (SEQ ID NO: 160);

~~QEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-37) (SEQ ID NO: 60);~~

EHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-36) (SEQ ID NO: 161);

HPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-35) (SEQ ID NO: 162);

PNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-34) (SEQ ID NO: 163);

NARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-33) (SEQ ID NO: 164);

ARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-32) (SEQ ID NO: 165);

RKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-31) (SEQ ID NO: 166);

KYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-30) (SEQ ID NO: 167);

YKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-29) (SEQ ID NO: 168);

KGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-28) (SEQ ID NO: 169);

GANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-27) (SEQ ID NO: 170);

ANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-26) (SEQ ID NO: 171);

NKKGLSKGCFGLKLDRIGSMSGLGC (CNP-25) (SEQ ID NO: 172);

KKGLSKGCFGLKLDRIGSMSGLGC (CNP-24) (SEQ ID NO: 173);

KGLSKGCFGLKLDRIGSMSGLGC (CNP-23) (SEQ ID NO: 174);

LSKGCFGLKLDRIGSMSGLGC (CNP-21) (SEQ ID NO: 175);

SKGCFGLKLDRIGSMSGLGC (CNP-20) (SEQ ID NO: 176);

KGCFGLKLDRIGSMSGLGC (CNP-19) (SEQ ID NO: 177);

GCFGLKLDRIGSMSGLGC (CNP-18) (SEQ ID NO: 178);

QEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP37(M32N)] (SEQ ID NO: 182);

PQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP37) (SEQ ID NO: 186);

MQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP37) (SEQ ID NO: 192);

~~GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP37) (SEQ ID NO: 75);~~

GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [Gly-CNP37(M32N)]) (SEQ ID NO: 181);

PGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-Gly-CNP37)) (SEQ ID NO: 145);

MGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-Gly-CNP37)) (SEQ ID NO: 191);

GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (HSA-CNP27) (SEQ ID NO: 144);

GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSNSGLGC [HSA-CNP27(M22N)) (SEQ ID NO: 183)];

PGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-HSA-CNP27)) (SEQ ID NO: 188);

MGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-HSA-CNP27)) (SEQ ID NO: 193);

~~GANRRGLSRGCFGLKLDRIGSMSGLGC [CNP27(K4,5,9R)] (SEQ ID NO: 36);~~

GANRRGLSRGCFGLKLDRIGSNSGLGC [CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);

PGANRRGLSRGCFGLKLDRIGSMSGLGC [Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189);

MGANRRGLSRGCFGLKLDRIGSMSGLGC [Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194);

~~PEG1K-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-CNP27(K4,5,9R)] (SEQ ID NO: 36);~~

~~PEG1K-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEG1K-CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);~~

~~PEG1K-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189);~~

~~PEG1K-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194);~~

~~PEO12-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-CNP27(K4,5,9R)] (SEQ ID NO: 36);~~

~~PEO12-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEO12-CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);~~

~~PEO12-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189);~~

~~PEO12-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194);~~

~~PEO24-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-CNP27(K4,5,9R)] (SEQ ID NO: 36);~~

~~PEO24-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEO24-CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);~~

~~PEO24-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189); and~~

~~PEO24-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194)~~.

32-34. (Cancel)

35. (Original) The host cell of claim 31, wherein the host cell is cultured in a medium under conditions suitable for expression of a fusion polypeptide encoded by the polynucleotides, wherein the fusion polypeptide comprises the CNP variant polypeptide directly linked to the cleavable peptide or protein or indirectly linked thereto via a linker.

36-38. (Cancel)

39. (Currently amended) A method of treating a CNP-responsive condition or disorder, comprising

administering a CNP peptide or variant to a subject, and

monitoring the level of at least one bone- or cartilage-associated biomarker in the subject,

wherein an increase in the level of the at least one bone- or cartilage-associated biomarker indicates a therapeutic effect of the CNP peptide or variant on the subject or the condition or disorder, wherein the CNP variant is selected from the group consisting of

GDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP53) (SEQ ID NO: 179);

PDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP53) (SEQ ID NO: 185);

MDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP53) (SEQ ID NO: 190)

DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP-53(M48N)] (SEQ ID NO: 180);

LRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-52) (SEQ ID NO: 146);

RVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-51) (SEQ ID NO: 147);

VDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-50) (SEQ ID NO: 148);

DTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-49) (SEQ ID NO: 149);

TKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-48) (SEQ ID NO: 150);

KSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-47) (SEQ ID NO: 151);

SRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-46) (SEQ ID NO: 152);

RAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-45) (SEQ ID NO: 153);

AAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-44) (SEQ ID NO: 154);

AWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-43) (SEQ ID NO: 155);

WARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-42) (SEQ ID NO: 156);

ARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-41) (SEQ ID NO: 157);

RLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-40) (SEQ ID NO: 158);

LLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-39) (SEQ ID NO: 159);

LQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-38) (SEQ ID NO: 160);

QEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-37) (SEQ ID NO: 60);

EHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-36) (SEQ ID NO: 161);

HPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-35) (SEQ ID NO: 162);

PNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-34) (SEQ ID NO: 163);

NARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-33) (SEQ ID NO: 164);

ARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-32) (SEQ ID NO: 165);

RKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-31) (SEQ ID NO: 166);

KYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-30) (SEQ ID NO: 167);

YKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-29) (SEQ ID NO: 168);

KGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-28) (SEQ ID NO: 169);

GANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-27) (SEQ ID NO: 170);

ANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-26) (SEQ ID NO: 171);

NKKGLSKGCFGLKLDRIGSMSGLGC (CNP-25) (SEQ ID NO: 172);

KKGLSKGCFGLKLDRIGSMSGLGC (CNP-24) (SEQ ID NO: 173);

KGLSKGCFGLKLDRIGSMSGLGC (CNP-23) (SEQ ID NO: 174);

LSKGCFGLKLDRIGSMSGLGC (CNP-21) (SEQ ID NO: 175);

SKGCFGLKLDRIGSMSGLGC (CNP-20) (SEQ ID NO: 176);

KGCFGLKLDRIGSMSGLGC (CNP-19) (SEQ ID NO: 177);

GCFGLKLDRIGSMSGLGC (CNP-18) (SEQ ID NO: 178);

QEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP37(M32N)] (SEQ ID NO: 182);

PQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP37) (SEQ ID NO: 186);

MQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP37) (SEQ ID NO: 192);

GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP37) (SEQ ID NO: 75);

GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [Gly-CNP37(M32N)]) (SEQ ID NO: 181);

PGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-Gly-CNP37)) (SEQ ID NO: 145);

MGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-Gly-CNP37)) (SEQ ID NO: 191);

GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (HSA-CNP27) (SEQ ID NO: 144);

GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSNSGLGC [HSA-CNP27(M22N)) (SEQ ID NO: 183)];

PGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-HSA-CNP27)) (SEQ ID NO: 188);

MGHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-HSA-CNP27)) (SEQ ID NO: 193);

~~GANRRGLSRGCFGLKLDRIGSMSGLGC [CNP27(K4,5,9R)] (SEQ ID NO: 36);~~

GANRRGLSRGCFGLKLDRIGSNSGLGC [CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);

PGANRRGLSRGCFGLKLDRIGSMSGLGC [Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189);

MGANRRGLSRGCFGLKLDRIGSMSGLGC [Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194);

~~PEG1K-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-CNP27(K4,5,9R)] (SEQ ID NO: 36);~~

PEG1K-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEG1K-CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);

PEG1K-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189);

PEG1K-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEG1K-Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194);

~~PEO12-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-CNP27(K4,5,9R)] (SEQ ID NO: 36);~~

PEO12-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEO12-CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);

PEO12-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189);

PEO12-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO12-Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194);

~~PEO24-GANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-CNP27(K4,5,9R)] (SEQ ID NO: 36);~~

PEO24-GANRRGLSRGCFGLKLDRIGSNSGLGC [PEO24-CNP27(K4,5,9R, M22N)]) (SEQ ID NO: 184);

PEO24-PGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-Pro-CNP27(K4,5,9R)]) (SEQ ID NO: 189); and

PEO24-MGANRRGLSRGCFGLKLDRIGSMSGLGC [PEO24-Met-CNP27(K4,5,9R)]) (SEQ ID NO: 194), wherein the CNP variant can be conjugated to a moiety selected from a hydrophobic acid and an amino acid sequence from 1 to 20 amino acids useful in bone targeting.

40. (Cancel)

41. (Previously presented) The method of claim 39, wherein the at least one bone- or cartilage-associated biomarker is selected from the group consisting of CNP, cGMP, propeptides of collagen type II and fragments thereof, collagen type II and fragments thereof, osteocalcin, proliferating cell nuclear antigen (PCNA), propeptides of type I procollagen (PINP) and fragments thereof, collagen type I and fragments thereof, aggrecan chondroitin sulfate, and alkaline phosphatase.

42-43. (Cancel)

44. (Previously presented) A method of increasing long bone growth, comprising administering a CNP variant according to Claim 1 to a subject in need thereof, where the administration increases long bone growth.

45. (Previously presented) A CNP variant according to Claim 1 useful for increasing long bone growth or treating achondroplasia, hypochondroplasia, short stature, dwarfism, or homozygous achondroplasia in a subject in need thereof.

46. (Previously presented) A CNP variant where the CNP variant is selected from
QEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP37(M32N)] (SEQ ID NO: 182);
PQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP37) (SEQ ID NO: 186);
MQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP37) (SEQ ID NO: 192);
GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [Gly-CNP37(M32N)]) (SEQ ID NO: 181);
PGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-Gly-CNP37)) (SEQ ID NO: 145); and
MGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-Gly-CNP37)) (SEQ ID NO: 191),
wherein the variant is conjugated to a moiety selected from a hydrophobic acid and an amino acid sequence from 1 to 20 amino acids useful in bone targeting.

REMARKS

In the Action, the examiner required restriction of pending claims 1-4, 7, 9, 10, 16, 18, 22-24, 30-31, 35, 39, 41 and 44-46 into Groups I-VI. Applicants hereby provisionally elect Group I (claims 1-4, 30, 45 and 46). In addition, Applicants elect the species Pro-CNP53 (SEQ ID NO: 185) for continued examination.

Applicants respectfully note that claim 45 should be in Group VI as the claim is directed to a composition for use in increasing long bone growth. Claims 1-4, 30 and 46 read on the elected subject matter.

Applicants request rejoinder of the non-elected method claims (Groups II-VI) upon notification of allowance of the elected composition claims.

Amendments to the Claims

Claims 1, 9, 30, 31 and 39 are amended recite particular CNP variants. The amendments include no new matter.

Conclusion

Applicants submit that the present application is in condition for allowance and respectfully request notification of the same.

This paper is accompanied by a Petition for One-Month Extension of Time and the requisite fee. The Director is hereby authorized to charge any deficiency in the fees filed to our Deposit Account No. 13-2855, under Order No. 30610/44556C1.

Dated: April 12, 2013

Respectfully submitted,

By/Katherine L. Neville/
Katherine L. Neville
Registration No.: 53,379
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PTO/SB/22 (03-13)
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PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a)	Docket Number (Optional) 30610/44556C1
Application Number 13/466,672-Conf. #1368	Filed May 8, 2012
For Variants of C-Type Natriuretic Peptide	
Art Unit 1647	Examiner Shulamith H. Shafer

This is a request under the provisions of 37 CFR 1.136(a) to extend the period for filing a reply in the above-identified application.

The requested extension and fee are as follows (check time period desired and enter the appropriate fee below):

	Fee	Small Entity Fee	Micro Entity Fee	
[X] One month (37 CFR 1.17(a)(1))	$200	$100	$50	$ 200.00
[ ] Two months (37 CFR 1.17(a)(2))	$600	$300	$150	$
[ ] Three months (37 CFR 1.17(a)(3))	$1,400	$700	$350	$
[ ] Four months (37 CFR 1.17(a)(4))	$2,200	$1,100	$550	$
[ ] Five months (37 CFR 1.17(a)(5))	$3,000	$1,500	$750	$

[ ] Applicant asserts small entity status. See 37 CFR 1.27.

[ ] Applicant certifies micro entity status. See 37 CFR 1.29.
Form PTO/SB/15A or B or equivalent must either be enclosed or have been submitted previously.

[ ] A check in the amount of the fee is enclosed.

[X] Payment by credit card.

[ ] The Director has already been authorized to charge fees in this application to a Deposit Account.

[X] The Director is hereby authorized to charge any fees which may be required, or credit any overpayment, to Deposit Account Number 13-2855 .

[X] Payment made via EFS-Web.

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

I am the

[ ] applicant/inventor.

[ ] assignee of record of the entire interest. See 37 CFR 3.71. 37 CFR 3.73(b) statement is enclosed (Form PTO/SB/96).

[X] attorney or agent of record. Registration number 53,379 .

[ ] attorney or agent acting under 37 CFR 1.34. Registration number _____ .

/Katherine L. Neville/	April 12, 2013
Signature	Date
Katherine L. Neville	(312) 474-6300
Typed or printed name	Telephone Number

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. Submit multiple forms if more than one signature is required, see below*.

[ ] *Total of 1 forms are submitted.

2220551

Electronic Patent Application Fee Transmittal

Application Number:	13466672
Filing Date:	08-May-2012
Title of Invention:	Variants of C-type Natriuretic Peptide
First Named Inventor/Applicant Name:	Daniel J. Wendt
Filer:	Katherine Lynn Neville/Tiffany Sevcik
Attorney Docket Number:	30610/44556C1

Filed as Large Entity

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Extension - 1 month with $0 paid	1251	1	200	200

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
		Total in USD ($)		200

Electronic Acknowledgement Receipt

EFS ID:	15504947
Application Number:	13466672
International Application Number:	
Confirmation Number:	1368
Title of Invention:	Variants of C-type Natriuretic Peptide
First Named Inventor/Applicant Name:	Daniel J. Wendt
Customer Number:	90849
Filer:	Katherine Lynn Neville
Filer Authorized By:	
Attorney Docket Number:	30610/44556C1
Receipt Date:	12-APR-2013
Filing Date:	08-MAY-2012
Time Stamp:	14:58:22
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Credit Card
Payment was successfully received in RAM	$200
RAM confirmation Number	1439
Deposit Account	132855
Authorized User	NEVILLE, KATHERINE L.
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		44556C1_response_to_restriction_1.pdf	61433 6fa7b7f729f6b130ed15349a29c1afe1513c0542	yes	24
	Multipart Description/PDF files in .zip description				
	Document Description		Start	End	
	Response to Election / Restriction Filed		1	1	
	Claims		2	23	
	Applicant Arguments/Remarks Made in an Amendment		24	24	
Warnings:					
Information:					
2	Extension of Time	One_Month_Request_for_Extension_of_Time_Under_37_CFR_1136a_2.pdf	21846 f0c89eaeea901ef9fa5fbe5e514bc0ccbd2281ca	no	1
Warnings:					
Information:					
3	Fee Worksheet (SB06)	fee-info.pdf	30284 078a9801ebb9e9137ed03e55c506e965ca9fedc8	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	113563		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

<u>New Applications Under 35 U.S.C. 111</u>
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

<u>National Stage of an International Application under 35 U.S.C. 371</u>
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

<u>New International Application Filed with the USPTO as a Receiving Office</u>
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Exhibit D

Examiner-Initiated Interview Summary	**Application No.** 13/466,672	**Applicant(s)** WENDT ET AL.	
	Examiner SHULAMITH H. SHAFER	**Art Unit** 1647	

All participants (applicant, applicant's representative, PTO personnel):

(1) *SHULAMITH H. SHAFER*. (3)_____.

(2) *Atty K.L.Neville*. (4)_____.

Date of Interview: *23 July 2013*.

Type: ☒ Telephonic ☐ Video Conference
☐ Personal [copy given to: ☐ applicant ☐ applicant's representative]

Exhibit shown or demonstration conducted: ☐ Yes ☒ No.
If Yes, brief description: _____.

Issues Discussed ☐101 ☐112 ☐102 ☐103 ☒Others
(For each of the checked box(es) above, please describe below the issue and detailed description of the discussion)

Claim(s) discussed: *1-4,10,16,18,22-24,30,44 and 45*.

Identification of prior art discussed: *N/A*.

Substance of Interview
(For each issue discussed, provide a detailed description and indicate if agreement was reached. Some topics may include: identification or clarification of a reference or a portion thereof, claim interpretation, proposed amendments, arguments of any applied references etc...)

Examiner indicated SEQ ID NO:185, the elected species is free of the prior art. Examiner indicated will search other sequences based on CNP53, SEQ ID NOs:179,190 and 180. These have been found free of the art. Examiner will rejoin and examine appropriate method claims:7, 10 and dependents and 44. Atty Neville authorized cancellation of non-elected sequences and claims to non-elected sequences..

Applicant recordation instructions: It is not necessary for applicant to provide a separate record of the substance of interview.

Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

☐ Attachment

/SHULAMITH H. SHAFER/ Primary Examiner, Art Unit 1647	

Exhibit E

US008598121B2

(12) **United States Patent**
Wendt et al.

(10) **Patent No.: US 8,598,121 B2**
(45) **Date of Patent: Dec. 3, 2013**

(54) **VARIANTS OF C-TYPE NATRIURETIC PEPTIDE**

(75) Inventors: **Daniel J. Wendt**, Walnut Creek, CA (US); **Shinong Long**, Milpitas, CA (US); **Sianna Castillo**, San Francisco, CA (US); **Christopher P. Price**, Kentfield, CA (US); **Mika Aoyagi-Scharber**, Mill Valley, CA (US); **Michel Claude Vellard**, San Rafael, CA (US); **Augustus O. Okhamafe**, Concord, CA (US)

(73) Assignee: **Biomarin Pharmaceutical Inc.**, Novato, CA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **13/466,672**

(22) Filed: **May 8, 2012**

(65) **Prior Publication Data**

US 2012/0316114 A1 Dec. 13, 2012

Related U.S. Application Data

(63) Continuation of application No. 12/784,117, filed on May 20, 2010, now Pat. No. 8,198,242.

(60) Provisional application No. 61/180,112, filed on May 20, 2009, provisional application No. 61/254,563, filed on Oct. 23, 2009.

(51) **Int. Cl.**

A61K 38/22	(2006.01)
A61K 38/00	(2006.01)
A61K 38/16	(2006.01)
C12P 21/04	(2006.01)

(52) **U.S. Cl.**

USPC **514/12.4**; 514/16.7; 514/21.3; 435/69.4; 435/69.7; 435/70.1; 530/324

(58) **Field of Classification Search**

None

See application file for complete search history.

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(57) **ABSTRACT**

The present disclosure provides variants of C-type natriuretic peptide (CNP), pharmaceutical compositions comprising CNP variants, and methods of making CNP variants. The CNP variants are useful as therapeutic agents for the treatment of diseases responsive to CNP, including but not limited to bone-related disorders, such as skeletal dysplasias (e.g., achondroplasia), and vascular smooth muscle disorders (e.g., restenosis and arteriosclerosis).

14 Claims, 61 Drawing Sheets

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What is claimed is:

1. A variant of C-type natriuretic peptide (CNP) selected from the group consisting of:

(SEQ ID NO: 179)
GDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMS GLGC (Gly-CNP53);

(SEQ ID NO: 185)
PDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMS GLGC (Pro-CNP53);

(SEQ ID NO: 190)
MDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMS GLGC (Met-CNP53)

(SEQ ID NO: 180)
DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNS GLGC [CNP-53(M48N)].

2. A pharmaceutical composition comprising a CNP variant of claim 1, and a pharmaceutically acceptable excipient, carrier or diluent.

3. The composition of claim 2, which is a lyophilized formulation prepared from a formulation that comprises a citric acid/citrate buffer or an acetic acid/acetate buffer having a pH from about 4 to about 6.

4. The composition of claim 3, wherein the lyophilized formulation is prepared from a formulation that further comprises (a) an isotonicity-adjusting agent or a bulking agent or (b) an antioxidant.

5. A method of treating a bone-related disorder or skeletal dysplasia, comprising administering a CNP variant to a subject in need thereof, wherein the CNP variant is a CNP variant according to claim 1, and wherein the bone-related disorder or skeletal dysplasia is selected from the group consisting of achondroplasia, hypochondroplasia, short stature, dwarfism and homozygous achondroplasia.

6. A method for recombinant production of a CNP variant, comprising culturing in a medium a host cell comprising a first polynucleotide encoding a CNP variant polypeptide linked to a second polynucleotide encoding a cleavable peptide or protein under conditions that result in expression of a fusion polypeptide encoded by the polynucleotides, wherein the fusion polypeptide comprises the CNP variant polypeptide directly linked to the cleavable peptide or protein or indirectly linked thereto via a linker, wherein the CNP variant is selected from the group consisting of:

(SEQ ID NO: 179)
GDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMS GLGC (Gly-CNP53);

(SEQ ID NO: 185)
PDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMS GLGC (Pro-CNP53);

(SEQ ID NO: 190)
MDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMS GLGC (Met-CNP53);

(SEQ ID NO: 180)
DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNS GLGC [CNP-53(M48N)].

7. The method of claim 6, wherein the cleavable peptide or protein is selected from the group consisting of histidine tags, human transcription factor TAF12, TAF12 fragments, TAF12 histone fold domain, mutants of TAF12 and fragments thereof, TAF12(C/A), TAF12(D/E), TAF12(4D/4E), TAF12 (6D/6E), TAF12(10D/10E), TAF12(C/A & D/E), TAF12 (C/A & 4D/4E), TAF12(C/A & 6D/6E), TAF12(C/A & 10D/10E), ketosteroid isomerase, maltose-binding protein, β-galactosidase, glutathione-S-transferase, thioredoxin, chitin-binding domain, BMP-2, BMP-2 mutants, BMP-2(C/A), and mutants and fragments thereof.

8. The method of claim 6, wherein the host cell is bacterial.

9. The method of claim 6, wherein the fusion polypeptide is expressed as a soluble protein or as an inclusion body.

10. The method of claim 6, further comprising isolating the expressed fusion polypeptide from the host cell or culture medium.

11. The method of claim 10, further comprising contacting the isolated fusion polypeptide with a cleaving agent selected from the group consisting of formic acid, cyanogen bromide (CNBr), hydroxylamine, protein self cleavage, Factor Xa, enterokinase, ProTEV, and SUMO protease.

12. A CNP variant produced according to the method of claim 6, wherein the CNP variant is selected from the group consisting of:

(SEQ ID NO: 179)
CDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMS
GLGC (Gly-CNP53));

(SEQ ID NO: 185)
PDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMS
GLGC (Pro-CNP53));

(SEQ ID NO: 190)
MDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMS
GLGC (Met-CNP53));

(SEQ ID NO: 180)
DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSG
LGC [CNP-53(M48N)]).

13. A method of increasing long bone growth, comprising administering a CNP variant according to claim 1 to a subject in need thereof, where the administration increases long bone growth.

14. A CNP variant according to claim 1 useful for increasing long bone growth or treating achondroplasia, hypochondroplasia, short stature, dwarfism, or homozygous achondroplasia in a subject in need thereof.

* * * * *

AMENDMENTS TO THE CLAIMS

1-43. (Canceled)

44. (New) A variant of C-type natriuretic peptide (CNP) comprising the amino acid sequence LQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (SEQ ID NO: 160).

45. (New) The CNP variant of claim 44 further comprising a synthetic polymeric group.

46. (New) The CNP variant of claim 45 wherein the synthetic polymeric group comprises a hydrophilic polymeric moiety.

47. (New) The CNP variant of claim 46 wherein the hydrophilic polymeric moiety comprises polyethylene glycol (PEG).

48. (New) The CNP variant of claim of 46 wherein the hydrophilic polymeric moiety is coupled to the amino acid sequence through a stable linkage.

49. (New) The CNP variant of claim of 46 wherein the hydrophilic polymeric moiety is coupled to the amino acid sequence through a hydrolysable linkage.

50. (New) A pharmaceutical composition comprising a variant of C-type natriuretic peptide (CNP) consisting of the amino acid sequence LQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (SEQ ID NO: 160) as the active agent.

51. (New) The pharmaceutical composition of claim 50 further comprising a pharmaceutically acceptable excipient, carrier, or diluent.

52. (New) A method of treating a bone-related disorder or skeletal dysplasia, comprising administering the pharmaceutical composition according to claim 50 to a subject, wherein the administering treats the bone-related disorder or skeletal dysplasia.

53. (New) The method of claim 52 wherein the bone-related disorder or skeletal dysplasia is achondroplasia and wherein administration treats achondroplasia.

54. (New) A method of increasing long bone growth comprising administering the pharmaceutical composition according to claim 50 to a subject in need thereof, wherein the administering increases long bone growth.

55. (New) A method of increasing long bone growth comprising contacting chondrocytes of a subject in need thereof with an effective amount of a CNP variant having the amino acid sequence LQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (SEQ ID NO: 160).

56. (New) The method of claim 55 wherein the chondrocytes are located within a bone growth plate of the subject.

57. (New) The method of claim 55 wherein the CNP variant binds to and activates natriuretic peptide receptor B (NPR-B) on the chondrocytes.

58. (New) A method of treating a bone-related disorder or skeletal dysplasia comprising contacting chondrocytes of a subject in need thereof with an effective amount of a CNP variant having the amino acid sequence LQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (SEQ ID NO: 160).

59. (New) The method of claim 58 wherein the chondrocytes are located within a bone growth plate of the subject.

60. (New) The method of claim 58 wherein the CNP variant binds to and activates natriuretic peptide receptor B (NPR-B) on the chondrocytes.

61. (New) The method of claim 58 wherein the bone-related disorder or skeletal dysplasia is achondroplasia.

EXHIBIT I

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Prolonging the Action of Protein and Peptide Drugs by a Novel Approach of Reversible Polyethylene Glycol Modification*

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Polyethylene glycol (PEG)-conjugated therapeutic peptides/proteins have been shown to exhibit clinical properties superior to those of their corresponding unmodified parent molecules. However, the desirable pharmacological features gained by protein PEGylation become irrelevant if conjugates are inactivated or cannot reach their target tissues. Here we describe the design and synthesis of MAL-FMS-OSU. This bifunctional agent enables PEG chains to be linked to peptides and proteins through a slowly hydrolysable chemical bond. PEG-FMS-peptide/protein conjugates thus formed undergo spontaneous hydrolysis at a slow rate upon incubation at pH 8.5, 37 °C with a $t_{1/2}$ value of 8–14 ± 2 h, generating the unmodified parent molecule. The validity of this approach was studied with exendin-4 and human growth hormone. A single subcutaneous administration of $PEG_{40,000}$-FMS-exendin-4 facilitated a prolonged and stable reduction in glucose levels in mice ($t_{1/2}$ = 30 ± 2 h) and exceeded the effect obtained by the same dose of the native hormone by 7–8 times.

Recently, several polyethylene glycol (PEG)[1]-conjugated therapeutic proteins have been shown to exhibit clinical properties superior to those of their corresponding unmodified parent proteins (1–3). Apparently, covalent binding of PEG to proteins produces steric interference, protecting conjugates from proteolysis and decreasing their rate of clearance from the circulation system via intracellular uptake and kidney filtration (2, 6, 7).

To achieve these desired pharmacological features, several low molecular weight PEG chains or a single high molecular weight PEG must be linked to a protein (8). However, this often drastically decreases the biological or pharmacological potency of the protein (4, 5). Therefore, to maximize protection while minimizing reductions in bioactivity, PEGylation is usually applied to higher molecular weight proteins that are less likely to be inactivated by the procedure. However even such modification may lead to marked reduction in activity. For example, a currently used 40-kDa PEG-interferon-α2a exhibits only 7% of the activity of the native cytokine, thus necessitating the administration of higher doses (9). Furthermore, in contrast to its parent protein this 40-kDa PEG-interferon-α2a does not readily penetrate all target tissues, as it is restricted to the blood and interstitial fluid (10).

Several attempts to overcome these obstacles through reversible protein/peptide PEG-assisted delivery (*e.g.* by employing detachable PEG chains) have been reported (11–15). They suffer, however, from major disadvantages including their dependence on enzymatic hydrolysis as the rate-determining step of PEG-parent molecule dissociation (11–13), potentially making the behavior of the molecules unpredictable and their utilization impractical because of steric considerations and inappropriate enzyme availability. An additional disadvantage is the nonspecific PEGylation of undesired protein/peptide functional moieties (*e.g.* hydroxyls, imidazole, thiols (14)), potentially causing post-PEGylation inter- and intramolecular reactions (14, 15) that could generate complex mixtures and immune-related risks.

In view of these issues, we tested a novel approach for designing PEG-peptide/protein conjugates from which PEG is released by spontaneous chemical hydrolysis. Reversible PEG-protein conjugation permits time-dependent reactivation of inactive PEGylated molecules. An appropriate conjugate would have to undergo slow and spontaneous hydrolysis under physiological conditions to yield the intact parent molecule (16–19). Our contributions are brought here in detail, using the antidiabetic peptide exendin-4 and human growth hormone (hGH) as *in vivo* and *in vitro* models, respectively.

EXPERIMENTAL PROCEDURES

Materials—Exendin-4 (HGEGTFTSDLSKQMEEEAVRLFIEWLKNGGPSSGAPPPS-NH_2) and a non-lysine-containing synthetic peptide of 27 amino acids (AEISGQLSYVRDVNSWQHIWTNVSIEN) were synthesized by the solid phase method using a multiple peptide synthesizer, AMS 422 (Abimed Analyser Technik, GmbH). An Fmoc (*N*-(9-fluorenyl)methoxycarbonyl) strategy was employed throughout the peptide chain assembly. α-Lactalbumin, 5,5′-dithiobis(2-nitrobenzoic acid), reduced glutathione, and trinitrobenzene sulfonic acid were purchased from Sigma. $PEG_{40,000}$-OSu (mPEG2-*N*-hydroxysuccinimide ester) was purchased from Shearwater (Huntsville, AL). All other materials used in this study were of analytical grade. A Centricon-50 ultrafiltration device for aqueous solutions was purchased from Millipore S.A.

Ultraviolet spectra were obtained by using a Beckman DU 7500 spectrophotometer in 1-cm path length UV cuvettes. Mass spectra were determined using MALDI-TOF and ESMS techniques (Bruker-Reflex-Reflectron model and VG-platform-II electrospray single quadropole mass spectrometer, Micro Mass, respectively).

HPLC analyses were performed using a Spectra-Physics SP8800 liquid chromatography system equipped with an Applied Biosystems 757 variable wavelength absorbance detector and a Spectra-SYSTEM

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[1] The abbreviations used are: PEG, polyethylene glycol; hGH, human growth hormone; MALDI-TOF, matrix-assisted laser desorption ionization time-of-flight; ESMS, electrospray single quadropole mass spectrometer; MAL, maleimide; FMS, 9-hydroxymethyl-7-sulfofluorene-*N*-hydroxysuccinimide; OSu, hydroxysuccinimide ester; HPLC, high pressure liquid chromatography; TLC, thin layer chromatography; PBS, phosphate-buffered saline.

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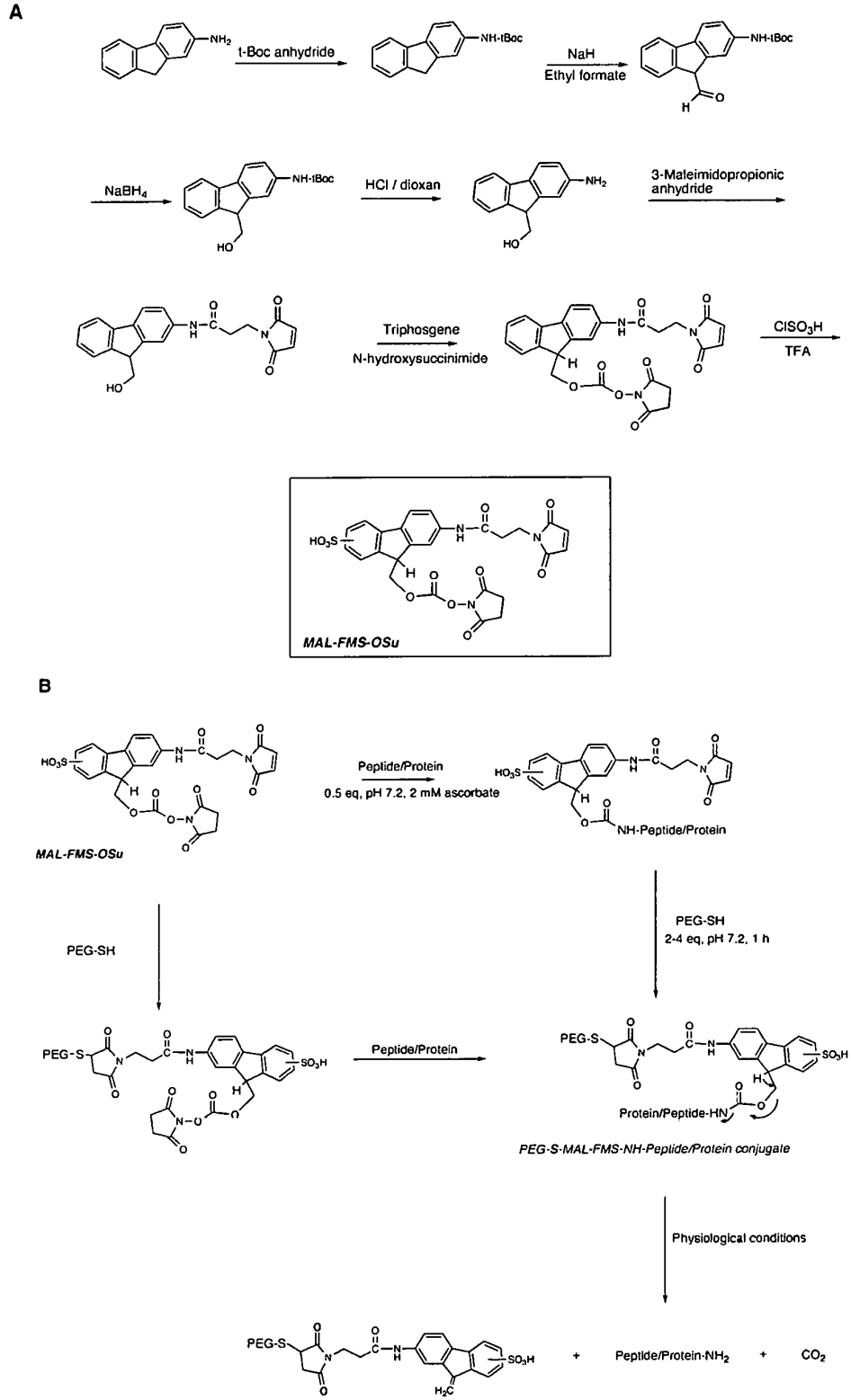

FIG. 1. **Schematic illustration of the synthesis and the structure of MAL-FMS-OSu (*A*) and of the two possible experimental procedures for preparing PEG-FMS-peptide/protein conjugates (*B*).**

P2000 liquid chromatography system equipped with a Spectra-SYSTEM AS100 autosampler and a Spectra-SYSTEM UV1000, all controlled by a ThermoQuest chromatography data system (ThermoQuest Inc., San Jose, CA). The column effluents were monitored by UV absorbance at 220 nm. Analytical reverse phase-HPLC was performed using a pre-packed Chromolith™ Performance RP-18e column (4.6 ×

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100 mm, Merck, Darmstadt, Germany). The column was eluted with a binary gradient of 10–100% solution B over 10 min with a flow rate of 3 ml/min (solution A was 0.1% trifluoroacetic acid in H_2O and solution B was 0.1% trifluoroacetic acid in acetonitrile:H_2O; 3:1, v/v). PEGylated compounds were analyzed using an RP-4 column (250 × 4 mm, 5-μm bead size, Vydac, Hesperia, CA) with a binary gradient of 10–100% solution B for 50 min at a flow rate of 1 ml/min.

Preparative separations were performed with pre-packed Vydac RP-18 or RP-4 columns (250 × 22 mm). The column was eluted with 10–100% solution B over 60 min (12 ml/min).

MAL-FMS-OSu—9-Hydroxymethyl-2-amino fluorene was synthesized with an 84% yield as described elsewhere (20). Conditions were as follows: TLC, chloroform:methanol, 9:1 (v/v); R_F = 0.38; calculated ESMS = 211.26 Da, and found ESMS for $[M+H]^+$ = 212.10 Da.

3-Maleimidopropionic Anhydride—3-Maleimidopropionic acid (1 g, 5.9 mmol) and *N,N'*-dicyclohexylcarbodiimide (0.69 g, 2.95 mmol) were dissolved in 10 ml of *N,N*-dimethylformamide and incubated for 4 h. Dicyclohexylurea was filtered out, and the anhydride formed was kept at 4 °C.

9-Hydroxymethyl-2-amino fluorene (0.4 g, 1.9 mmol) and $NaHCO_3$ (0.74 g, 8.85 mmol) were dissolved in water and 3-maleimidopropionic anhydride in *N,N*-dimethylformamide (12 ml, 2.95 mmol) was added. The reaction mixture was stirred for 40 min, and product formation was monitored by analytical HPLC on a Chromolith column (R_t = 4.46 min, 10–100% solution B in 10 min, 3 ml/min). The crude product was purified using preparative HPLC (RP-18 column, 10–100% acetonitril: water (75:25; v/v), 60 min, 12 ml/min). The yield was 57%, 1.08 mmol, 0.39 g; calculated ESMS = 362.38 Da, and found ESMS = 362.42 Da.

Pyridine (0.167 ml, 2 mmol) was added dropwise to a stirred solution of 9-hydroxymethyl-2-(amino-3-maleimidopropionate)fluorene (0.37g, 1.02 mmol) and triphosgene (0.425 g, 1.43 mmol) in dry tetrahydrofuran (10 ml). After 20 min the precipitated pyridine hydrochloride salt was filtered out, and the tetrahydrofuran was removed by vacuum. The oil obtained was dissolved in 10 ml of dry tetrahydrofuran with *N*-hydroxysuccinimide (0.61g, 5.3 mmol). Pyridine (0.26 ml, 3.2 mmol) was then added, and the solution was stirred for 20 min. Some additional precipitated pyridine hydrochloride salt was filtered out, and the tetrahydrofuran was removed by vacuum. The oil obtained was dissolved in chloroform (100 ml) and washed with an aqueous $NaHCO_3$ solution (0.5 N, 3 × 50 ml), HCl (0.1 N, 3 × 50 ml), water (2 × 50 ml), and brine. The chloroform was removed by vacuum, and the product was desiccated. Conditions were as follows: yield was 89%, 0.9 mmol, 0.45 g; HPLC (Chromolith column) R_t = 5.7 min (10–100% solution B in 10 min, 3 ml/min). Calculated ESMS = 503 Da, found ESMS for $[M+Na]^+$ = 526.38 Da, and found ESMS for $[M+K]^+$ = 542.30 Da.

9-Hydroxymethyl-2-(amino-3-maleimidopropionate)-7-sulfofluorene N-Hydroxysuccinimide—To a solution of 9-hydroxymethyl-2-(amino-3-maleimidopropionate)-fluorene *N*-hydroxysuccinimide (0.2 mmol, 0.1 g) in trifluoroacetic acid (10 ml), chlorosulfonic acid (0.5 ml) was added. After 15 min, cold ether (90 ml) was added, and the precipitated product was washed three times with ether and desiccated. Conditions were as follows: yield: 95%, 0.11 g, 0.19 mmol; HPLC (Chromolith column) R_t = 2.65 min (10–100% solution B in 10 min, 3 ml/min). The calculated ESMS = 583 Da; found ESMS for $[M-H]^+$ = 582.24 Da, for $[M+H]^+$ = 584.52 Da, and for $[M+Na]^+$ = 606.47 Da.

$PEG_{40,000}$-SH—$PEG_{40,000}$-OSu was dissolved at a concentration of 40 mg/ml in an aqueous solution of cystamine-di-HCl (1 M) and brought to pH 8.5 with $NaHCO_3$. Reaction was carried out for 2 h at 25 °C. The product thus obtained was dialyzed overnight against 0.1 M $NaHCO_3$, treated with 30 mM dithiothreitol (25 °C, 1 h), and redialyzed against 0.01 M HCl. $PEG_{40,000}$-SH was obtained with a 93% yield. It contained 1 mol sulfhydryl moiety per mol of $PEG_{40,000}$ as determined with 5,5'-dithiobis(2-nitrobenzoic acid) in the presence of ascorbate.

Preparation of PEG_{5000}-2-Sulfo-9-fluorenylmethoxycarbonyl N-Hydroxysuccinimide—MAL-FMS-OSu (1.8 mg, 3 μmol) was added to a solution of PEG_{5000}-SH (15 mg, ~3 μmol) in phosphate-buffered saline (PBS) (0.5 ml, pH 7.4), and the reaction solution was stirred for 30 min. The product PEG_{5000}-FMS-OSu was purified by HPLC on an RP-4 column and lyophilized.

PEG_{5000}-FMS-Exendin-4—PEG_{5000}-FMS-OSu (2.3 mg, ~0.42 μmol) was added to a solution of exendin-4 (1.6 mg, 0.4 μmol) in PBS (0.5 ml, pH 8), and the reaction solution was stirred for 2 h. The product PEG_{5000}-FMS-exendin-4 was purified by HPLC on an RP-4 column and lyophilized.

Preparation of $PEG_{40,000}$-FMS-Exendin-4—To a stirred solution of exendin-4 (1 mg in 1.0 ml of 0.1 M phosphate buffer, 2 mM ascorbate, pH 7.2, 0.239 mM) was added 280 μg of MAL-FMS-OSu (28 μl from a fresh solution of MAL-FMS-OSu in *N,N*-dimethylformamide, 10 mg/ml, 2.0 molar excess over the peptide). After 7 min, solid $PEG_{40,000}$-SH was added to a final concentration of 0.5 mM (2.1 molar excess over the peptide). The reaction was carried out for 1 h, dialyzed overnight against H_2O, and then further filtered through Centricon with a molecular mass cutoff of 50 kDa to remove any residual exendin-4 or MAL-FMS-exendin-4 that had not linked to $PEG_{40,000}$-SH. The concentration of the conjugate was determined by amino acid analysis according to the content of aspartic acid (two residues), alanine (two residues) and valine (one residue), or calculated from the absorbance of the conjugate at 280 nm. The calculated extinction coefficient at 280 nm was 26,940 ± 100, a value that is the additive absorbance of exendin-4 (ϵ_{280} = 5740) and $PEG_{40,000}$-FMS (ϵ_{280} = 21,200). A solution of 20 μM $PEG_{40,000}$-FMS-exendin had A = 0.51. Thus the conjugate had a 1:1 $PEG_{40,000}$-FMS/exendin stoichiometry.

Preparation of $PEG_{40,000}$-Exendin-4—Exendin-4 (0.3 mg, 75 nmol) was dissolved in PBS, pH 7.5, and reacted with $PEG_{40,000}$-OSu (20 mg, 470 nmol) for 3 h, filtered (7 times) through Centricon (cut off, 50 kDa), and characterized by MALDI-TOF mass spectrometry (found: 48,074 Da).

$PEG_{40,000}$-FMS-peptide 27 was prepared as described for $PEG_{40,000}$-

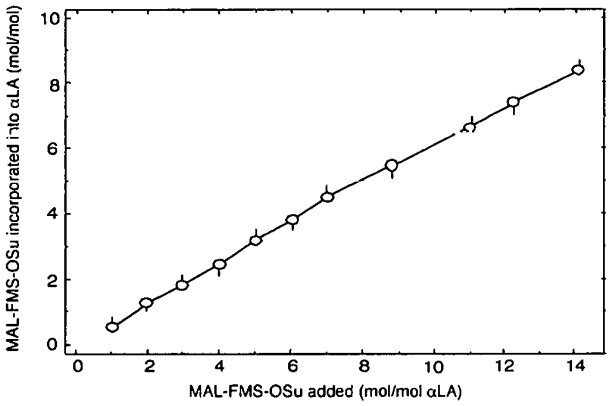

FIG. 2. **Degree of incorporation of MAL-FMS-OSu at pH 7.2 into α-lactalbumin as a function of the amount of added reagent.** To samples of α-lactalbumin (1.0 ml of 1 mg/ml in 0.1 M phosphate buffer, pH 7.2) MAL-FMS-OSu was added at concentrations ranging from 1 eq up to 14 molar eq of MAL-FMS-OSu. For each treatment the amount incorporated into the protein was determined by the absorbance at 280 nm after dialysis and by quantitating the amount of unmodified amino side chain moieties with trinitrobenzene sulfonic acid.

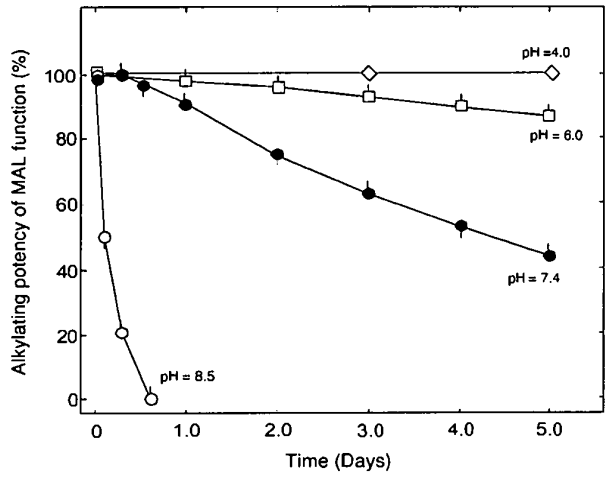

FIG. 3. **Stability of the maleimide functional moiety in MAL-FMS-OSu in aqueous solutions having different pH values.** MAL-FMS-OSu (1 mM) was incubated at room temperature in H_2O (pH 6.0), in 0.007 M acetic acid (pH~4.0), in 0.1 M phosphate buffer (pH 7.4), and in 0.1 M $NaHCO_3$ (pH 8.5). At the indicated time points, aliquots were allowed to react with a slight excess of reduced glutathione (15 min at pH 7.2), and the concentration of unreacted reduced glutathione was determined with 5,5'-dithiobis(2-nitrobenzoic acid).

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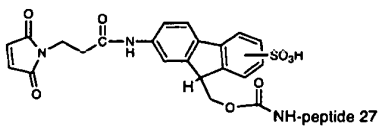
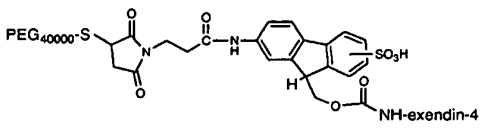
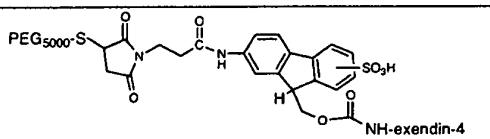
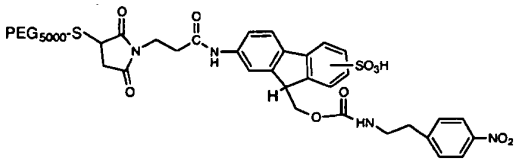
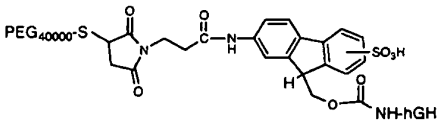
TABLE I
Structures and half-lives of PEG-FMS conjugates

	Structure	k (h^{-1})[b]	$t_{1/2}$ (h)[c]
Mal-FMS-Peptide 27[a]		0.082	8.4
PEG_{40000}-FMS-exendin-4		0.058	11.9
PEG_{5000}-FMS-exendin-4		0.050	13.8
PEG_{5000}-FMS-4-nitro-phenethylamine		0.074	9.4
PEG_{40000}-FMS-human growth hormone (hGH)		0.059	11.8

[a] Rate of hydrolysis was followed by trinitrobenzene sulfonic acid assay.
[b] k is the slope constant derived from the plot of ln(PEG-FMS conjugate) against time (h).
[c] $t_{1/2}$ was determined from the formula $t_{1/2} = \ln 2/k$.

FMS-exendin-4. MAL-FMS-OSu (280 μg) was added to a solution of hGH (4.5 mg, 0.225 μmol) in 0.5 ml of PBS, pH 7.4. After 10 min $PEG_{40,000}$-SH (10 mg) was added, and the reaction mixture was stirred for 1 h and dialyzed overnight against water. An hGH displacement assay was performed as described elsewhere (21).

Glucose-lowering Assay of $PEG_{40,000}$-FMS-Exendin-4—Three groups of CD1 mice (n = 6 per group) subcutaneously received saline, native exendin-4 (4 μg/mouse), or $PEG_{40,000}$-FMS-exendin-4 (4 μg of peptide/mouse). Circulating glucose levels were measured with a glucose analyzer (Beckman Instruments) by the glucose oxidase method. Blood samples for the blood glucose analyses were taken from the tail veins. The level of glucose in normal healthy CD1-mice was 140 ± 7 mg/dl (7.77 mM).

Glucose-lowering Assay of Released Exendin-4—$PEG_{40,000}$-FMS-exendin-4 (0.36 mg containing 30 μg of exendin-4) was incubated for 24 h at pH 8.5, 37 °C. The released peptide was separated from the $PEG_{40,000}$-sulfodibezofulvene using HPLC on a Chromolith column (10–100% solution B in 10 min, 3 ml/min), and the peptide solution was lyophilized. The peptide concentration was determined by its absorbance at 220 nm. Native exendin-4 and the released exendin-4 were dissolved in saline and administered subcutaneously at identical doses to five groups of CD1 mice (n = 3 per group). Circulating glucose levels were then determined as described above.

RESULTS

We have designed, synthesized, and characterized MAL-FMS-OSu, Fig. 1*A*). This is a water-soluble heterobifunctional reagent consisting of a sulfonated fluorenyloxycarbonyl *N*-hydroxysuccinimide ester that reacts with peptide and protein amino groups. A maleimide group was attached to the fluorenyl backbone to enable coupling to sulfhydryl-containing PEG-SH (Fig. 1*B*). At 320 nm, where peptides or proteins absorb negli-

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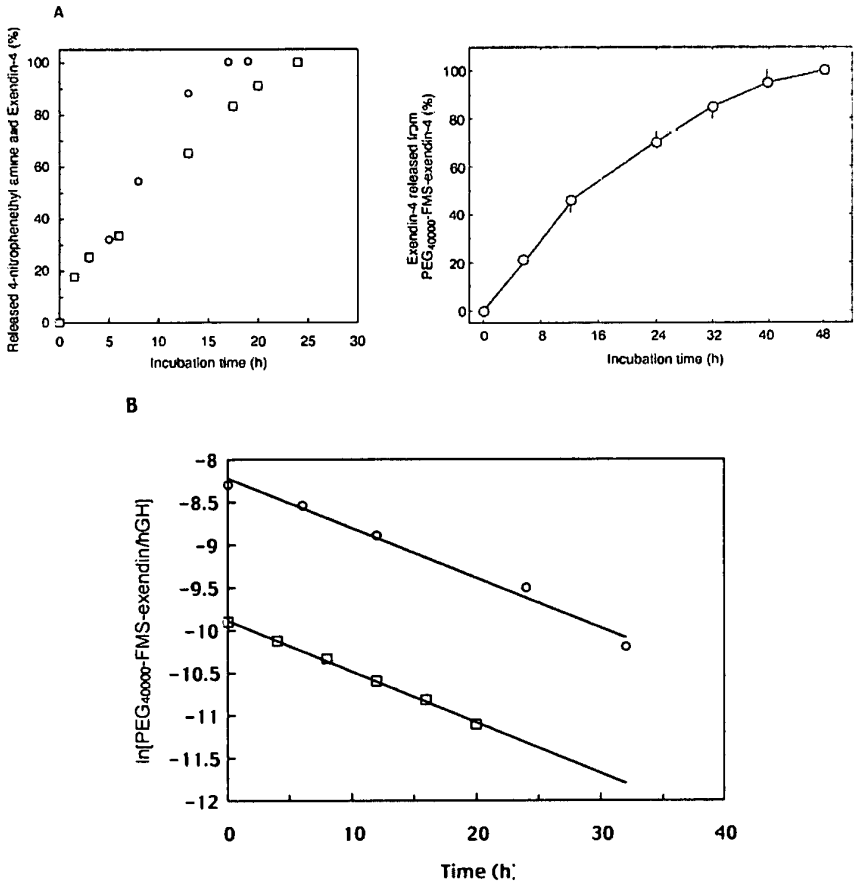

FIG. 4. The rate of hydrolysis of PEG-FMS conjugates upon incubation at pH 8.5, 37 °C is shown in *A*. Solutions of $PEG_{40,000}$-FMS-exendin-4 (*right*, O), PEG_{5000}-FMS-exendin-4 (*left*, O), and PEG_{5000}-FMS-4-nitro-phenethyl amine (*left*, □) were incubated in PBS at pH 8.5, 37 °C. At the indicated time points, aliquots (50 μl) were analyzed using HPLC on an RP-4 column. Results are expressed as percent of the maximal peak area of released exendin-4 and 4-nitrophenethylamine as a function of time. *B*, the concentration of $PEG_{40,000}$-FMS-exendin-4 (O) and $PEG_{40,000}$-FMS-hGH (□) was determined for each time point by HPLC. The linear plot obtained indicates that the rate of hydrolysis is of first order reaction. The half-life time of the conjugates was calculated from $t_{1/2} = \ln 2/k$, when k is the slope of the linear plot (h^{-1}).

gibly, MAL-FMS-OSu (either free or covalently bound to proteins) absorbs with a molar extinction coefficient (ϵ_{320}) of 16,100 ± 150 mol^{-1} cm^{-1}.

Degree of MAL-FMS Incorporation as a Function of the Spacer Added—In Fig. 2, samples of α-lactalbumin (1 mg/ml) were modified with increasing concentrations of MAL-FMS-OSu in 0.1 M PBS, pH 7.2, for 30 min at 25 °C. That is, under experimental conditions where the maleimide-alkylating capacity of the spacer remains nonmodified (see the next paragraph). About 1 mol of MAL-FMS was incorporated into α-lactalbumin per 2.1 mol of reagent added, up to a 14 molar excess over the protein (Fig. 2).

Stability of the MAL Function in Aqueous Solutions—Fig. 3 demonstrates the potency of the reaction of MAL-FMS-OSu with reduced glutathione as a function of time at different pH values. MAL-FMS-OSu was found fully stable at pH 4–6 over a period of a day or two, and at pH 7.2 it is stable over a period of several hours. At pH 8.5 the maleimido moiety is destroyed at an accelerated rate ($t_{1/2}$ = 2.7 ± 0.3 h, Fig. 3).

Two possible pathways for the related PEGylation of target peptides, proteins, and small molecules are suggested (Fig. 1*B*). One involves attaching MAL-FMS-OSu to the amine component of the target and then substituting PEG-SH for the maleimide moiety. The second option involves preparing PEG-FMS-OSu, which then reacts with the amine component of the target (Fig. 1*B*). This pathway is suitable for sulfhydryl- or disulfide-containing peptides and proteins.

Rate of Hydrolysis of PEG-FMS Conjugates—Four PEG-FMS conjugates and one MAL-FMS conjugate were prepared using the described pathways (see Table I and "Experimental Procedures"). The hydrolysis rates and reaction orders of PEG_{5000}-FMS-4-nitro-phenethylamine, PEG_{5000}-FMS-exendin-4, $PEG_{40,000}$-FMS-exendin-4, and $PEG_{40,000}$-FMS-hGH were evaluated at pH 8.5, 37 °C (Table I). The hydrolysis rate at pH 8.5 is equivalent to the hydrolysis rate in serum as shown previously (16–19). The release of 4-nitro-phenethylamine, exendin-4, and hGH was monitored by reverse phase-HPLC and was determined to be a first order reaction (Table I and Figs. 4 and 5). As shown (Fig. 4 and Table I), the peptides and protein were released from the conjugates in a slow homogenous fashion, with $t_{1/2}$ of 9.4, 13.8, 11.9, and 11.8 h. Exendin-4 was fully released from the $PEG_{40,000}$ conjugate after 48 h of incubation.

Demonstrating the Validity of Reversible PEGylation with hGH and Exendin-4—In vitro, $PEG_{40,000}$-FMS-hGH exhibits no receptor binding affinity. Upon incubation, recognition of the released hGH by the native hGH receptor was preserved (Fig. 5).

Exendin-4 is an insulinotropic glucagon-like peptide-1 agonist that associates with β-pancreatic cells, elevates endogenous cAMP levels, enhances secretion of insulin, and lowers circulating glucose levels (22–25). A profound pharmacological advantage of this glucagon-like peptide-1 agonist is that (following administration at any dosage) the circulating blood glucose level never falls below a threshold that in non-diabetic healthy CD1 mice is 74 ± 7 mg/dl (26). Fig. 6 shows the glucose-lowering profile of subcutaneously administered native exendin-4 and of $PEG_{40,000}$-FMS-exendin-4 at a dose of 4 μg of peptide/mouse relative to a saline-treated group of mice. With the native peptide, blood glucose level declined 26–28% (from 140 to 104–101 mg/dl) with the largest percent change in blood glucose level occurring 0.5–1 h after administration. Glucose concentrations then returned to their initial levels with a $t_{1/2}$ of

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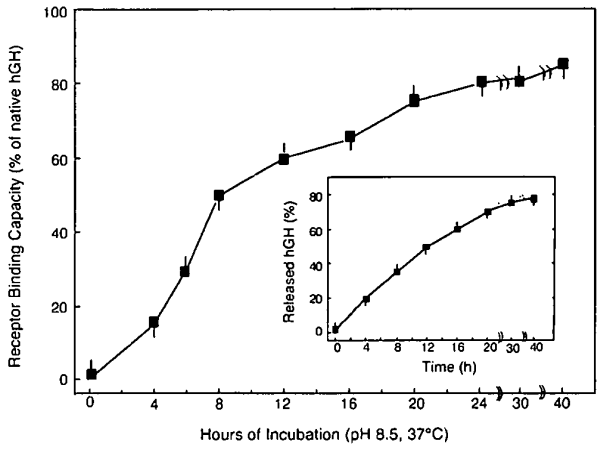

FIG. 5. **Time course of reactivation of $PEG_{40,000}$-FMS-hGH upon incubation at pH 8.5, 37 °C.** $PEG_{40,000}$-FMS-hGH (1 mg/ml) was incubated in 0.1 M phosphate buffer, 0.6% bovine serum albumin, and 2 mM NaN_3 at 37 °C. Aliquots were withdrawn at the indicated time points and analyzed for their potencies in displacing ^{125}I-hGH from enriched hGH receptor preparation extracted from rabbit liver plasma membranes. Native hGH displaces ^{125}I-hGH in this assay half-maximally at a concentration of 0.3 ± 0.03 nM. An hGH derivative exhibiting half-maximal displacement in this assay at a concentration of 3.0 ± 0.3 nM is considered to have 10% of the binding potency of the native receptor. The rate of release of hGH from PEG_{40}-FMS-hGH upon incubation at pH 8.5, 37 °C is shown in the *inset*. $PEG_{40,000}$FMS-hGH (1 mg of protein) was incubated as described above. At the indicated time points, 0.1-ml aliquots were withdrawn and subjected to analytical HPLC analysis.

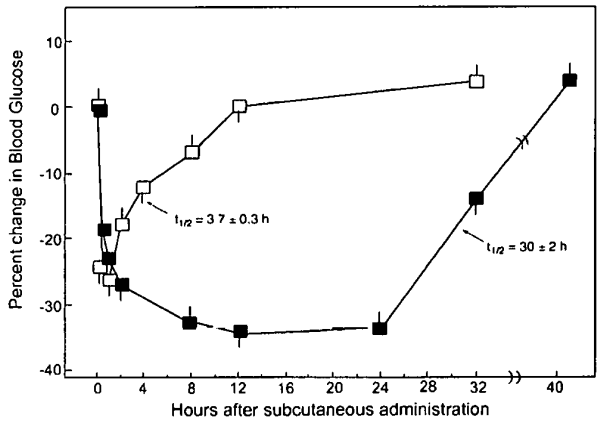

FIG. 6. **Glucose-lowering patterns of native exendin-4 and $PEG_{40,000}$-FMS-exendin-4 following a single subcutaneous administration to CD1-mice.** Three groups of CD1 mice (n = 6 per group) underwent one subcutaneous administration of saline, native exendin-4 (□, 4 μg/mouse), or $PEG_{40,000}$-FMS-exendin-4 (■, 4 μg of peptide/mouse). Circulating glucose levels were then monitored. Results are expressed as percent decrease in plasma glucose concentration in the groups treated with exendin-4 or $PEG_{40,000}$-FMS-exendin-4 relative to that found in the saline-treated group measured at the same time point during the day.

3.7 ± 0.3 h. Following the subcutaneous administration of $PEG_{40,000}$-FMS-exendin-4, the decrease in blood glucose level took place at a more moderate rate. Circulating glucose reached its lowest concentration 8–12 h after administration (92 mg/dl, 33%). Stable, low circulating glucose concentrations were then maintained for a further 12 h. Return to initial glucose levels took place with a $t_{1/2}$ of 30 ± 2 h, which is 7.5 times longer than that obtained by the same dose of the native hormone.

Irreversibly conjugated $PEG_{40,000}$-exendin-4 has only ≤1% of

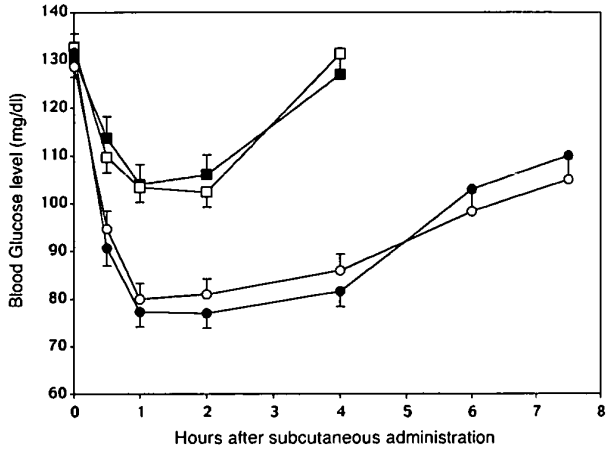

FIG. 7. **Glucose-lowering patterns of exendin-4 released from $PEG_{40,000}$-FMS-exendin-4.** Five groups of CD1 mice (n = 3 per group) underwent one subcutaneous administration of saline, native exendin-4, or released exendin-4 (1 and 0.1 μg of peptide/mouse, respectively). Circulating glucose levels were then determined. Results are expressed as plasma glucose levels (mg/dl) measured following subcutaneous administration. ■, native exendin-4 (0.1 μg); □, released exendin-4 (0.1 μg); ●, native exendin-4 (1 μg); ○, released exendin-4 (1 μg).

the activity of native exendin-4 (IC_{50} = 250 ± 30 pmol/mouse *versus* IC_{50} = 2.5 ± 0.24 pmol/mouse for the native exendin-4, not shown).

Biological Potency of Exendin-4 that Was Released from $PEG_{40,000}$-FMS-Exendin-4 Conjugate—Fig. 7 shows the glucose-lowering pattern of exendin-4 released from the $PEG_{40,000}$-FMS-exendin-4 conjugate by incubation at pH 8.5, 37 °C for 24 h. The glucose-lowering pattern of the released exendin-4 was indistinguishable from that obtained by the native peptide; both reduced blood glucose levels by 30 and 50% 1 h after subcutaneous administration of 0.1 and 1 μg of peptide/mouse, respectively.

DISCUSSION

PEGylation's previously mentioned side effect of drastically decreasing drug bioactivity has meant that only a limited number of such conjugates have found clinical application (5). This happens despite the profound advantages that steric protection confers in elongating the lifetimes of the drugs *in vivo*, protecting them from proteolysis and shielding them from the immune system (4–8). Theoretically, decreased bioactivity can be overcome by linking the PEG chains via a chemical bond sensitive to mild alkaline and/or acid hydrolysis or enzymatically able to be cleaved by serum proteases or esterases. Obviously, an inconsistent rate of hydrolysis would render such an approach impractical. A prerequisite condition is therefore that the hydrolysis of the PEG chains from the conjugate takes place at a slow rate and in a homogenous fashion under the strictly homeostatic pH and temperature conditions of the mammalian circulatory system (27).

Previously, we prepared FMS-OSu (17–19) as a reversible protein modifier. Protein-linked FMS moieties undergo slow and spontaneous hydrolysis under physiological conditions, generating the unmodified parent molecule. Hydrolysis of FMS-protein conjugates at 37 °C in normal human serum, in aqueous buffers of pH 8.5, or in the circulatory system *in vivo* takes place with a $t_{1/2}$ of 5–7 h. Using the FMS moiety as the scaffold for the suggested reversible PEGylation technology enables the hydrolysis rates of various PEG-FMS-protein conjugates to be predicted. The $t_{1/2}$ for the hydrolysis of modified

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small molecules and PEG-conjugated polypeptides and proteins falls within a relatively narrow range of 8–14 h (Table I). The constant hydrolysis rate for PEG-FMS-protein conjugates is caused by the β-elimination reaction, which occurs at position 9 of the fluorenyl moiety (Fig. 1*B*) and is solely dependent on the pH of the surrounding medium. Thus, in contrast to what occurs in studies based on enzymatic hydrolytic bond cleavage, similar PEG-FMS hydrolysis rates are expected from all conjugates regardless of the identity of the protein/peptide moiety conjugated. Using the present approach one can further control the hydrolysis rate by substituting the fluorenyl moiety with electron-withdrawing or electron-inducing groups that increase or decrease, respectively, the hydrolysis rate. The number of PEG-FMS chains attached to a drug should also affect the rate at which the native drug is released.

Exendin-4 contains one His N^{α} amino function and two Lys N^{ϵ} amino groups, enabling modification at these three positions. Indeed, N-terminal amino acid sequencing revealed that, although the $PEG_{40,000}$-FMS-exendin-4 product eluted as a single peak on HPLC, it was actually a mixture containing primarily the N^{α}-modified hormone. Similarly, hGH contains several amino functions that may be conjugated. However, in view of the reversibility of the PEGylation reaction, which regenerate the native peptide and protein hormones, this point deserves only minor consideration.

Calculations based on the hydrolysis rates of FMS-exendin-4 and $PEG_{40,000}$-FMS-exendin-4 at 37 °C in normal sera and PBS, pH 8.5, respectively, revealed that ~4% of exendin-4 is released from the conjugate each hour *in vivo* (19). We further hypothesized that this release rate if combined with prolonged maintenance of the conjugate in the circulatory system prior to exendin-4 hydrolysis should yield a long lasting glucose-lowering signal in mice, as indeed is found following a single subcutaneous administration of $PEG_{40,000}$-FMS-exendin-4 conjugate (Fig. 6).

It should be noted that the covalently linked exendin-4 is fully active following its release from $PEG_{40,000}$-FMS-exendin-4 by incubation (Fig. 7), suggesting that the conjugation *per se* does not lead to irreversible modification and/or denaturation of the peptide.

The sulfonated fulvene moiety is not toxic as previously shown (19). High molecular weight PEG is known to be safe in terms of toxicity and immunogenicity and is widely used in the food, cosmetic, and pharmaceutical industries (28, 29).

In summary a conceptual approach termed "reversible PEGylation" was implemented here with low molecular weight polypeptides and proteins. This novel approach enables the desirable pharmacological features associated with PEGylation to be conferred on low molecular weight peptide and protein drugs that would otherwise have been fully or partially inactivated by this technique. A pharmacologically "silent" conjugate that is "trapped" in the circulatory system releases the covalently linked parent peptide or protein with a desirable pharmacokinetic profile. This new development is expected to extend the lifetime, bioavailability, and efficacy of existing peptide drugs and to extend the same in peptide drug candidates that may yet be discovered by genomics and proteomics.

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Prolonging the Action of Protein and Peptide Drugs by a Novel Approach of Reversible Polyethylene Glycol Modification

Haim Tsubery, Marina Mironchik, Mati Fridkin and Yoram Shechter

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EXHIBIT G1

The opinion in support of the decision being entered today is *not* binding precedent of the Board.

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

Ex parte JOACHIM WERRES

Appeal 2006-2966
Application 09/148,152[1]
Technology Center

Decided: July 3, 2007

Before BRADLEY R. GARRIS, Administrative Patent Judge, FRED E. MCKELVEY, Senior Administrative Patent Judge, and ALLEN R. MACDONALD, *Administrative Patent Judge.*

GARRIS, *Administrative Patent Judge.*

---

[1] Application filed September 3, 1998, for reissue of U.S. Patent No. 5,656,177, which issued August 12, 1997 from Application No. 08/352,097, filed November 30, 1994.

DECISION ON APPEAL

Statement of the Case

This is an appeal under 35 U.S.C. § 134 from the Examiner's final rejection of claims 1-39 in the above-identified reissue application. We have jurisdiction under 35 U.S.C. § 6(b).

We AFFIRM.

Appellant claims a method of decreasing microbial growth and preventing slime formation in an aqueous medium which comprises incorporating into the aqueous medium an effective microbial growth retarding and slime formation preventing amount of an oil-in-water emulsion. Reissue claim 1, which corresponds to Patent claim 1, defines the method in terms of the species elected without traverse during patent prosecution. Reissue claim 10 defines the method in terms which are generic to the elected species. These claims adequately represent the claimed subject matter on appeal and are reproduced below:

1. A method of decreasing microbial growth and preventing slime formation in an aqueous medium comprising the step of incorporating in said aqueous medium an effective microbial growth retarding and slime formation preventing amount of an oil-in-water emulsion comprising an oil phase and at least one emulsifier for the oil phase, wherein the oil phase comprises saturated or unsaturated, open-chain, normal or isomeric hydrocarbons containing 8 to 30 carbon atom [sic]; and wherein said emulsion is free of toxic microbiocides.

10. A method of decreasing microbial growth and preventing slime formation in an aqueous medium comprising the step of incorporating in said aqueous medium an effective microbial growth retarding and slime formation preventing amount of an oil-in-water emulsion comprising an oil phase and at least one emulsifier for the oil phase, wherein the oil phase comprises at least one substance selected from the group consisting of:

(i) saturated or unsaturated, open-chain or cyclic, normal or isomeric hydrocarbons containing 8 to 30 carbon atoms;

(ii) saturated or unsaturated fatty alcohols, saturated or unsaturated fatty acids, fatty acid monoalkyl esters, fatty acid amides, or monoalkyl amides of saturated or unsaturated fatty acids, wherein the fatty alcohol, fatty acid or fatty acid residue contains 8 to 30 carbon atoms;

(iii) monoesters or polyesters of saturated or unsaturated fatty acids containing 4 to 30 carbon atoms and monoalcohols or polyols except for polyethylene glycols;

(iv) polyamides of saturated or unsaturated fatty acids containing 8 to 30 carbon atoms and aliphatic polyamines containing 2 to 6 nitrogen atoms;

(v) acyclic, monocyclic or bicyclic terpenes; and

(vi) polyoxyalkylene compounds formed from alkylene oxides and at least one substance selected from the group consisting of C_{12}-C_{18} fatty alcohols, C_{12}-C_{18} fatty acids and glycerides of C_{12}-C_{18} fatty acids;

wherein when the aqueous medium is a water piping or a cooling or heating cycle or an industrial process water as a water carrying system and a substance of group (v) is used, said substance of group (v) is always used in admixture with at least one substance of the groups (i) – (iv) or (vi), and wherein said emulsion is free of toxic microbiocides.

Claims 1-39 are rejected under 35 U.S.C. § 251 as being based upon a defective reissue declaration. According to the Examiner, the declaration is defective "because none of the errors which are relied upon to support the reissue application are errors upon which a reissue can be based" (Final Office Action, mailed Oct. 7, 1999, 2).

Claims 10-39 are rejected under 35 U.S.C. § 251 "as being an improper recapture of claimed subject matter deliberately canceled in the application for the patent upon which the present reissue is based" (*id.* at 3).

The Issues

(1) Regarding claims 1-39, is the Reissue Declaration of record defective for failing to establish an error that is correctable by reissue under 35 U.S.C. § 251?

(2) Do claims 10-39 violate the recapture rule by reciting subject matter that was deliberately surrendered in an effort to obtain allowance of the '177 patent claims?

The Reissue Declaration

The Reissue Declaration, filed November 16, 1998, presents two errors in support of reissue. These errors and the undisputed facts which engendered the errors are copied below from pages 1-2 of the Declaration.

1. Through error and without deceptive intent, the patent was allowed to issue without a generic claim. The facts are as follows:

> a. On October 4, 1995, the Examiner issued an Action making two election of species requirements, the first being a choice from among 19 defined "species" of compounds and the second being a choice from among six defined "species" of uses;
>
> b. On January 3, 1996, a provisional election was made;
>
> c. On June 28, 1996, an Amendment was filed in response to an Office Action dated April 9, 1996, inserting a number of claims, including a generic claim numbered "17".

d. A final rejection issued September 23, 1996, and a response was filed December 23, 1996.

e. On January 17, 1997, the Examiner faxed a proposed amendment to my attorney, proposing to amend Claim 17 to limit it only to the elected species. *That proposal was erroneously agreed to.* [Emphasis added.] The Examiner then entered an Examiner's Amendment making the proposed changes and allowed the claims. The patent issued on August 12, 1997.

f. Since the Examiner found the elected "species" allowable, the generic claims were then *per se* allowable unless and until the Examiner would conduct a further search of the remaining "species". Considering the number of permutations of the denoted "species" (19 x 6 = 114), it was error to have agreed to the cancellation of the generic claims [sic].

2. Through error and without deceptive intent, references cited in a Search Report dated July 14, 1994, from the German Patent Office in a corresponding German application were not brought to the attention of the U.S. Examiner. The facts, as established by a Declaration of Dr. Gerald Kloepsch accompanying this Reissue Declaration, are as follows:

a. The Search Report from the German Patent Office was mailed July 14, 1994, and received July 21, 1994. As was Dr. Kloepsch's practice, he reported the German Search Report to his client. As was the usual practice in his office, since no response to a Search Report is necessary, the Search Report was merely placed in the respective file, to wait for an Action on the merits.

b. The application Dr. Kloepsch prepared for filing in other countries was more detailed and of a different scope then [sic] that of the German application. It was his opinion at that time that the references cited in the German Search Report were not material to the scope of the coverage to be obtained in the other countries.

c. U.S. Application serial no. 08/352,097 was filed on November 30, 1994, about four months after receipt of the German Search Report. At that time, the issuance of the German Search report was forgotten.

d. During the prosecution of the U.S. application, Dr. Kloepsch sent to his U.S. associates the Search Report of the PCT Application identical in scope with the U.S. application. The references of the PCT Search Report were brought to the U.S. Examiner's attention in an IDS of January 3, 1996. But, apparently, Dr. Kloepsch still did not remember the German Search report so the references cited in it were not brought to the attention of the U.S. Examiner.

e. At least some of the references cited in the German Search Report may be considered material to the patentability of the generic claims of the U.S. application [i.e., the subject reissue application].

Principles of Law

Regarding Issue (1):

Under 35 U.S.C. § 251 (1952), reissue is permissible "[w]henever any patent is, through error without any deceptive intention, deemed totally or partly inoperative or invalid, by reason of a defective specification or drawing, or by reason of the patentee claiming more or less than he had a right to claim in the patent."

The deliberate action on an inventor or attorney during prosecution generally fails to qualify as a correctible error under § 251. *In re Serenkin*, 479 F.3d 1359, 1362, 81 USPQ2d 2011, 2014 (Fed. Cir. 2007). There is no correctable § 251 error when an inventor or attorney deliberately agrees with an Examiner's requirement that non-elected claims be prosecuted in a divisional application rather than in the application containing the elected

claims. *In re Orita*, 550 F.2d 1277, 1280, 193 USPQ 145, 148 (CCPA 1977). This is so regardless of the propriety of the requirement. *In re Watkinson*, 900 F.2d 230, 231-32, 14 USPQ2d 1407, 1408-09 (Fed. Cir. 1990).

Regarding Issue (2):

The recapture rule prevents a patentee from regaining through reissue the subject matter that the patentee/applicant surrendered (e.g., via claim amendment) in an effort to obtain allowance of the original patent claims. *In re Clement*, 131 F.3d 1464, 1468, 45 USPQ2d 1161, 1164 (Fed. Cir. 1997). However, the recapture rule does not apply in the absence of evidence that the applicant's amendment was an admission that the scope of the amended claim was not in fact patentable. *Id.* In determining whether surrender of subject matter has occurred, the proper inquiry is whether an objective observer viewing the prosecution history would conclude that the purpose of the applicant's amendment (or argument) was to overcome the prior art and secure the patent. *Kim v. Conagra Foods, Inc.*, 465 F.3d 1312, 1323, 80 USPQ2d 1495, 1502 (Fed. Cir. 2006).

Analysis of The Defective Reissue Declaration Rejection

Reissue Declaration Error 1

In the Reissue Declaration, the Appellant states that, through error and without deceptive intent, the '177 patent was allowed to issue without a generic claim (Declaration 1, Item 1). The undisputed facts presented in support of this statement reveal that the asserted "error" was occasioned by deliberate actions on the part of Appellant (or his attorney).

These deliberate actions included agreement with the Examiner's requirement for an election of species to be prosecuted in the '177 patent application with non-elected species to be prosecuted in divisional applications (id. at Items 1.a. and 1.b.).[2] Similarly, Appellant (or his attorney) deliberately agreed to an Examiner's amendment that limited or narrowed generic claim 17 to the elected species only (id. at Item 1.e.).

A correctable § 251 error does not include these deliberate decisions by Appellant whereby the '177 patent was allowed to issue with elected species claims only (i.e., without a generic claim). *Orita,* 550 F.2d at 1280, 193 USPQ at 148. These deliberate choices do not comprise error correctable by reissue regardless of the propriety of the election requirement or claim amendment made by the Examiner. *Watkinson*, 900 F.2d at 231-32, 14 USPQ2d at 1408-09. There is a distinction between a § 251 error and a deliberate choice subsequently found to be disadvantageous. *Serenkin*, 479 F.3d at 1364, 81 USPQ2d at 2015.

Appellant cites *Ex parte Holt*, 214 USPQ 381 (Bd. App. 1982), "as standing for the proposition that failure to take other remedial action, such as filing a divisional or continuation application, should not adversely affect an applicant's right to reissue" (Supple. Appeal Brief, filed May 22, 2007, 1; *see also* Revised Appeal Brief, filed April 25, 2000, (hereinafter Br.) 10). *Holt* involved an improper rejection of a Markush claim under 35 U.S.C. § 121. The Board found that applicant's failure to recognize the impropriety

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[2] It might be presumed that no such divisional applications were filed and that such non-filing occurred by inadvertence and without deceptive intent as in *Orita*, 550 F.2d 1277, 193 USPQ 145. However, the record is silent on these matters.

of the rejection was a correctable error under § 251 and that applicant's failure to file a timely divisional application in response to the improper rejection should not foreclose § 251 relief.

Interestingly, *Holt* was similarly relied on by the appellant in *Watkinson*. As explained in *Watkinson*, 900 F.2d at 232, 14 USPQ2d at 1409, there is a legally significant difference between the rejection in *Holt* and a restriction requirement. That is, a restriction requirement is "proper" under 35 U.S.C. § 121, whereas the rejection of a Markush claim is not. *Id.* Moreover, the merit of a restriction requirement is not reviewable on appeal since the requirement is a matter within an Examiner's discretion and not tantamount to a rejection of claims. *Id.*, 900 F.2d at 233, 14 USPQ2d at 1409. For these reasons, the court in *Watkinson* found *Holt* inapplicable to the facts of that appeal.

The facts of the *Watkinson* appeal parallel the facts of the subject appeal. The applicants in both cases acquiesced in a restriction or election requirement, canceled (or permitted cancellation of) the non-elected claims and accepted issuance of a patent containing elected claims only. Therefore, we likewise find *Holt* inapplicable to the facts of this appeal.

In an apparent attempt to analogize the facts of this appeal to those of *Holt*, Appellant argues that the election of species requirement "results in 114 permutations of combinations of 'species' [, and] [t]he result is a *de facto* rejection of Claim 17 resulting in a denial of generic coverage such that Applicant could not be made whole through the filing of multiple applications to each species" (Br. 12). Thus, Appellant argues that the "cancellation" of generic claim 17 (i.e., amendment of claim 17 to limit it to

elected species only) was not in response to an election of species requirement but rather was in response to a *de facto* rejection of a generic claim (Br. 11).

This argument is unpersuasive. The Examiner's election of species requirement can not be properly considered a *de facto* rejection which is analogous to the improper Markush rejection in *Holt.* Indeed, as expressly stated in *Watkinson*, 900 F.2d at 233, 14 USPQ2d at 1409, "a restriction requirement is a matter within the discretion of the Examiner and not tantamount to a rejection of claims."

As further support for his position, Appellant cites *In re Doyle*, 293 F.3d 1355, 1358, 63 USPQ2d 1161, 1163 (Fed. Cir. 2002), wherein the court determined that failure to present a so-called linking claim (i.e., a generic claim broad enough to read on or link two or more groups of claims subject to a restriction requirement) is an error correctable by reissue. (Further Supple. Br., filed July 1, 2002, 1-2; Reply to Supple. Examiner's Answer, filed July 11, 2006, 3-4). According to Appellant, "[t]he facts of the present case fall into the situation described by the court in *Doyle* [since] [t]he new claims in the instant reissue application are generic, or "linking," claims which are broader than any nonelected claims and could have been prosecuted with the elected claims" (Further Supple. Br. 2). As a matter of clarification, we point out that the "new claims" referred to by Appellant are newly added reissue claims 10-39 and that Appellant characterizes independent reissue claim 10 as a generic claim corresponding to generic claim 17 of the '177 patent application (Br. 6-7 and 23-24 (Appendix II)).

The Appellant is incorrect in stating that "[t]he facts of the present case fall into the situation described by the court in Doyle" (Further Supple. Br. 2). As a basis for its holding in *Doyle*, the court repeatedly emphasized that the generic or linking claims of Dr. Doyle's reissue application had never been submitted for prosecution in the original patent application. *See Doyle*, 293 F.3d at 1358, 63 USPQ at 1164 ("Dr. Doyle simply seeks a broadening reissue of his '595 patent to cover material that he invented and disclosed, but inadvertently *failed to claim* in his issued patent;" emphasis added) and 293 F.3d at 1360, 63 USPQ at 1165 ("the applicant [Dr. Doyle] never asserted the reissue claims or anything similar to them in his original application").

In contrast, Appellant submitted generic claim 17 for prosecution in the '177 patent application and deliberately authorized an amendment by the Examiner of the claim to limit or narrow its coverage to the elected species only. By now seeking reissue of appealed generic claim 10 which corresponds to former generic claim 17, the Appellant clearly is attempting to retract his deliberate decision made during prosecution of the '177 patent application. This deliberate decision is not an error that is correctable under § 251. *Serenkin*, 479 F.3d at 1362-63, 81 USPQ2d at 2014.

For the above-stated reasons, the facts presented in the Reissue Declaration do not establish that issuance of the '177 patent without a generic claim was due to an error which can be corrected by reissue under 35 U.S.C. § 251.

Reissue Declaration Error 2

As an additional reissue error, Appellant states: "Through error and without deceptive intent, references cited in a Search Report dated July 14, 1994, from the German Patent Office in a corresponding German application were not brought to the attention of the US Examiner" (Decl. 2, Item 2). Concerning the relevancy of these references, Appellant states only that "[a]t least some of the references cited in the German Search Report may be considered material to the patentability of the generic claims of the US application [i.e., the subject reissue application]" (Decl. 2, Item 2.e). Indeed, Appellant acknowledges that these references would not raise a substantial new question of patentability of the patented claims but urges that they would have been relevant to generic claim 17 of the original '177 patent application and are relevant to generic claim 10 of this reissue application (Br. 6).

As previously explained, § 251 permits the reissue of defective patents "[w]henever any patent is, through error without any deceptive intention, deemed wholly or partly inoperative or invalid, by reason of a defective specification or drawing, or by reason of the patentee claiming more or less than he had a right to claim in the patent" (1952). Here, the failure to bring the above-noted references to the attention of the US Examiner has not rendered the '177 patent defective in any perceptible way. As the Appellant has acknowledged, these references do not raise a substantial new question of patentability of the '177 patent claims. Furthermore, it is simply irrelevant to Appellant's request for reissue under

§ 251 that these references may be pertinent to other claims such as former generic claim 17 and current generic reissue claim 10.

As support for his belief that the above discussed failure is correctable by reissue, Appellant cites *In re Harita*, 847 F.2d 801, 6 USPQ2d 1930 (Fed. Cir. 1988). However, unlike the circumstances before us, the references cited in the reissue application of *Harita* rendered at least some of Harita's patent claims invalid. *Id.,* 847 F.2d at 802, 6 USPQ2d at 1930. Although the reissue claims patentably distinguished over this prior art, they were subjected to an "inequitable conduct" rejection which was reversed. *Harita*, 847 F.2d at 802, 6 USPQ2d at 1930. Because these facts are completely dissimilar to those before us, the Appellant's position is not supported by *Harita.*

Summary of Analysis of Defective Reissue Declaration Rejection

For the above-stated reasons, the Reissue Declaration of record is defective in that it fails to present any error which is correctable by reissue under 35 U.S.C. § 251.

Analysis of The Recapture Rejection

This rejection of newly added reissue claims 10-39 is based on the Examiner's determination that generic reissue claim 10 is an attempt by Appellant to recapture subject matter which was surrendered when Appellant authorized the Examiner to amend generic claim 17 of the '177 patent application to narrow its coverage to the elected species only. However, there is no evidence that Appellant authorized this narrowing

amendment in order to avoid a prior art (or any other kind of) rejection, and the Examiner does not argue otherwise. Instead, the Examiner believes his recapture rejection is proper simply because Appellant authorized the Examiner to amend claim 17 so as to remove from its coverage subject matter generic to the elected species.

The Examiner's theory for this rejection is not well-taken. As explained earlier, the recapture rule does not apply in the absence of evidence that a claim amendment was an admission that the scope of the unamended claim was not in fact patentable. *Clement,* 131 F.3d at 1468, 45 USPQ2d at 1164. Here, there is no evidence that Appellant's authorization of the Examiner's amendment was an admission that the scope of generic claim 17 was not patentable. Additionally, the Examiner points to nothing and we find nothing in the prosecution history of the '177 patent application which would lead an objective observer to conclude that the purpose of the claim 17 amendment was to overcome prior art and secure the patent. In the absence of such prosecution history, no basis exists for determining that the subject matter defined by the reissue claims had been surrendered during prosecution of the original application. *Kim v. Conagra Foods, Inc.*, 465 F.3d at 1323, 80 USPQ2d at 1502.

Conclusions of Law

(1) With respect to claims 1-39, the Appellant has failed to establish on this record any error which is correctable by reissue under 35 U.S.C. § 251.

(2) With respect to claims 10-39, the Examiner has failed to establish on this record that these claims recapture subject matter which was surrendered by Appellant during prosecution of the '177 patent application.

Order

The rejection of claims 1-39 under 35 U.S.C. § 251 based upon a defective reissue declaration is AFFIRMED.

The rejection of claims 10-39 under 35 U.S.C. § 251 based on recapture is REVERSED.

No time period for taking any subsequent action in connection with this appeal may be extended under 37 C.F.R. § 1.136(a)(iv)(effective Sept. 13, 2004).

AFFIRMED

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EXHIBIT G2

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

Ex parte EDWARD BEVERLY MORRISON,
PETR VALENTINOVICH KUZMIN, and PAVEL TISHIN

Appeal 2012-012266
Application 12/827,650
Patent 7,157,914 B2
Technology Center 2800

Before MAHSHID D. SAADAT, DENISE M. POTHIER,
and JEFFREY S. SMITH, *Administrative Patent Judges*.

SAADAT, *Administrative Patent Judge*.

DECISION ON APPEAL[1]

Appellants appeal under 35 U.S.C. § 134(a) from the rejection of claims 28-36 as lacking an error correctable by reissue. We have jurisdiction under 35 U.S.C. § 6(b).

We affirm.

---

[1] An Oral Hearing for this appeal was held on February 4, 2013.

STATEMENT OF THE CASE

Introduction

Appellants' invention relates to a Time Domain Electromagnetic (TDEM) surveying method having improved sensor resolution. (*See* Spec. col. 3, ll. 25-26).

Exemplary Claim

> 28. A method for providing a tow assembly for an electromagnetic airborne survey system at an assembly location, comprising:
>
> providing a plurality of hollow transmitter support frame sections;
>
> providing a transmitter coil for generating a primary magnetic field;
>
> providing connector cables and a tow cable; and
>
> assembling the transmitter support frame sections and the transmitter coil and the cables to provide a tow assembly having a flexible hollow transmitter support frame that is circular or approximates a circle having the transmitter coil disposed within the hollow transmitter support frame and formed from serially connecting the transmitter support frame sections, with the connector cables each having a first end connected to a respective location on a circumference of the transmitter support flame and a second end connected to a first end of die tow cable.

The Rejection

Claims 28-36 stand rejected under 35 U.S.C. § 251 for lack of defect or error in the original patent and as not being an error correctable by reissue. (*See* Ans. 5-6).

FINDINGS OF FACT

Prosecution History

1. Appellants filed U.S. Application No. 10/716,813 ("813 Application") on Nov. 20, 2003.

2. On May 8, 2006, the Examiner required restriction to three patentably distinct groups of claims as follows:

> I. Claims 1, 3-10, 12, 13, 15-28, 32, and 33, drawn to a method and apparatus using a tow assembly, the assembly comprising a flexible frame, the receiver flexibly connected to a receiver support frame and a dual mode gain amplifier, classified in class 324, subclass 330.
>
> II. Claim 29, drawn to a computer program comprising a computer readable medium and instructions to operate a general electromagnetic antenna assembly, classified in class 702, subclass 5.
>
> III. Claims 30, 31, 34, and 35, drawn to trailing airborne surveying systems having specific antenna structures, classified in class 343, subclass 707.

3. On August 18, 2006, Appellants filed a response to the Examiner's Restriction Requirement and elected to prosecute the claims of group I. Appellants did indicate that "[t]his election/restriction is made by the Applicant without prejudice to the right to file one or more divisional applications comprising the subject matter of species (II) or (III)." However, no such divisional application was filed.

4. On January 2, 2007, Application 10/716,813 issued as U.S. Patent 7,157,914 ("914 Patent") including claims 1, 3-10, 12, 13, 15-28, 32, and 33 (renumbered as claims 1-27).

5. On December 23, 2008, Appellants filed reissue U.S. Application No. 12/343,321 ("Reissue Application").

6. In U.S. Application No. 12/343,321, claims 1-27 were claims 1, 3-10, 12, 13, 15-28, 32, and 33 of 813 Application and newly added claims 28-33 were directed to a method for providing a tow assembly.

7. On December 10, 2009, the Examiner required restriction to one of the following two inventions:

> I. Claims 1-27, drawn to a method and apparatus for airborne electromagnetic surveying and circuitry therefor, classified in class 324, subclass 330.
>
> II. Claim 28-33, drawn to an antenna for a tow assembly, classified in class 343, subclass 707.

8. On March 10, 2010, Appellants filed a response to the Examiner's Restriction Requirement and elected to prosecute the claims of group I.

9. On June 30, 2010, Appellants filed (as a divisional of Application 12/343,321) reissue U.S. Application No. 12/827,650 containing claims 28-36 alleging error as follows:

> in overlooking embodiments of my invention which pertain to a method for providing a tow assembly for an electromagnetic airborne survey system at an assembly location, comprising: providing a plurality of transmitter support frame sections, a transmitter coil for generating a primary magnetic field, connector cables and a tow cable and assembling the transmitter support frame sections and the transmitter coil and the cables to provide a tow assembly having a transmitter support frame that is circular or approximates a circle bearing the transmitter coil and formed from serially connecting the transmitter support flame sections, with the connector cables each having a first end connected to a respective location on a circumference of the transmitter support frame and a second end connected to a first end of the tow cable.

10. On August 24, 2011, the Examiner finally rejected claims 28-36 under 35 U.S.C. § 251 as being improper for reissue.

11. On February 23, 2012, Appellants filed an appeal of the Examiner's final rejection.

The Examiner's Rejection

12. The Examiner found that a restriction requirement had been made during prosecution of application for the 914 Patent between Groups I, II, and III, where Appellants elected without traverse to prosecute the invention of claims Group I including claims 1, 3-10, 12, 13, 15-28, 32, and 33 of the 813 Application and "never filed any divisional applications covering such non-elected species" (Ans. 5).

13. The Examiner found further that

> The claims of the present divisional reissue application read on or are broader versions of the claims in Group III (claims 30, 31, 34 and 35) of the '813 application and thus cover the primary features of the Group, i.e., the specific features of the antenna structures of the towed assembly. Alternatively, *if these divisional reissue claims were presented in the '813 application during prosecution thereof, they would also have been subject to the same restriction by either being placed in Group III or in a separate group* for similar reasons for restriction from Group I. Thus, the present claims should have been presented in a divisional application to the parent application.

(Ans. 5-6) (Emphasis added).

14. The Examiner concluded that "failure of Applicants to file a divisional application during the pendency of the '813 application (which later issued to the '914 patent) does not amount to an error correctable by reissue" (Ans. 6).

Issues on Appeal

1. Is the failure to present claims 28-36 during the prosecution of the 914 Patent an error that can be corrected by reissue?

2. Are appealed claims 28-36 of reissue U.S. Application No. 12/827,650 substantially identical to claims of the non-elected groups identified in the Examiner's restriction requirement during the prosecution of the 914 Patent?

PRINCIPLES OF LAW

The section of the patent statute providing for correction of errors in a patent by reissue of the patent reads, in relevant part:

> Whenever any patent is, through error, deemed wholly or partly inoperative or invalid . . . by reason of the patentee claiming more or less than he had a right to claim in the patent, the Director shall, on the surrender of such patent and the payment of the fee required by law, reissue the patent for the invention disclosed in the original patent.

35 U.S.C. § 251, first paragraph.

"[T]he whole purpose of the [reissue] statute, so far as claims are concerned, is to permit limitations to be added to claims that are too broad or to be taken from claims that are too narrow." *In re Weiler*, 790 F.2d 1576, 1580 (Fed. Cir. 1986) (quoting *In re Handel*, 312 F.2d 943,948 (CCPA 1963)).

The *"Orita* doctrine" precludes applicants from obtaining by reissue claims which, because of a requirement for restriction in which they had acquiesced, they could not claim in their patent. *In re Orita,* 550 F.2d 1277, 1280 (CCPA 1977).

When applicants acquiesce in the Examiner's requirement for restriction, such action manifestly is not "error" causing patentee to claim "less than he had a right to claim in the patent" in the language of 35 U.S.C. § 251. *Id.* 550 F.2d at 1280.

The so-called *Orita* doctrine precludes reissue applicants from obtaining substantially identical claims to those of non-elected groups identified in an Examiner's restriction requirement when such claims could not have been prosecuted in the application from which they were restricted. *In re Doyle,* 293 F.3d 1355, 1359 (Fed. Cir. 2002).

ANALYSIS

We have reviewed the Examiner's rejection in light of Appellants' contentions that the Examiner has erred. We disagree with Appellants' conclusions. We agree with the Examiner that with respect to claims 28-36 on appeal, the patent resulting from the prosecution of the 813 Application was error-free.

Appellants argue that the Examiner erred in failing to recognize that the present claims "to the 'method for providing a tow assembly,' which were never part of the subject matter claimed during the prosecution of the original '914 patent . . . [and] were never held to be patentably distinct during the prosecution of the '813 application" (App. Br. 5). Appellants conclude that, contrary to the decision in *Weiler*, 790 F.2d at 1579, failure to file a divisional application to the non-elected Groups in the original '813 application has no bearing on the present reissue claims which were "not 'non-elected' in that application" (*id.*).

According to Appellants (App. Br. 11), "in the instant reissue application, claims to an overlooked invention were never filed in the original application," and therefore, relate to a situation different from that of *Orita*, 550, F.2d at 1280 (the failure to file a timely divisional application to non-elected subject matter is not error correctable by reissue). Moreover,

Appellants argue that "*Orita* does not address the situation in which an invention that was fully disclosed in an original application for patent . . . was overlooked by the applicant through error without deceptive intent" (Reply Br. 11).

We disagree. The critical issue is the relation of the subject matter of newly presented claims 28-36 to the subject matter covered by Groups I - III of the restriction requirement of the 813 Application. Contrary to Appellants' arguments that appealed claims 28-36 "could never be a part of any restriction requirement promulgated in that original application, because there were no claims to that invention" (*id.*), appealed claims 28-36 are substantially identical claims to those of nonelected Group III (claims 30, 31, 34, and 35) identified in the Examiner's restriction requirement of the 813 Application. As stated above, the reissue claims asserted in *In re Orita* did not just read on nonelected subject matter, but rather were "substantially identical to those non-elected in [the] application." *Orita*, 550 F.2d at 1280. Therefore, presently appealed claims 28-36 could not have been prosecuted in the 813 Application from which their invention (Group III, including claims 30, 31, 34, and 35) was restricted.

In the following table, we compare the subject matter of claim 28 of the present reissue Application to the subject matter of claim 34 of the 813 Application, grouped in the non-elected Group III. We note the nominal recitation of "a method for providing" followed by steps of "providing" and "assembling" in claim 28 of the present application, but see no substantial difference with respect to the scope and the structural features between the claims.

	Reissue Application No. 12/827,650 Claim 28	Application No. 10/716,813 Non-Elected Claim 34
1	A method for providing a *tow assembly for* an electromagnetic *airborne survey system* at an assembly location, comprising:	A *tow assembly apparatus for airborne* time domain electromagnetic *surveying* comprising: . . . the transmitter section can be assembled and disassembled . . .
2	providing *a plurality of hollow transmitter support frame sections*; . . . to provide *a tow assembly having a flexible hollow transmitter support frame* . . .	(a) A *flexible support frame* including: (i) *A transmitter section* having a central axis and *consisting of a plurality of interconnectable transmitter section frame members*, such that the transmitter section can be assembled and disassembled, and the transmitter section frame members define a polygonal profile; and
3	providing *a transmitter coil* for generating a primary magnetic field;	Wherein the transmitter section houses an elastically suspended *transmitter coil*
4	providing *connector cables* and a tow cable;	Wherein the transmitter section and the receiver section arc connected by means of *a plurality of connecting cables*
5	with *the connector cables each having a first end connected to a respective location on a circumference of the transmitter support frame and a second end connected to a first end of the tow cable.*	Wherein *the tow assembly apparatus is connected to the aircraft by means of at least one cable means connected to the transmitter section at a plurality of points*;

A comparison of the subject matter of method claim 28 of the present reissue Application 12/827,650 to the subject matter of apparatus claim 34 of the 813 Application leads us to a similar invention in that there is no substantial difference between the claims even though they are directed to different statutory categories. In this case, these claims are simply not patentably distinct. Under *Orita*, of course, there is no correctable error in failing to prosecute divisional applications on inventions of the non-elected groups identified by the Examiner in the original restriction requirement.

We also disagree with Appellants that failure to file a divisional application to the non-elected Groups in the original 813 Application has no bearing on the present reissue claims which were "not 'non-elected' in that application" (App. Br. 5). That is, had they been presented in the original application, appealed claims 28-36 would also have been subjected to the same restriction by either being placed in Group III or in a separate group for similar reasons for restriction from Group I. Consistent with the decision in *Weiler*, 790 F.2d at 1579, even if the present reissue claims were "not 'non-elected'" in the 813 Application, we agree with the Examiner that failing to file divisional applications on the subject matter to which the *claims had never been presented*, similar to failing to file divisional applications on subject matter to which *claims had been made in the original application* would not have been "error" as set forth in § 251 (*see* Ans. 10).

We conclude that as a matter of law, the "Orita doctrine" precludes Appellants from obtaining by reissue the claims on appeal.

CONCLUSION

The Examiner did not err in rejecting claims 28-36 as being improper for reissue.

No time period for taking any subsequent action in connection with this appeal may be extended under 37 C.F.R. § 1.136(a)(1)(iv).

AFFIRMED

ELD

EXHIBIT G3

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

Ex parte BEKA SOLOMON

Appeal 2012-002100
Application 11/358,951
Technology Center 1600

Before DONALD E. ADAMS, DEMETRA J. MILLS, and
JEFFREY N. FREDMAN, *Administrative Patent Judges*.

FREDMAN, *Administrative Patent Judge*.

DECISION ON APPEAL

This is an appeal under 35 U.S.C. § 134 involving claims to a method of inhibiting aggregation of soluble β-amyloid in a human. The Examiner rejected the claims as lacking an error correctable by reissue, as obvious, as claiming new matter and as failing to satisfy the written description requirement. We have jurisdiction under 35 U.S.C. § 6(b). We affirm.

Statement of the Case

Background

"Aggregated amyloid β-protein (βA4) is a major constituent of the abnormal extracellular amyloid plaque that characterizes the brains of victims of Alzheimer's disease (AD)" (Spec. col. 3, ll. 8-10). The Specification teaches that "a method is provided of selecting anti-aggregation molecule such as a monoclonal antibody" (Spec. col. 3, ll. 42-43). The Specification teaches that after "the identification of the anti-aggregation molecules has been completed; it is possible to utilize two or more to prevent or reverse aggregation" (Spec. col. 5, ll. 40-42).

The Claims

Claims 5, 7-9, 17, 19-21, and 31-38 are on appeal. Claim 5 is representative and reads as follows:

> 5. A method of inhibiting aggregation of soluble β-amyloid in a human subject, comprising causing an effective amount of an anti-β-amyloid antibody, or an antigen-binding fragment thereof, to come into contact with the soluble β-amyloid, said antibody or fragment being effective to bind β-amyloid and to inhibit aggregation of the soluble β-amyloid in said subject to an extent at least as great as that obtainable with antibody AMY-33, thereby inhibiting aggregation of the soluble β-amyloid, wherein said anti-β-amyloid antibody is one that is obtainable using an immunogen consisting of a peptide consisting of residues 1-28 of β-amyloid, and said antigen binding fragment is an antigen binding fragment of said antibody, and wherein said antibody or fragment is not conjugated with a detectable moiety.

The issues

A. The Examiner rejected claims 5, 7-9, 17, 19-21, and 31-38 under 35 U.S.C. § 251 for lack of defect or error in the original patent and as not being an error correctable by reissue (Ans. 5-7).

B. The Examiner rejected claims 5, 7-9, 17, 19-21 and 31-38 under 35 U.S.C. § 103(a) as obvious over Walker,[1] Hanan,[2] Bacskai,[3] Friden,[4] and Becker[5] (Ans. 8-14).

C. The Examiner rejected claims 17, 19-21 and 35-38 under 35 U.S.C. § 103(a) as obvious over Becker, Cordell,[6] Spillantini,[7] and Kirschner[8] (Ans. 14-22).

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[1] Walker et al., *Labelling of Cerebral Amyloid In Vivo with a Monoclonal Antibody*, 53 J. NEUROPATHOLOGY AND EXPERIMENTAL NEUROLOGY 377-383 (1994).
[2] Eilat Hanan and Beka Solomon, *Inhibitory effect of monoclonal antibodies on Alzheimer's β-amyloid peptide aggregation*, 3 INT. J. EXP. CLIN. INVEST. 130-133 (1996).
[3] Bacskai et al., *Imaging of amyloid-β deposits in brains of living mice permits direct observation of clearance of plaques with immunotherapy*, 7 NATURE MEDICINE 369-372 (2001).
[4] Friden, P., US 5,182,107, issued Jan. 26, 1993.
[5] Becker et al., EP 0 613 007 A2, published Aug. 31, 1994.
[6] Cordell, B., *β-Amyloid Formation as a Potential Therapeutic Target for Alzheimer's Disease*, 34 ANNU. REV. PHARMACAL. TOXICOL. 69-89 (1994).
[7] Spillantini et al., *Different configurational states of β-amyloid and their distributions relative to plaques and tangles in Alzheimer disease*, 87 PROC. NAT'L ACAD. SCI. USA 3947-3951 (1990).
[8] Kirschner et al., *Synthetic peptide homologous to β protein from Alzheimer disease forms amyloid-like fibrils in vitro*, 84 PROC. NAT'L ACAD. SCI. USA 6953-6957 (1987).

D. The Examiner rejected claims 5, 7-9, and 31-34 under 35 U.S.C. § 112, first paragraph, new matter, as failing to satisfy the written description requirement (Ans. 22-24).

E. The Examiner rejected claims 5, 7-9, and 31-34 under 35 U.S.C. § 112, first paragraph, as failing to satisfy the written description requirement (Ans. 24-28).

A. 35 U.S.C. § 251 for lack of defect or error

The Examiner finds that:

> Appellant's failure to timely file divisional application(s) for non-elected claims from the original application is not an error correctable by reissue under 35 U.S.C. 251, since appellant, by acquiescing in restriction requirement, canceling non-elected claims at allowance (Paper No. 16, mailed 03/03/1997), and allowing the patent to issue on remaining claims, lost her opportunity to challenge propriety of the restriction requirement, and since holding otherwise would permit appellant to undermine copendency requirements of 35 U.S.C. 120 and 121 by using reissue statute to avoid restriction requirement.

(Ans. 6.)

Appellant contends that the "present claims are not the same as or substantially identical to the claim in the original patent application that was cancelled subsequent to a restriction requirement" (App. Br. 15).

Appellant contends that

> the present claims are not of "substantially similar scope" as claim 24 of the original application, as is required by the above-quoted portion of Doyle to invoke the Orita Doctrine. The present claims all require that the aggregating protein be β-amyloid, while claim 24 is generic to any aggregated protein. The examiner has conceded, in the restriction

> requirement of September 20, 2006, in this case that every species within the scope of the above-quoted claim 24 is patentably distinct from every other species within the scope of that claim

(App. Br. 16).

The issue with respect to this rejection is: Does the evidence of record support the Examiner's conclusion that there is a lack of defect or error in the original patent and where the asserted error is not an error correctable by reissue?

Findings of Fact

The following findings of fact ("FF") are supported by a preponderance of the evidence of record.

1. The Examiner restricted the original claims of US application 08/358,786 into three groups, Group I, drawn to selection of anti-aggregation molecules, Group II, drawn to treating a protein aggregation disease using an expression vector encoding an anti-aggregation molecule, and Group III, drawn to treating a protein aggregation disease with a monoclonal antibody (*see* US 08/358,786, Restriction, filed Nov. 12, 1995).

2. Appellant elected Group I (*see* US 08/358,786, Election, filed Feb. 8, 1996).

3. Group III comprised claim 24, which reads as follows:

> The method of treating a protein aggregation disease including the steps of preparing at least one human monoclonal antibody that binds to an aggregated protein which is the cause of a disease and which reverses aggregation allowing bioactivity, and administering the monoclonal antibody.

4. Originally filed reissue claim 5 reads as follows:

> A method of preventing or reducing aggregation of a soluble aggregating protein in a subject, comprising causing an effective amount of an anti-aggregation molecule, wherein said anti-aggregation molecule is effective to prevent or reduce aggregation of the soluble aggregating protein in said subject, to come into contact with the soluble aggregating protein, thereby preventing or reducing aggregation of the soluble aggregating protein.

5. Originally filed reissue claim 7 reads as follows:

> A method in accordance with claim 5, wherein said anti-aggregation molecule is a monoclonal antibody.

Principles of Law

"The board held that, under the circumstances of this case, failure to file a timely divisional application cannot be corrected by reissue. . . . We are in substantial agreement with the board's analysis of this case." *In re Orita*, 550 F.2d 1277, 1279-1280 (CCPA 1977).

Analysis

In the instant case, Appellant stated that the error was that "all the parties involved, i.e., Applicant, Assignee, and the attorney . . . were concentrating their efforts on the patenting of the presently claimed method of selecting an anti-aggregation molecule having the chaperone-like activity of anti-aggregation that issued in the 5,688,651 patent as claims 1-4, the only claims in the patent" (Reissue Dec. 2, Feb. 22, 2006). Appellant further stated that "[i]t was only recently that the Applicant/Patentee and the Assignee realized that the concepts fully disclosed in the specification directed to . . . methods of disaggregating an aggregate of or preventing the

aggregation of an aggregating protein . . . were not claimed *per se*, and further they could be and should be now so claimed" (Reissue Dec 2-3, Feb. 22, 2006).

In the instant reissue, Appellant filed new claims 5-28 drawn to either a "method of preventing or reducing aggregation of a soluble aggregating protein in a subject" as in originally filed claim 5 or a "method of disaggregating an aggregate of a soluble aggregating protein in a subject" as in originally filed claim 17 (*see* Prelim. Amdt., filed Feb. 22, 2006; FF 4). In response to an Examiner's restriction requirement in the reissue, Appellant elected the narrower claims under examination limited to a "method of inhibiting aggregation of soluble β-amyloid in a human subject" (Claim 5, Claims Appx., App. Br. 82).

In the originally filed application, US 08/358,786, Appellant elected Group I drawn to methods of selecting anti-aggregation molecules without traverse (FF 1-2). Appellant did not elect claims drawn to treatment, as both of the non-elected Groups II and III in US 08/358,786 were drawn to methods of treating protein aggregation diseases (FF 1).

We agree with the Examiner that whether we consider the originally filed reissue claims or the narrower elected and currently pending reissue claims, all of these claims are drawn to methods of *treating* protein aggregation diseases. All of these claims are substantially similar to the claims 7-24 which were not elected in US 08/358,786. Therefore, these claims would all fall into either nonelected Group II or III of the original restriction, which were both drawn to types of treatment. None of these

claims would fall within the originally elected Group I of *selecting* anti-aggregation molecules.

This fact pattern precisely matches that in *Orita*, where the Applicants "had forgotten to timely file a divisional application covering the originally non-elected subject matter. Appellants further averred that their failure to file the divisional application was an error without any deceptive intention." *Orita*, 550 F.2d at 1279. Here, while Appellant does not directly state that the error was a failure to file divisional applications, the subject matter of the reissue claims was present in claims 7-24 of the originally filed US 08/358,786 application and was non-elected as Groups II and III of that application (FF 1-3).

Appellant contends that

> the present claims are not of "substantially similar scope" as claim 24 of the original application, as is required by the above-quoted portion of Doyle to invoke the Orita Doctrine. The present claims all require that the aggregating protein be β-amyloid, while claim 24 is generic to any aggregated protein. The examiner has conceded, in the restriction requirement of September 20, 2006, in this case that every species within the scope of the above-quoted claim 24 is patentably distinct from every other species within the scope of that claim

(App. Br. 16).

We are not persuaded. The originally filed reissue claims were of substantially the same scope as that of claim 24 of US 08/358,786, differing only in that claim 24 was limited to monoclonal antibodies as the anti-aggregation molecule, a limitation found in claim 7 of the originally filed reissue claims (*see* FF 3-5).

We also are not persuaded by Appellant's reliance on *Doyle*. *Doyle* found that the case involved "a matter of first impression: whether failure to present a so-called linking claim, a claim broad enough to read on—or link—two or more groups of claims subject to a restriction requirement, is an error correctable by reissue" laid out by the Court of Customs and Patent Appeals. *In re Doyle*, 293 F.3d 1355, 1358 (Fed. Cir. 2002).

In *Doyle*, the Court found that

> it was crucial that the applicant explicitly agreed to the requirement of independent prosecution of the disputed claims (or claims substantially similar to the disputed claims) in a divisional, and not as a part of the application directed towards the elected group. When the applicants returned in reissue seeking to add the disputed claims, the examiner, the Board, and this court rightly held them to the terms of their original agreements. The case is different where, as here, the applicant never asserted the reissue claims or anything similar to them in his original application, and also never agreed to prosecute the reissue claims in a divisional application.

Doyle, 293 F.3d at 1360. The instant facts track much more closely to *Orita* than to *Doyle*. In *Doyle*, there was a linking claim that was broader than all of the original claims and encompassed both groups. *Id.* at 1358. In this case however, no linking claim was filed. Instead, Appellant agreed to the restriction requirement, and then, in the reissue, introduced new claims which would have properly been placed into the non-elected Group III, and not into the elected Group I which was examined and issued. Unlike the new claims in *Doyle*, the newly introduced claims 5-28 filed in US reissue 09/441,140 were not so broad that they would serve as linking claims between Groups I and III, but were treatment claims which would clearly

fall within non-elected Group III. The newly introduced claims 5-28 filed in US reissue 09/441,140 did not encompass nor relate to methods of selecting anti-aggregation molecules of the elected Group I which was prosecuted to issuance as US 5,688,651.

Appellant also contends that "present claims 5, 17, 31 and 35 are not identical to claim 24 as had been deleted from the original application, nor are they of 'substantially similar scope' to that nonelected claim" (App. Br. 17).

We are not persuaded. While there is no doubt that the instant claims, as amended, are narrower than the originally filed claim 24 which was not limited to treatment of β-amyloid disease, we do not find that Appellant has overcome the *Orita* doctrine for two reasons. First, the asserted error permitting reissue identified in Appellant's declaration was drawn to the originally filed claims 5-28, where claim 7, for example, is of substantially identical scope to originally filed claim 24 in US 08/358,786. Thus, the originally identified error is not susceptible to cure by reissue under the *Orita* doctrine.

Second, even the narrower claims now in prosecution, if presented to the Examiner in the original US 08/358,786 application, would minimally have been restricted into either the original Group III or a different Group other than the elected Group I. Unlike the situation in *Doyle*, where the new claims were broader than the groups elected, in this case, the claims are narrower and would have properly fallen either into Group III or into further restriction Groups as in the Restriction requirement in 11/358,951 mailed Sep. 20, 2006. Consonant with *Orita*, should Appellant "prevail, the

copendency requirement would become meaningless, for should an applicant fail to file a divisional application while maintaining copendency as required by section 120, he could simply revert to section 251 in order to cure his mistake." *Orita*, 550 F.2d at 1281. Here, permitting Appellant to correct the failure to file divisional applications by reissue would defeat the copendency requirement of 35 U.S.C. § 120, the result found undesirable by the CCPA in *Orita*.

Conclusion of Law

The evidence of record supports the Examiner's conclusion that there is a lack of defect or error in the original patent and where the asserted error is not an error correctable by reissue.

B. 35 U.S.C. § 103(a) over Walker, Hanan, Bacskai, Friden, and Becker

The Examiner finds that "Walker's method of *in vivo* administration meets the instantly recited step of causing an effective amount of anti-β-amyloid antibody, or an antigen-binding fragment thereof, to come into contact with β-amyloid" (Ans. 9). The Examiner finds that "[a]dministration of the antibody to a subject would be expected to inherently result in inhibition of soluble β-amyloid aggregation and/or disaggregation of aggregated β-amyloid, as evidenced by Bacskai et al. who demonstrate that *in vivo* administration of the 10D5 antibody . . . results in reduction of brain amyloid deposits in aged PDAPP transgenic mice" (*id.* at 10).

The Examiner finds it obvious "to genetically engineer the 10D5 monoclonal antibody to create a less immunogenic antibody molecule, such as a single chain antibody, for use as a delivery vehicle in therapeutic

applications in humans as suggested by Walker et al. and in view of both Friden and Becker et al" (*id.* at 12).

Appellant contends that "the present invention is based on the discovery that the 'naked' antibody (i.e., without a linked drug) has therapeutic properties: reduction of the amyloid plaque and inhibition of the aggregation of soluble β-amyloid" (App. Br. 34). Appellant contends that "the newly discovered fact that the naked antibody can itself inhibit aggregation of soluble β-amyloid and/or cause disaggregation of aggregated β-amyloid confers on the antibody a 'direct' therapeutic capacity which is far more significant than the mere binding property" (*id.* at 39).

The issues with respect to this rejection are:

(i) Does the evidence of record support the Examiner's conclusion that Walker, Hanan, Bacskai, Friden, and Becker render claim 5 obvious?

(ii) If so, has Appellant presented evidence of secondary considerations, that when weighed with the evidence of obviousness, is sufficient to support a conclusion of non-obviousness?

Findings of Fact

6. Walker teaches that for "all studies, we used monoclonal antibody (MAb) 10D5, a murine IgG_1 kappa light chain (whole IgG and/or Fab fragments) to amino acids 1-16 of Aβ" (Walker 377, col. 2).

7. Walker teaches that "[a]ntibody 10D5 or nonimmune IgG (in sterile saline) was injected directly into the CSF . . . . In the two experimental squirrel monkeys. 450 μg (3 μg/μl) of Fab fragments were injected" (Walker 378, col. 1).

8. Walker teaches that "β-Amyloid in untreated, fresh-frozen tissue sections was robustly labeled by antibody 10D5, whereas normal (non Aβ-immune) mouse IgG did not bind to native Aβ in this tissue (data not shown). The 10D5 whole IgG and Fab fragments showed comparable immunostaining *in vitro*" (Walker 379, col. 1).

9. Walker teaches that:

> our studies in aged nonhuman primates demonstrate that it is possible to label Aβ selectively *in vivo* by a monoclonal antibody infused into the CSF. It will be necessary to increase the sensitivity of the method and to determine the safety of the procedure before this approach can be assessed for efficacy in a clinical setting. In particular, the specific etiology of the leukocytic reaction to intracisternal injection requires further study. However, *in vivo* labeling has considerable potential for delivering therapeutic agents that could prevent or reverse Aβ deposition in the brains of patients with cerebrovascular amyloidosis or Alzheimer's disease. Furthermore, this strategy eventually might be useful in diagnostic and experimental approaches to β-amyloidogenesis.

(Walker 382, col. 1.)

10. Hanan teaches that "[a]ggregation of β-amyloid was monitored using a commercially available monoclonal antibody, called mAb AMY 33, raised against the peptide 1-28 of β-amyloid (Zymed, San Francisco, CA) and another four monoclonal antibodies called 6C6 and 10D5, raised against peptide 1-28 of β-amyloid" (Hanan 131, col. 1).

11. Bacskai teaches "clearance of amyloid-β deposits after initial treatment of the cortex with direct application of 10D5. . . . These data indicate that clearance of amyloid-β after exposure to 10D5 is a specific

response to anti-amyloid-β antibodies rather than a nonspecific response to injury" (Bacskai 371, col. 1).

12. Friden teaches that "[a]nti-amyloid antibodies or antibody fragments immunoreactively bind with amyloid plaques that are characteristic of Alzheimer's Disease. For clinical diagnostic detection purposes, a detectable group such as gadolinium or technitium is often attached to the anti-amyloid antibody" (Friden, col. 22, ll. 61-67).

13. Friden teaches that the "conjugate was localized to the brain vasculature in a manner similar to that for OX-26 alone, indicating that the attachment of this anti-amyloid antibody to OX-26 did not affect its ability to target to brain capillary endothelial cells in vivo" (Friden, col. 23, ll. 18-22).

14. Becker teaches that the "antibodies of the present invention are especially preferred in the diagnosis and/or treatment of Alzheimer's disease in mammals, preferably humans" (Becker, col. 7, ll. 49-52).

15. Becker teaches that the

> Winter technology involves the replacement of complementarity determining regions (CDRs) of a human antibody with the CDRs of a murine monoclonal antibody thereby converting the specificity of the human antibody to the specificity of the murine antibody which was the source of the CDR regions. This "CDR grafting" technology affords a molecule containing minimal murine sequence and thus is less immunogenic.

(Becker, col. 7, ll. 2-10.)

16. The Specification teaches that the "present invention provides a method of selecting monoclonal antibodies, genetically engineered antibody

fragments and small peptides which mimic binding sites of the antibodies and which prevent aggregation and yet do not inhibit bioactivity" (Spec. col. 4, ll. 62-66).

17. The Specification teaches in a "preferred embodiment the human monoclonal antibody that binds to an aggregating protein and which prevents aggregation is utilized. In a further preferred embodiment the monoclonal antibody is an anti-β-amyloid and is designated AMY-33 which recognizes amino acids 1-28 of β-amyloid" (Spec. col. 6, ll. 21-26).

18. The Specification teaches, in Example 2, that "[b]inding of mAb AMY-33 to βA4 prevents self-aggregation of the β-amyloid, probably by recognizing the sequence 25-28 located in the proposed aggregation fragment comprising the amino acids between 25-28" (Spec. col. 16, ll. 5-8).

Principles of Law

"In proceedings before the Patent and Trademark Office, the Examiner bears the burden of establishing a prima facie case of obviousness based upon the prior art." *In re Fritch*, 972 F.2d 1260, 1265 (Fed. Cir. 1992).

"[R]ejections on obviousness grounds cannot be sustained by mere conclusory statements; instead, there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness." *KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. 398, 418 (2007).

Analysis

Prima facie obviousness

Walker and Becker provide some suggestion, however bare, to treat β-amyloid plaques in Alzheimer's disease with monoclonal antibodies (FF 6-9, 14, 15). However, none of the prior art references suggest inhibiting

aggregation. In particular, neither Walker, Becker, nor Friden teach the use of any particular level of inhibition of aggregation where the level is "to an extent at least as great as that obtainable with antibody AMY-33" as required by claim 5. There is also no teaching of a method "of disaggregating an aggregate of soluble β-amyloid in a human subject" as required by claim 17 in Walker, Becker or Friden.

While the postfiling date Hanan and Bacskai evidence that 10D5 inherently had the capacity of inhibiting aggregation of soluble β-amyloid in a human subject (FF 10-11), these references cannot properly be relied upon to provide a reason or motivation to administer the antibodies of Walker, Becker or Friden to a patient. Nor can these post filing date references provide a reasonable expectation of success in such an administration.

While we agree with the Examiner that Walker and Becker provide some suggestion to treat patients with anti-β-amyloid antibodies, the Examiner has not established that the Becker antibody or the Walker murine 10D5, even when humanized, would have reasonably been expected to be safe for patients and capable of treating β-amyloid aggregation. The essence of the rejection is based on an improper obvious to try argument, since there is no evidence that the combination had a reasonable expectation of success in treating Alzheimer's disease. Walker is not concerned with safety, since the animals in Walker are sacrificed (FF 6-9). While Becker may be more success oriented, Becker does not identify an antibody which would fall within the scope of the claim nor does Becker provide any evidence of any successful treatment of β-amyloid aggregation. Therefore, in the absence of

any suggestion of a reasonable expectation of success, we see the invention as obvious to try at best, in view of the combined teachings of Walker, Becker and Friden. That is not a proper standard of obviousness. *In re O'Farrell*, 853 F.2d 894, 904 (Fed. Cir. 1988).

Secondary Considerations

Appellant contends that "even if, *arguendo*, the examiner has established a *prima facie* case of obviousness, the evidence of record is sufficient to rebut such *prima facie* case and establish the patentability of the present invention." (App. Br. 32.)

The Examiner finds that "Appellant's argument is not persuasive because the evidence of unexpected results is not commensurate in scope with the entire range of the claimed invention" (Ans. 43). The Examiner also finds that several antibodies cited did not function (*id.* at 44).

We find that Appellant has the better position. In this case, the functional attribute of the antibody to have anti-aggregating or disaggregating properties for therapeutic use to treat Alzheimer's disease is an unexpected result which overcomes the prima facie case of obviousness. We conclude that Appellant's unexpected result is reasonably commensurate with the scope of the claim which is limited to antibodies to amino acids 1-28 of β-amyloid and which inhibit aggregation at least as well as AMY-33.

To the extent the Examiner may be concerned that the claims may embrace possibly inoperative antibody embodiments, we point to *Atlas Powder Co. v. E.I. du Pont de Nemours & Co.*, 750 F.2d 1569, 1576 (Fed. Cir. 1984), where the court stated that "[e]ven if some of the claimed combinations were inoperative, the claims are not necessarily invalid. 'It is

not a function of the claims to specifically exclude ... possible inoperative substances....'"

Conclusion of Law

(i) The evidence of record does not support the Examiner's conclusion that Walker, Hanan, Bacskai, Friden, and Becker render claim 5 obvious.

(ii) Appellant has presented evidence of secondary considerations, that when weighed with the evidence of obviousness, is sufficient to support a conclusion of non-obviousness.

C. 35 U.S.C. § 103(a) over Becker, Cordell, Spillantini, and Kirschner

The Examiner finds that Becker teaches "therapeutic use of anti-β-amyloid antibodies for the treatment of humans having Alzheimer's disease" (Ans. 15). The Examiner finds that "antibodies specific for β-amyloid peptide in the β-sheet conformation are 'functional as direct therapeutic and diagnostic aids'" (*id.* at 16). The Examiner finds that Cordell teaches "compounds that bind β-amyloid and block its ability to seed further molecular addition would be of therapeutic advantage . . . . Figure 4 on page 82 exemplifies this therapeutic approach, wherein compounds that bind to and interrupt β-amyloid protein aggregation are indicated as therapeutic targets" (*id.* at 16-17). The Examiner finds that Spillantini teaches that "antibodies that recognize epitopes within β-amyloid 1-28 are capable of staining β-amyloid proteins that are in a more soluble state and have not yet formed dense core aggregates" (*id.* at 18). The Examiner finds that Kirschner teaches that "the N-terminal region of β-amyloid (i.e., residues 1-28), and in particular the region comprising residues 18-28, is crucial for β-

sheet fibril formation and subsequent aggregation of β-amyloid peptide" (Ans. 18).

The Examiner finds it obvious that "the ordinarily skilled artisan would have recognized the value of inhibitory antibodies directed to β-amyloid 1-28, and in particular residues 14-28 of β-amyloid, as therapeutic agents for diseases associated with abnormal β-amyloid aggregation and deposition, such as Alzheimer's disease" (Ans. 20).

Appellant contends that "the examiner has pointed to no reference of record that suggests that such antibodies would be useful as a therapeutic because of an expectation that they would disaggregate plaque" (App. Br. 47-48). Appellant contends that the "fact that an antibody may bind to amyloid plaque would not necessarily create an expectation, at the time the present invention was made, that such binding will automatically cause disaggregation of the plaque. Indeed, even today it is known that not all antibodies that bind to amyloid plaque will cause disaggregation" (*id.* at 47).

The issue with respect to this rejection is: Does the evidence of record support the Examiner's conclusion that Becker, Cordell, Spillantini, and Kirschner render claim 17 obvious?

Findings of Fact

19. The Examiner finds that "Cordell adds, compounds that bind β-amyloid and block its ability to seed further molecular addition would be of therapeutic advantage" (Ans. 16).

20. Spillantini teaches:

> that antisera against peptides in β-amyloid can be obtained that react predominantly with one of a number of different objects-namely, amyloid plaques without cores, amyloid

> plaques with cores, or tangle-bearing bodies and cells. This suggests that the β-amyloid sequence exists in different configurations and/or in different states of aggregation in amyloid plaques and neurofibrillary tangle-bearing cells.

(Spillantini 3949, col. 2.)

21. Kirschner teaches that "[w]e have demonstrated that a synthetic peptide identical in sequence to the first 28 amino acids of the 4-kDa amyloid protein can assemble *in vitro* into fibrils and bundles having very similar structural and antigenic features as those observed *in situ*" (Kirschner 6957, col. 1).

Analysis

Prima facie obviousness

Becker teaches treating β-amyloid plaques in Alzheimer's disease with monoclonal antibodies (FF 14, 15). The Examiner finds that "Cordell adds, compounds that bind β-amyloid and block its ability to seed further molecular addition would be of therapeutic advantage" (Ans. 16; FF 19). Spillantini and Kirschner teach antibodies which can bind β-amyloid (Ans. 20-21).

Even if we agree with the Examiner that the prior art teaches treatment of β-amyloid plaques with antibodies, none of the prior art references suggest disaggregating an aggregate of β-amyloid using an antibody, either *in vivo* or *in vitro*. There is no evidence of record that the prior art recognized that the anti-β-amyloid antibodies would disaggregate β-amyloid plaques.

Conclusion of Law

The evidence of record does not support the Examiner's conclusion that Becker, Cordell, Spillantini, and Kirschner render claim 17 obvious.

D. 35 U.S.C. § 112, first paragraph, new matter

The Examiner finds that there "is no *verbatim* support in the specification as originally filed for anti-β-amyloid antibodies which inhibit aggregation of β-amyloid to a particular specified degree, nor does this language flow naturally from the disclosure as originally filed" (Ans. 23). The Examiner finds that the "specification as filed thus does not support the genus of antibodies that is described in terms of meeting or exceeding the ability of the antibody AMY-33 to inhibit β-amyloid aggregation" (*id.*).

Appellant contends that the "concept of using any antibody that binds to β-amyloid and prevents aggregation is present in the present specification as filed, for example, at column 16, line 21-26" (App. Br. 59). Appellant contends that "one of ordinary skill in the art would certainly have understood that, once one raises and tests other antibodies for these properties, those that behave even better than the antibodies shown in the assay of Figure 7A might be found" (*id.* at 59-60).

The issue with respect to this rejection is: Does the evidence of record support the Examiner's finding that the limitation "to inhibit aggregation of the soluble β-amyloid in said subject to an extent at least as great as that obtainable with antibody AMY-33" in claim 5 represents new matter?

Findings of Fact

22. The Specification teaches that "the formation of the immuno-complexes with selected, highly specific monoclonal antibodies, should provide a general and convenient method to prevent aggregation of the proteins without affecting their biological properties" (Spec. col. 16, ll. 17-21).

Principles of Law

"[I]t is the specification itself that must demonstrate possession. And while the description requirement does not demand any particular form of disclosure, ... or that the specification recite the claimed invention *in haec verba*, a description that merely renders the invention obvious does not satisfy the requirement" *Ariad Pharmaceuticals, Inc. v. Eli Lilly and Co.*, 598 F.3d 1336, 1352 (Fed. Cir. 2010).

Analysis

While the Examiner is correct that the specific language of the claims was not disclosed *ipsis verbis* in the Specification (Ans. 23), *ipsis verbis* support is not required. *Fujikawa v. Wattanasin*, 93 F.3d 1559, 1570 (Fed. Cir. 1996).

The Specification teaches that the antibody AMY-33 functions to prevent self-aggregation of β-amyloid (FF 18), and teaches that AMY-33 is preferred monoclonal antibody from the class of all monoclonal antibodies which bind aggregating proteins (FF 17-18, 22).

We agree with Appellant that the person of ordinary skill, in reading the Specification, would have found the Specification demonstrating possession of monoclonal antibodies or fragments thereof which are

generated by an immunogen that is amino acids 1-28 of β-amyloid. The ordinary artisan would also find that the Specification teaches that the monoclonal antibodies have an activity which effectively functions to prevent self-aggregation of β-amyloid, where the standard for determining that function is the known AMY-33 antibody disclosed by the Specification (FF 17-18, 22). We therefore conclude that the claim limitation is supported by the Specification as filed.

Conclusion of Law

The evidence of record support the Examiner's finding that the limitation "to inhibit aggregation of the soluble β-amyloid in said subject to an extent at least as great as that obtainable with antibody AMY-33" in claim 5 represents new matter.

E. 35 U.S.C. § 112, first paragraph, written description

The Examiner finds that "present claims are drawn to the use of a genus of antibody molecules having a specific functional property, for which appellant has only disclosed a single species within the genus" (Ans. 24). The Examiner finds that "there is insufficient guidance and direction of the genus of antibodies broadly encompassed by the claimed invention" (*id.*).

The Examiner finds that

> the specification does not provide adequate written description of the claimed genus nor guidance as to which of myriad of antibody molecules that are encompassed by the claimed invention would be capable of inhibiting β-amyloid aggregation to a level that meets or exceeds the ability of the single disclosed species of AMY-33 to inhibit aggregation

(*id.* at 27).

Appellant contends that "the inventor was fully in possession of the concept of raising monoclonal antibodies in the standard manner and using those that bind to the immunogen in the same assays in which the commercial antibodies were used in the remainder of the specification" (App. Br. 65-66).

Appellant contends that the "claims do not recite the antibodies merely by means of a functional statement but rather recite specific properties that the antibodies possess. These properties include binding to β-amyloid and either inhibiting aggregation of soluble β-amyloid or causing disaggregation of aggregated β-amyloid" (*id.* at 68).

The issue with respect to this rejection is: Does the evidence of record support the Examiner's conclusion that the disclosure of the Specification failed to demonstrate possession and descriptive support for Claim 5?

Principles of Law

> [T]he hallmark of written description is disclosure.... [T]he test requires an objective inquiry into the four corners of the specification from the perspective of a person of ordinary skill in the art. Based on that inquiry, the specification must describe an invention understandable to that skilled artisan and show that the inventor actually invented the invention claimed.

Ariad Pharmaceuticals, Inc. v. Eli Lilly and Co., 598 F.3d at 1351.

Analysis

We agree with the Examiner that the Specification does not adequately describe the antibody or a fragment which "binds beta-amyloid and inhibits aggregation of soluble beta-amyloid to an extent at least as great as that obtainable with antibody AMY-33" of claim 5 as broadly claimed.

It is well settled that:

> the inventor cannot lay claim to that subject matter unless he can provide a description of the compound sufficient to distinguish infringing compounds from non-infringing compounds, or infringing methods from non-infringing methods. As the district court observed, "[t]he claimed method depends upon finding a compound that selectively inhibits PGHS-2 activity. Without such a compound, it is impossible to practice the claimed method of treatment."

University Of Rochester v. G.D. Searle & Co., 358 F.3d 916, 926 (Fed. Cir. 2004).

Claim 5, as interpreted in light of the Specification, is broadly drawn to any method of using an antibody, where the antibody "binds beta-amyloid and inhibits aggregation of soluble beta-amyloid to an extent at least as great as that obtainable with antibody AMY-33." The Specification therefore must adequately describe that genus of compounds.

The written description requirement can be met by disclosing "complete or partial structure, other physical and/or chemical properties, *functional characteristics when coupled with a known or disclosed correlation between function and structure*, or some combination of such characteristics." *Enzo Biochem, Inc. v. Gen-Probe Inc.*, 323 F.3d 956, 964, (Fcd. Cir. 2002).

In this case, the Specification has a single working example of an antibody, AMY-33 (*see* FF 18). In addition, the Specification does not describe any of the specific structural features, physical or chemical properties, or other information which would permit identification of antibodies which would bind beta-amyloid and which would have the

functional capacity to inhibit aggregation as well as the AMY-33 antibody. The claim is entirely functional.

Indeed, while the post-filing date evidence demonstrates that the 10D5 antibody, a known prior art antibody (FF 6), shares the functional requirements of the claim (FF 11), the Specification provides no description which would have permitted the ordinary artisan to recognize that the 10D5 antibody satisfied the functional requirements of claim 5.

The present case is therefore analogous to *Rochester* and *Centocor*. In *Rochester*, the patent "describes in detail how to make cells that express either COX-1 or COX-2, but not both ..., as well as 'assays for screening compounds, including peptides, polynucleotides, and small organic molecules to identify those that inhibit the expression or activity of the PGHS-2 gene product.'" *Rochester*, 358 F.3d at 927.

Rochester held that even if a DNA sequence might support a claim to hybridizing nucleic acids, the "same is not necessarily true in the chemical arts more generally. Even with the three-dimensional structures of enzymes such as COX-1 and COX-2 in hand, it may even now not be within the ordinary skill in the art to predict what compounds might bind to and inhibit them." *Rochester*, 358 F.3d at 925.

Thus, in *Rochester*, the court found unpersuasive the instant argument by Appellant that "the generation of antibodies, but also the selection of those antibodies with the enumerated specific properties by appropriate assays is well known in the existing art" (App. Br. 69). The court found that the ordinary artisan could not predict which compounds might satisfy the

functional requirements of the claim, the same situation which applies in the instant fact pattern.

Appellant also relies upon the USPTO written description guidelines, and in particular, Example 13, drawn to support for a "claim drawn to 'an isolated antibody capable of binding to antigen X'" (*see* App. Br. 70). Appellant also relies upon a Board decision (*see* Reply Br. 35-37). We find that the decision of the Federal Circuit in *Centocor* is more closely related to this issue.

In *Centocor*, the Court addressed Example 13 of the USPTO written description guidelines. The Court found that the "PTO guidelines conclude that characterization of the protein alone may be sufficient under circumstances where 'one of skill in the art would have recognized that the disclosure of the adequately described [protein] X put the applicant in possession of antibodies which bind to [protein] X.'" *Centocor Ortho Biotech, Inc. v. Abbott Laboratories*, 636 F.3d 1341, 1351 (Fed. Cir. 2011).

Centocor distinguished the USPTO written description guideline situation, where the claim simply required binding an antigen, from the more complicated situation where specific properties beyond simply binding of antigen by the antibody are required. *Id.* at 1352. *Centocor* found that "[c]laiming antibodies with specific properties, e.g., an antibody that binds to human TNF-α with A2 specificity, can result in a claim that does not meet written description even if the human TNF-α protein is disclosed because antibodies with those properties have not been adequately described." *Id.* at 1352.

The instant situation is virtually identical to the *Centocor* situation, since in both cases the antigen of interest was known, but there was no description in the Specification or prior art of the specific properties required. In the instant case, there was no description of any "genetically-engineered" antibodies which were "effective to bind β-amyloid and to inhibit aggregation of the soluble β-amyloid in said subject to an extent at least as great as that obtainable with antibody AMY-33."

As *Ariad* points out, the "written description requirement also ensures that when a patent claims a genus by its function or result, the specification recites sufficient materials to accomplish that function—a problem that is particularly acute in the biological arts." *Ariad*, 598 F.3d at 1352-3. That is the central issue here, where at best a single species of antibody which satisfies the functional requirements of claim 5 is disclosed. We conclude that Appellant's claims "merely recite a description of the problem to be solved while claiming all solutions to it and ... cover any compound later actually invented and determined to fall within the claim's functional boundaries— leaving it to the pharmaceutical industry to complete an unfinished invention." *Ariad*, 598 F.3d at 1353.

Conclusion of Law

The evidence of record supports the Examiner's conclusion that the disclosure of the Specification failed to demonstrate possession and descriptive support for Claim 5.

SUMMARY

In summary, we affirm the rejection of claims 5, 7-9, 17, 19-21, and 31-38 under 35 U.S.C. § 251 for lack of defect or error in the original patent and as not being an error correctable by reissue.

We reverse the rejection of claims 5, 7-9, 17, 19-21 and 31-38 under 35 U.S.C. § 103(a) as obvious over Walker, Hanan, Bacskai, Friden, and Becker.

We reverse the rejection of claims 17, 19-21 and 35-38 under 35 U.S.C. § 103(a) as obvious over Becker, Cordell, Spillantini, and Kirschner.

We reverse the rejection of claims 5, 7-9, and 31-34 under 35 U.S.C. § 112, first paragraph, new matter, as failing to satisfy the written description requirement.

We affirm the rejection of claims 5, 7-9, and 31-34 under 35 U.S.C. § 112, first paragraph, as failing to satisfy the written description requirement.

No time period for taking any subsequent action in connection with this appeal may be extended under 37 C.F.R. § 1.136(a).

AFFIRMED

cdc

EXHIBIT G4

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

Ex parte MING CHANG WU, PEI-YU CHIOU, and AARON T. OHTA

Appeal 2016-000348
Application 13/926,101
Technology Center 2800

Before JAMES C. HOUSEL, JENNIFER R. GUPTA, and LILAN REN, *Administrative Patent Judges.*

GUPTA, *Administrative Patent Judge.*

DECISION ON APPEAL[1]

Appellants[2] appeal under 35 U.S.C. § 134(a) from the Examiner's decision finally rejecting claims 5, 9–27, 29–52, 54–61, and 63–72. We have jurisdiction under 35 U.S.C. § 6(b).

We AFFIRM.

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[1] In this Decision, we refer to the Non-Final Office Action mailed October 2, 2014 ("Non-Final Act."), the Appeal Brief filed March 17, 2015 ("App. Br."), the Examiner's Answer mailed August 4, 2015 ("Ans."), and the Reply Brief filed October 5, 2015 ("Reply Br.").

[2] Appellants identify the real party in interest as Regents of the University of California and Berkeley Lights, Inc. App. Br. 1.

Claim 5, reproduced below, is illustrative of the claims on appeal.

> 5. A process of manipulating cells or particles by light induced dielectrophoresis in an apparatus comprising a first layer, a second layer, a light source, and a microvision-based pattern recognition subsystem, said process comprising:
>
> applying a biasing voltage between said first layer and said second layer at least one of which comprises a photoconductive material configured for conversion of received light to a local electric field in a vicinity of said received light, wherein said cells or particles are disposed in a volume between said first layer and said second layer; and
>
> controlling with said microvision-based pattern recognition subsystem, in response to registering positions of said particles or cells as determined from microscopic imaging, a pattern of light projected from said light source onto said photoconductive material to create a corresponding pattern of virtual electrodes at said surface of said photoconductive material, each said virtual electrode attracting or repelling ones of said cells or particles in said volume by dielectrophoresis.

DISCUSSION

The application on appeal is a division of U.S. Patent Application Serial No. 13/287,093, which was filed on November 1, 2011, and issued as U.S. Patent No. RE 44,711 on January 21, 2014, which is a reissue of U.S. Patent Application Serial No. 11/105,304 (hereinafter "the '304 application"), which was filed April 12, 2005, and issued as U.S. Patent No. 7,612,355 B2 (hereinafter "the '355 patent") on November.3, 2009.

During prosecution of the '304 application, the Examiner issued Restriction Requirement. The restriction set forth two groups of claims as follows:

I: Claims 7–37, drawn to apparatus for manipulating cells or particles by light induced dielectrophoresis, classified in class 204, subclass 643; and

II: Claims 40–43 and 48–52, drawn to a method of manipulating particle or cellular objects retained within a liquid, classified in class 435, subclass 173.1.

The Restriction Requirement determined Inventions I and II (hereafter referred to as "Groups I and II") to be related, but patentably distinct.

In response to the Restriction Requirement, Applicants elected to prosecute Group I, apparatus claims 7–37. Applicants' response did not dispute the propriety of the Examiner's restriction requirement, and canceled the non-elected claims.

On November 3, 2009, the '304 application issued as the '355 patent, containing four apparatus claims. A divisional application presenting the non-elected claims was never filed during the pendency of the '304 application.

On November 1, 2011, Applicants filed Reissue Application Serial No. 13/287,093. The Examiner issued a two-way restriction between claims directed to an apparatus (Group I, claims 1–25, 67–82, and 88–94), and claims directed to a process (Group II, claims 26–66, 83–87, 95–104, and 105–111).

In response to the restriction, Applicants elected the apparatus claims (Group I), and canceled the non-elected claims.

On June 25, 2013, Applicants filed the instant reissue application, which is a division of Reissue Application Serial No. 13/287,093. All original patent claims were canceled and reissue claims 5, 9–27, 29–61, and 63–72, directed to a process are pending.

Claims 5, 9–27, 29–52, 54–61, and 63–72 stand rejected as being based upon a defective reissue declaration under 35 U.S.C. § 251.

Appellants argue the claims as a group. *See generally* App. Br. 3–9. We select claim 5 as representative of the rejected claims, and the remaining claims on appeal will stand or fall with claim 5.

The requirements for reissuance of a patent are set forth in 35 U.S.C. § 251. The first paragraph of Section 251 reads as follows:

> Whenever any patent is, through error, deemed wholly or partly inoperative or invalid, by reason of a defective specification or drawing, *or by reason of the patentee claiming more or less than he had a right to claim in the patent*, the Director shall, on the surrender of such patent and the payment of the fee required by law, reissue the patent for the invention disclosed in the original patent, and in accordance with a new and amended application, for the unexpired part of the term of the original patent. No new matter shall be introduced into the application for reissue. [Emphasis added].

The Federal Circuit has explained that section 251 "is remedial in nature, based on fundamental principles of equity and fairness, and should be construed liberally." *In re Weiler*, 790 F.2d 1576, 1579 (Fed. Cir. 1986). Nevertheless, the court has also made it clear that "not every event or circumstance that might be labeled 'error' is correctable by reissue." *Id.*

Appellants argue that the Examiner erred in determining that the "process claims, regardless of their subject matter, are precluded from reissue due to the Restriction and subsequent Election in the Original Application." App. Br. 6. Specifically, Appellants contend that our reviewing court's decision in *In re Doyle*, 293 F.3d 1355 (Fed. Cir. 2002), not *In re Orita*, 550 F.2d 1277 (CCPA 1977), is instructive in deciding whether Appellants' failure to present the process claims on appeal is an error correctable by reissue. *Id.* at 7; *see also* Reply Br. 2–3.

Doyle involved a matter of first impression: "whether failure to present a so-called linking claim, a claim broad enough to read on—or link—two or more groups of claims subject to a restriction requirement, is an error correctable by resissue." *Doyle*, 293 F.3d at 1358. The Federal Circuit answered this question in the affirmative. *Id.* at 1360. In reaching its conclusion, the court found that the "*Orita* doctrine"[3] did not apply to the facts involved in *Doyle* for two reasons: (1) the new claims were neither identical nor substantially similar to the nonelected claims, and (2) the new claims could have prosecuted with the elected group without running afoul of the restriction requirement. *Id.*

We agree with the Examiner that the facts and circumstances in the present reissue application are distinguishable from those of *Doyle*, which carved out a narrow exception to the *Orita* doctrine. As the Examiner points out, the claims on appeal in the present case are distinguishable from the claims at issue in *Doyle* because they are not directed to genus claims that are broad enough to read on or link both the elected and non-elected claims. The fact that the claims presented in *Doyle* were linking claims was at the heart of the matter decided in *Doyle*. *Doyle*, 293 F.3d at 1358; *see also Landmark Screens LLC v. Morgan, Lewis, & Bockius, LLP*, 676 F.3d 1354, 1364 (Fed. Cir. 2012) (explaining that *Doyle* is properly invoked "[w]hen the reissue claims are *broader* than the issued claims" (emphasis added)). Thus, despite Appellants' contentions, we do not consider this difference to be "immaterial." *See* Reply Br. 2.

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[3] The "*Orita* doctrine" precludes applicants from obtaining substantially identical claims to those of non-elected groups identified in an examiner's restriction requirement when such claims could not have been prosecuted in the application from which they were restricted. *Doyle*, 293 F.3d at 1359.

As discussed above, in the present case Appellants acquiesced to the restriction requirement in the '304 application, electing apparatus claims (Group I), and cancelling the non-elected claims (Group II, method claims), and then, in this reissue, introduced new process claims 5, 9–27, 29–52, 54–61, and 63–72. Unlike the new claims in *Doyle*, the process claims on appeal are not so broad that they would read on or link the claims of Groups I and II in the restriction requirement made in the '304 application. Rather, Appellants' new process claims are narrower than the original non-elected group of method claims, which are not limited to being performed in an apparatus comprising "a microvision-based pattern recognition subsystem" configured to control light projected from a light source "in response to registering positions of said particles or cells as determined from microscopic imaging."

Even if the claims are not viewed as narrower than the original non-elected group of method claims, as the Examiner point outs, the process claims on appeal are patentably distinct from the apparatus claims elected during prosecution of the '304 application. Non-Final Act. 4; Ans. 6–7. As a result, if presented to the Examiner during the prosecution of the '304 application, the new process claims would have been restricted into either original Group II or a group other than the elected Group I. Consequently, Appellants could not have prosecuted the process claims on appeal with the issued claims.

Moreover, as well-stated by the Examiner in the Answer (Ans. 8), Appellants lost their ability for rejoinder by voluntarily cancelling the withdrawn claims before rejoinder occurred. *Id.* (citing *Manuel of Patenting Examining Procedure* § 821.04(b) ("MPEP")).

The facts in the instant appeal track more closely to *Orita* than to *Doyle*. *Orita*, 550 F.2d at 1278–1279. Although the appealed reissue claims are not identical to the canceled, non-elected claims of the original patent application, they are narrower than, and encompass the subject matter of, each of the canceled, non-elected claims. Appellants, here, acquiesced to the restriction requirement in the '304 application by cancelling the non-elected claims (method claims), and are now trying to obtain claims of the same type as the canceled claims via reissue. *Orita*, 550 F.2d at 1280; *see also In re Smyser*, 135 F.2d 747, 749–50 (CCPA 1943), and *In re Cornell*, 150 F.2d 702, 704 (CCPA 1945). Under the rationale set forth in *Smyser and Cornell* (cases upon which *Orita* rests), however, Appellants are not entitled to obtain method claims *per se*, regardless of scope, because they conceded by their acquiescence in the restriction requirement that they were not entitled to claim method claims in their original application. *Smyser*, 135 F.2d at 751; *see also Cornell*, 150 F.2d at 704.

Finally, as pointed out by the court in *Orita*:

> . . . granting by reissue claims substantially identical to those non-elected in application I would be ignoring the proper restriction requirement set forth in that application in which appellants acquiesced. Indeed, appellants' misapplication of section 251 would, if permitted, circumvent the copendency requirement of section 120, incorporated by reference in section 121 (see note 2 supra). Should appellants prevail, the copendency requirement would be meaningless, for should an applicant fail to file a divisional application while maintaining copendency as required by section 120, he could simply revert to section 251 in order to cure his mistake. Section 251 is not a pancea designed to cure every mistake which might be committed by an applicant or his attorney . . . [Footnote omitted].

Orita, 550 F.2d at 1280–1281. The same concerns of *Orita* are equally applicable here.

In sum, a preponderance of the evidence supports the Examiner's conclusion that Appellants' asserted error is not an error correctable by reissue. Non-Final Act. 5; *see also* Reply Br. 4 (where Appellants state that their mistake was "failure to add — in the Response of February 26, 2009 — method claims having each and every limitation of apparatus claims 12 and 24."). Accordingly, we sustain the Examiner's rejection of claims 5, 9–27, 29–52, 54–61, and 63–72.

DECISION

For the above reasons, the Examiner's rejection of claims 5, 9–27, 29–52, 54–61, and 63–72 is affirmed.

No time period for taking any subsequent action in connection with this appeal may be extended under 37 C.F.R. § 1.136(a).

AFFIRMED

EXHIBIT H1

2014-1050
(Serial No. 12/827,650)

UNITED STATES COURT OF APPEALS FOR THE FEDERAL CIRCUIT

IN RE EDWARD BEVERLY MORRISON, PAVEL TISHIN, PETR VALENTINOVICH KUZMIN

Appeal from the United States Patent and Trademark Office,
Patent Trial and Appeal Board.

BRIEF FOR APPELLEE—DIRECTOR OF THE UNITED STATES PATENT AND TRADEMARK OFFICE

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February 18, 2014

REPRESENTATIVE CLAIM

28. A method for providing a tow assembly for an electromagnetic airborne survey system at an assembly location, comprising:

providing a plurality of hollow transmitter support frame sections;

providing a transmitter coil for generating a primary magnetic field;

providing connector cables and a tow cable; and

assembling the transmitter support frame sections and the transmitter coil and the cables to provide a tow assembly having a flexible hollow transmitter support frame that is circular or approximates a circle having the transmitter coil disposed within the hollow transmitter support frame and formed from serially connecting the transmitter support frame sections, with the connector cables each having a first end connected to a respective location on a circumference of the transmitter support frame and a second end connected to a first end of the tow cable.

A3; A200.

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STATEMENT OF RELATED CASES

The Director is not aware of any other appeal in connection with this case that has previously been before this Court, or that is currently pending in any other court. The Director is also unaware of any related cases pending in this or any other court that will directly affect, or be directly affected by, this Court's decision in the pending appeal.

I. STATEMENT OF THE ISSUE

Edward Beverly Morrison, Pavel Tishin, and Petr Valentinovich Kuzmin ("Morrison") are the inventors of U.S. Patent No. 7,157,914 ("the '914 patent"). Morrison originally claimed multiple, distinct inventions in the application leading to the '914 patent. Accordingly, the Examiner restricted the claims into three groups. Morrison elected the first group and prosecuted those claims to allowance, but did not file divisional application(s) to the nonelected claims or to any other subject matter prior to issuance of the '914 patent. Morrison now attempts to prosecute subject matter distinct from his elected invention by filing a reissue application. The issue before this Court is whether the Board properly determined that Morrison's failure to timely file a divisional application is not correctable by reissue.

II. STATEMENT OF THE CASE

The '914 patent, entitled "Airborne Electromagnetic Time Domain System, Computer Product and Method," issued on January 2, 2007 with twenty-seven claims. A18, A20-35. On June 30, 2010, Morrison filed a reissue application[1] adding new claims. A17, A36-37, A134. The Examiner finally rejected the new claims under 35 U.S.C. § 251 for lack of defect or error in the

[1] The present reissue application is a divisional of reissue application no. 12/343,321, filed November 16, 1999, which issued on March 15, 2011 as U.S. RE42,217.

original patent correctable by reissue. A242-57 (Examiner's Answer). Morrison appealed to the Board of Patent Appeals and Interferences ("Board"), which affirmed. A1-12. This appeal followed.

A. Morrison Discloses a Method and Apparatus for Geological Mapping Using Time Domain Electromagnetic Surveying

Time domain electromagnetic ("TDEM") surveying allows for the geological exploration of the earth. In simplistic terms, TDEM surveying involves electromagnetic pulses that penetrate the earth's surface. The feedback information produced from the electromagnetic pulses allows geophysicists to accurately map underground features such as water sources, mineral deposits, soil features, *etc.*, from above the earth. *See generally* United States Environmental Protection Agency, "Time-Domain Electromagnetic Methods," http://www.epa.gov/esd/cmb/GeophysicsWebsite/pages/reference/methods/Surface_Geophysical_Methods/Electromagnetic_Methods/Time-Domain_Electromagnetic_Methods.htm (last visited Jan. 8, 2014); *see also* A20 (col. 1, ll. 14-35).

The airborne TDEM system disclosed in the '914 patent consists of a transmitter **16** and a receiver **18** assembly towed from, *e.g.*, a helicopter. A21 (col. 4, ll. 12-19); A23 (Fig. 1). The transmitter includes a wire coil **28** attached to a support frame **20**. A21 (col. 4, ll. 26-32); A22 (col. 5, ll. 13-20); A25 (Fig. 3). The transmitter coil **28** carries a time-varying (on-off) current that in turn

generates periodic electromagnetic field pulses. A20 (col. 1, ll. 26-29); A33 (col. 7, ll. 11-16). The receiver, equipped with a sensor coil **50**, measures the earth's response to the pulses. A20 (col. 1, ll. 26-35); A22 (col. 6, l. 9). A computer program analyzes the data the receiver obtains to produce useable survey data. A22 (col. 6, ll. 64-66).

While airborne TDEM surveying was known in the art, the '914 patent states that its system provides improved sensor resolution over known systems through the use of a dual-mode gain amplifier linked to the sensor. A21 (col. 3, ll. 25-26); *see also* A33 (col. 7, ll. 10-25). The dual-mode gain amplifier limits signal amplification when the transmitter is pulsed "on," but allows for high signal amplification when the transmitter is pulsed "off." A33 (col. 7, ll. 10-25). The resulting increased signal-to-noise ratio and reduced electrical interference improves overall sensor resolution. *Id.*

B. Prosecution of the Original Application

The '914 patent originated as U.S. patent application no. 10/716,813, which was filed on November 20, 2003. During prosecution of the application, the Examiner issued a restriction requirement,[2] separating the pending claims

[2] Section 121 of the Patent Act grants the Director the authority to limit a patent to one invention. 35 U.S.C. § 121. In a so-called "restriction requirement," the examiner typically organizes the claims into groups (*i.e.*, independent or distinct inventions) and requires the applicant to elect one group for prosecution. 37 C.F.R. § 1.142(a). Claims to the invention(s) not elected are

into three groups for purposes of examination:

- Group I, claims 1, 3-10, 12-13, 15-28, and 32-33, drawn to *a method and apparatus using a tow assembly*, the assembly comprising a flexible frame, the receiver flexibly connected to a receiver support frame and a dual mode gain amplifier, classified in class 324, subclass 330;

- Group II, claim 29, drawn to *a computer program* comprising a computer readable medium and instructions to operate a general electromagnetic antenna assembly, classified in class 702, subclass 5; and

- Group III, claims 30-31 and 34-35, drawn to *trailing airborne surveying systems* having specific antenna structures, classified in class 343, subclass 707.

A205-10 (emphases added).

The Examiner explained that the independent claims of each group had non-overlapping features and thus represented distinct subcombinations. A207-08; *see also* Manual of Patent Examining Procedure ("MPEP") § 806.05(d) (discussing restriction of claims to distinct subcombinations). The claims of Group III were separately grouped from the claims of Groups I and II because the Group III-claims required specific antenna (*i.e.*, transmitter and receiver) structure not found in the claims of Groups I and II. A207-08.

Specifically, independent claims 30 and 34 of Group III required a receiver support frame and a transmitter support frame connected to one another

---

withdrawn from consideration, and if not later subject to a rejoinder process, are canceled prior to issuance. 37 C.F.R. § 1.142(b).

by way of a plurality of connecting cables (shown, for example, as tension rope **40** in Figure 4) evenly distributed along the circumference of the support frames. A316-17, 319-20 (nonelected claims 30 and 34); *see also* A22 (col. 5, ll. 40-47); A26 (Fig. 4) (reproduced below with red annotation).

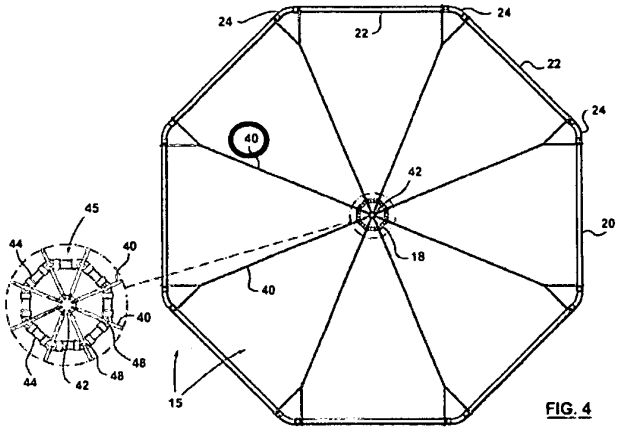

FIG. 4

Independent claim 31 of Group III specified that the receiver support frame consist of a plurality of interconnected frame members that are easily assembled and disassembled. A317-18 (nonelected claim 31).

In contrast, the independent claims of Groups I and II recited none of these features. A208. The claims of Group I focused on other features—specifically, the dual-mode gain amplifier and a sensor means flexibly connected to the receiver support frame. *Id.* And the claim of Group II recited a computer readable medium. *Id.* The Examiner also noted that the art recognized the claims of each group as having distinct subject matter and

separate classification, further supporting restriction of the claims into the three groups. *Id.*

In response to the restriction requirement, Morrison elected without traverse the claims of Group I, and withdrew claims 29-31 and 34-35 "without prejudice to the right to file one or more divisional applications comprising the subject matter of species (II) or (III)." A322; A6 (FF12). Immediately thereafter the Examiner allowed claims 1, 3-10, 12-13, 15-28, and 32-33 of Group I (renumbered as claims 1-27), and with Morrison's permission, canceled the nonelected claims of Groups II and III. *See* A324-27 (Notice of Allowability and Examiner's Amendment).

The '914 patent issued on January 2, 2007. A18. Independent claims 1 and 26 recite various embodiments of "[a]n airborne time domain electromagnetic surveying system." A33 (col. 8, ll. 15-42); A34 (col. 10, l. 51-col. 11, l. 13). Remaining independent claims 20 and 27 recite methods for "producing survey data." A34 (col. 9, l. 64-col. 10, l. 31); A35 (col. 11, l. 13-col. 12, l. 23). Morrison did not file any divisional application(s) to the nonelected subject matter or to any other non-claimed subject matter prior to issuance. *See, e.g.*, A6 (FF12).

C. Prosecution of the Reissue Applications

As described in his opening Brief, Br. at 5-6, Morrison filed two reissue applications from the '914 patent. The first, U.S. Application No. 12/343,321, contained the complete set of patented claims 1-27 from the '914 patent, as well as new claims 28-33 directed to a method for providing a tow assembly. *See* A328-29 (new claims 28-33). Consistent with the original prosecution, the Examiner restricted the new claims from the patented claims. A220-24. The Examiner explained that because the new claims do not require the dual-mode gain amplifier and the flexible connection between the sensor and receiver support frame characteristic of the patented claims, they are properly restricted from the patented claims. A221.

Morrison again elected to prosecute claims 1-27 to allowance, but this time filed a divisional application—the second reissue application and the application underlying this appeal—containing the nonelected reissue claims 28-33. A36-37. In his reissue declaration, A58-61, Morrison attested that the '914 patent was "wholly or partly inoperative by reason of an error in claiming less than I had a right to claim in the '914 patent," A59. Specifically, Morrison declared that he had claimed his invention too narrowly by overlooking embodiments that pertain to a method for providing a tow assembly at an assembly location. *Id.*

The Examiner rejected claims 28-33 and later-added claims 34-36 under 35 U.S.C. § 251 for lack of defect or error in the original patent correctable by reissue. *See* A242-57. Comparing the proposed reissue claims with the nonelected claims of Group III of Morrison's original application, the Examiner found that the reissue claims relate to subject matter not distinct from the nonelected claims, as both sets of claims are directed to the specific features of the transmitter and receiver structures of the towed assembly. A245. The Examiner explained that, had the reissue claims been presented in the original prosecution, those claims could not have been prosecuted in that application because they would have been grouped with the nonelected claims of Group III, or alternatively, in a separate group of claims. A245-46. Relying on MPEP § 1412.01, the Examiner concluded that Morrison's failure to file a divisional application to the nonelected subject matter prior to issuance of the '914 patent is not an error correctable by reissue. A246.

D. The Board's Decision

The Board affirmed the Examiner's rejection of all of the pending claims under 35 U.S.C. § 251 for lack of defect or error in the '914 patent correctable by reissue. A12. First, the Board found independent claim 28, as follows, representative:

> 28. A method for providing a tow assembly for an electromagnetic airborne survey system at an assembly location, comprising:
>
> providing a plurality of hollow transmitter support frame sections;
>
> providing a transmitter coil for generating a primary magnetic field;
>
> providing connector cables and a tow cable; and
>
> assembling the transmitter support frame sections and the transmitter coil and the cables to provide a tow assembly having a flexible hollow transmitter support frame that is circular or approximates a circle having the transmitter coil disposed within the hollow transmitter support frame and formed from serially connecting the transmitter support frame sections, with the connector cables each having a first end connected to a respective location on a circumference of the transmitter support frame and a second end connected to a first end of the tow cable.

A3; *see also* A200.

The Board then addressed the rejection under § 251, and found that the evidence of record supported the Examiner's conclusion that the '914 patent does not contain an error correctable by reissue. A4-12. The Board agreed with the Examiner that Morrison's reissue claims are substantially similar to the nonelected claims of Morrison's original application. A9. Specifically, the Board compared the subject matter of representative reissue claim 28 to that of original, nonelected claim 34 of Group III and found no substantial difference between the scope and structural features recited in the claims. A9-11. The

Board concluded that the reissue claims would have been grouped with nonelected Group III. A11. And because Morrison chose not to file a divisional application to the nonelected subject matter, the Board explained, he is precluded from pursuing those claims under the *Orita* doctrine. *Id.* (citing *In re Orita*, 550 F.2d 1277 (CCPA 1977)).

The Board also found unpersuasive Morrison's argument that his reissue application is proper because the reissue claims contain subject matter not presented in any of the original claims. A11. Even assuming that the reissue claims were never expressly "non-elected," they would have been grouped into another nonelected group had they been presented and lost through Morrison's failure to file a divisional application to those claims. *Id.* The Board explained that under this Court's decision in *In re Weiler*, 790 F.2d 1576, 1579 (Fed. Cir. 1986), Morrison's failure to file divisional applications to subject matter not previously claimed does not constitute error correctable under § 251. A11.

III. SUMMARY OF THE ARGUMENT

The *Orita-Weiler-Doyle* line of cases prohibits a patentee from using a reissue proceeding to obtain claims that the patentee could not have prosecuted in his or her original application. These cases are based on the rationale that, in acquiescing to an examiner's restriction requirement, the applicant has chosen to pursue claims only to the elected invention. In other words, the applicant has

agreed to independently prosecute any invention distinct from that elected in divisional application(s). This Court accordingly does not treat a patentee's failure to file those divisional application(s) as an "error" correctable under the reissue statute. Otherwise, the patentee who failed to file a divisional application could use a reissue proceeding to circumvent the co-pendency requirement of 35 U.S.C. § 120.

Morrison attempts to do exactly what the *Orita-Weiler-Doyle* line of cases rejects. In response to the Examiner's restriction requirement, Morrison elected without traverse the claims of Group I and prosecuted them to allowance. During pendency of his original application, Morrison did not file divisional application(s) to the nonelected claims or to any other distinct subject matter he disclosed. Morrison now seeks to prosecute an invention that he chose not to pursue in a divisional application. But that invention, as Morrison admits, is distinct from his patented claims and accordingly could not have been prosecuted in his original application. Put differently, because Morrison claimed exactly what he had a right to claim in his original application—*i.e.*, the elected method and apparatus claims—the Board correctly determined that the '914 patent lacks an error correctable under the reissue statute. For this reason, the Board's rejection should be affirmed.

IV. ARGUMENT

A. Standard of Review

Morrison has the burden to show that the Board committed reversible error. *In re Watts*, 354 F.3d 1362, 1369 (Fed. Cir. 2004). Whether the claims of a reissue application are unpatentable under 35 U.S.C. § 251 is a question of law based on underlying findings of fact. *In re Serenkin*, 479 F.3d 1359, 1361 (Fed. Cir. 2007). This Court reviews the Board's legal conclusions *de novo* but affirms the Board's factual findings that are supported by substantial evidence. *In re Jolley*, 308 F.3d 1317, 1320 (Fed. Cir. 2002); *see also* 5 U.S.C. § 706(2)(E). Substantial evidence is "such relevant evidence as a reasonable mind might accept as adequate to support" the conclusion reached. *Consol. Edison Co. v. NLRB*, 305 U.S. 197, 229 (1938). It is "something less than the weight of the evidence but more than a mere scintilla of evidence." *In re Kotzab*, 217 F.3d 1365, 1369 (Fed. Cir. 2000). "[W]here two different, inconsistent conclusions may reasonably be drawn from the evidence in record, an agency's decision to favor one conclusion over the other is the epitome of a decision that must be sustained upon review for substantial evidence." *Jolley*, 308 F.3d at 1329.

B. The Board Correctly Concluded That Morrison's Patent Lacks an Error Correctable by Reissue

Section 251 of the 1952 Patent Act provides for the reissue of a patent that "through error without any deceptive intention, [is] deemed wholly or partly

inoperative or invalid . . . by reason of the patentee claiming more or less than he had a right to claim in the patent." 35 U.S.C. § 251 (2006), *amended by* 35 U.S.C. § 251(a) (2011) (adding paragraph letters and deleting "without any deceptive intention"). Fundamental principles of equity and fairness guide the application of the reissue statute, as Morrison notes. *See* Br. at 19. But "[b]y its terms section 251 restricts reissue to situations in which an error occurred." *In re Doyle*, 291 F.3d 1355, 1358 (Fed. Cir. 2002). And not just any "error" qualifies under the statute. *E.g.*, *Weiler*, 790 F.2d at 1579 ("[N]ot every event or circumstance that might be labeled 'error' is correctable by reissue."). This Court and its predecessor court have generally refused to call "error" the deliberate choices a patent applicant makes (albeit later regretted) to obtain allowance. *See e.g.*, *In re Yamazaki*, 702 F.3d 1327, 1333 (Fed. Cir. 2012) (holding that patentee's failure to prevent a patent from issuing with terminal disclaimer not an error correctable by reissue); *In re Mostafazadeh*, 643 F.3d 1353, 1358 (Fed. Cir. 2011) (reiterating that intentional surrender of subject matter during original prosecution to overcome prior art not an error); *Serenkin*, 479 F.3d at 1363 (holding that a "conscious decision" to accept a later filing date for an application not a correctable error); *In re Mead*, 581 F.2d 251, 257 (CCPA 1978) (holding that patentee's "conscious choice" not to file a

continuing application not correctable error). The *Orita*, *Weiler*, and *Doyle* decisions, relevant to this appeal and discussed in detail below, stand in accord.

1. The *Orita-Weiler-Doyle* line of cases prohibits a patentee from using a reissue proceeding to obtain claims that the patentee could not have prosecuted in his or her original application

Orita, *Weiler*, and *Doyle* hold that when an applicant makes a deliberate choice in response to a restriction requirement to prosecute a single invention and fails to file divisional application(s) to the remaining, nonelected inventions—whether originally claimed or not—an error correctable by § 251 does not exist. These cases are based on the rationale that once an applicant acquiesces to a restriction requirement, any invention distinct from the one elected and prosecuted to allowance can only be pursued in a timely-filed divisional application.

In *Orita*, the CCPA addressed the issue of whether an applicant's failure to file a divisional application to nonelected claims constituted an "error" under § 251. 550 F.2d at 1280. During the prosecution of Orita's original application, the examiner issued a restriction requirement separating the claims into two groups. *Id.* at 1278. The applicants elected the first group and canceled the nonelected claims of the second. *Id.* After a patent issued, applicants filed a reissue application to claims "substantially identical to the originally non-elected claims," and stated in a declaration that they had claimed less than they had a

right to claim because "they had forgotten to timely file a divisional application covering the originally non-elected subject matter." *Id.* at 1278-79.

The court upheld the Board's denial of the reissue, holding that applicants' acquiescence to the examiner's restriction requirement did not constitute an "error" under § 251. The court explained that while "appellants undoubtedly erred by failing to file a timely divisional application," the resulting patent was error-free: "Patentees claimed exactly what they had a right to claim in the patent, no more nor less, and appellants' failure to timely re-file does not change this fact." *Id.* at 1280.

The court also noted that allowing reissue claims substantially identical to those not elected would render the co-pendency requirement of § 120 "meaningless." The court continued:

> for should an applicant fail to file a divisional application while maintaining copendency as required by section 120, he could simply revert to section 251 in order to cure his mistake. Section 251 is not a panacea designed to cure every mistake which might be committed by an applicant or his attorney, and the case at bar exemplifies a mistake which this section cannot cure.

Id. at 1281.

The CCPA's decision gave rise to what is now commonly referred to as "the *Orita* doctrine." It finds its roots in two pre-1952 Patent Act cases: *In re Cornell*, 150 F.2d 702 (CCPA 1945) and *In re Smyser*, 135 F.2d 747 (CCPA 1943). In both of those cases, the CCPA held that the reissue statute did not

authorize claiming of subject matter in a reissue that could not have been included in the original application as a result of applicants' acquiescence in the examiner's requirement for "division." *Cornell*, 150 F.2d at 704-05; *Smyser*, 135 F.2d at 751.

Following *Orita*, which dealt with *claimed* subject matter, this Court held in *Weiler* that error correctable by § 251 does not lie in failing to file divisional applications to *non-claimed* subject matter distinct from the subject matter that the applicant elected. *Weiler*, 790 F.2d at 1580. The examiner in *Weiler* restricted the original claims into three groups. Weiler chose the first group without traverse and prosecuted those claims to allowance, but did not file divisional applications directed to the nonelected subject matter or to any other disclosed subject matter. *Id.* at 1578. After issuance, Weiler filed a reissue application with claims to subject matter disclosed but never claimed in the original application. *Id.* at 1578-79. This Court upheld the Board's rejection of the reissue application for lack of error. In doing so, this Court noted that the subject matter of the claims on appeal was "clearly independent of and distinct" from that of both the elected and nonelected claims, and accordingly, "Weiler would thus have had no right to insert and present [the reissue claims] in the original application after the examiner's requirement for restriction." *Id.* at 1581. Following the logic of *Orita*, this Court held that by acquiescing in an

examiner's restriction requirement, "it cannot on the present record have been error to forego divisional applications on subject matter to which claims had never been made." *Id.* at 1582.

While *Orita* and *Weiler* addressed specific claims to distinct inventions, *Doyle* addressed a "linking" claim, *i.e.*, "a claim broad enough to read on—or link—two or more groups of claims subject to a restriction requirement." 293 F.3d at 1358. This Court characterized the issue in *Doyle* as "whether failure to present a so-called linking claim . . . is an error correctable by reissue." *Id.* Doyle's original application included both compound and method claims. *Id.* at 1356. In response to the examiner's nine-way restriction requirement, Doyle elected the claimed invention of using a genus of catalysts to insert carbenes into certain chemical bonds, and canceled the remaining claims. *Id.* Doyle did not file any divisional applications to the nonelected claims. But following issuance of the patent, Doyle filed a reissue application with claims directed to a method of using a genus of catalysts to insert any prochiral molecule (*i.e.*, not just carbene) into the chemical bonds. *Id.* at 1357. Doyle conceded that the new claims, while broader in scope than the elected invention, read on some of the nonelected claims. *Id.*

The Board upheld the rejection of the claims based on lack of error correctable by reissue. *Id.* at 1358. This Court reversed. In doing so, the Court

first noted that "[t]he so-called *Orita* doctrine . . . precludes a reissue applicant from obtaining substantially identical claims to those of nonelected groups identified in an examiner's restriction requirement when such claims could not have been prosecuted in the application from which they were restricted." *Id.* at 1359. The Court then explained that *Weiler* prevents a reissue applicant from pursuing claims to "a completely separate invention from the issued claims or any of the nonelected claims." *Id.* at 1362. Such claims, the *Doyle* Court explained, if presented during the original prosecution, "would have been subject to the original restriction requirement and the applicant would not have been allowed to prosecute them with the original patent—he would have been required to assert them in a divisional." *Id.*

But in *Doyle*, the Court held that neither *Orita* nor *Weiler* applied because Doyle's reissue claims were "not to an invention distinct from that of the issued claims." *Id.* at 1363. Unlike Orita and Weiler, Doyle could have—and indeed *should have*—prosecuted the broader linking claims (linking the elected and nonelected claims) in the original application. *Id.* And importantly, because Doyle never acquiesced to prosecuting the broader linking claims in a divisional, the estoppel rationale underlying *Orita* and *Weiler* did not apply. *Id.*

The *Orita* and *Weiler* cases stand for the "established principle that applicants are 'estopped from obtaining by reissue claims which, because of a

requirement for restriction in which they had acquiesced, they could not claim in their patent.'" *In re Watkinson*, 900 F.2d 230, 232 (Fed. Cir. 1990) (quoting *Orita*, 550 F.2d at 1280). *Doyle* comports with that principle, as Doyle could have prosecuted his reissue claims in his original application. That he failed to prosecute his reissue claims in his original application constituted a correctable error of claiming less than Doyle had a right to claim under § 251. In contrast, the applicants in *Orita* and *Weiler* could not have pursued their reissue claims in their original applications because those claims were distinct from the elected invention. Again, by acquiescence to a restriction requirement, those applicants agreed to limit their original application to the elected invention. Therefore they were estopped from submitting other, distinct subject matter in reissue that they failed to timely submit in a divisional application during the pendency of their original applications.

2. The '914 patent lacks an error because the now-claimed subject matter could not have been prosecuted in Morrison's original application

In this case, the Board correctly found that Morrison is estopped from prosecuting via reissue claims directed to an invention distinct from his elected—and ultimately patented—invention. A4-12; *see also* A252 (stating that "appellants' new claims [are] directed to an invention that is completely different/patentably distinct from the elected invention of the original patent").

The Examiner restricted the claims of the original application into three independent groups (inventions).[3] A207. Morrison elected Group I without traverse, thereby acquiescing to the Examiner's determination that the *only* invention that the '914 patent could claim was the assembly and method of Group I. A6 (FF12). Put differently, Morrison agreed to prosecute all subject matter distinct from the claims of Group I in timely-filed divisional applications.

Instead of filing divisional applications prior to issuance of the '914 patent, however, Morrison now attempts to claim that nonelected subject matter through reissue. But this Court's precedent of almost 70 years forecloses him from doing so. Specifically, Morrison's reissue claims represent an invention substantially similar to previously-claimed subject matter that he deliberately abandoned after the restriction requirement, and therefore this case squarely fits into the facts of *Orita*, or alternatively, Morrison's reissue claims represent a distinct but "overlooked" invention that he failed to originally claim at all, and this case squarely fits into the facts of *Weiler*. *See* A9-11; *infra* at 24-36.

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[3] Morrison does not challenge the propriety of the original restriction requirement. Nor could he, as "neither this court nor the board has jurisdiction in this proceeding to review the merits of a requirement for restriction under section 121, as a restriction requirement is a matter within the discretion of the examiner and not tantamount to a rejection of claims." *Watkinson*, 900 F.2d at 233.

But whichever case applies directly to the facts at hand makes no difference in the end. It is dispositive here that the reissue claims are distinct from Morrison's elected invention, *i.e.*, the claims of Group I, as Morrison has consistently acknowledged throughout prosecution. *See* A155 (Response to Non-Final Office Action) (stating that the reissue claims are "independent and distinct from the restricted group of claims" and "not properly grouped within the prior restriction"); A187-88 (Appeal Brief) (stating that the reissue claims "do[] not fall within the Group I invention that was elected in the . . . application that matured into the original '913 [*sic*] patent").[4] Morrison also admits to this Court that his reissue claims are distinct from the claims of his elected invention. *See* Br. at 14 ("these inventions are separate and distinct, even though related as subcombinations"). Morrison accordingly could not have prosecuted the distinct reissue claims in his original application because of his acquiescence to the restriction requirement, and his resulting patent lacks an error correctable by reissue. *Doyle*, 293 F.3d at 1361; *Watkinson*, 900 F.2d at 232; *Weiler*, 790 F.2d

[4] Morrison's position was also made clear in oral argument before the Board. *See* A300 (Oral Argument Transcript) (JUDGE SAADAT: So I just want to make the record clear, had these claims been part of the original application do you think they would have been restricted into a different group, or they would be part of group I? MR. MARCUS: In my opinion, they would have been restricted into a different group . . . . JUDGE SAADAT: So, if you accept that as a plausible situation, these claims would have been restricted into a different group than group I. MR. MARCUS: In my view, yes.").

at 1582, *Orita*, 550 F.2d at 1280; *Cornell*, 150 F.2d at 704; *Smyser*, 135 F.2d at 751.

Specifically, § 251 requires error in the issued patent. *See, e.g.*, *Doyle*, 293 F.3d at 1361. The thrust of the *Orita-Weiler-Doyle* line of cases is that when a patentee has claimed exactly that to which he is entitled in a patent because he has agreed to claim only the elected invention—no error exists in that patent. *Orita*, 550 F.2d at 1280. And here, Morrison claimed exactly what he had a right to claim—the elected claims of Group I that issued as claims 1-27 of the '914 patent. Thus, Morrison's repeated assertion that his "error" lies not in failing to file a divisional application with his current reissue claim, but in failing to present those claims during prosecution of the original application, *see, e.g.*, Br. at 25, 26, 36, lacks merit. *Even if* Morrison had presented the reissue claims during the original prosecution of the '914 patent *and* the Examiner had entered those claims, the result is still a dead end. After having been presented, those new claims would have been restricted and withdrawn as directed to nonelected subject matter, *see* MPEP § 821.03, and consequently would have been lost through Morrison's failure to file a timely divisional application, *Doyle*, 293 F.3d at 1362.

Put differently, no matter how Morrison characterizes the "error" he made during the original prosecution, his issued patent lacks an error correctable by

reissue "because the matter sought on reissue could not have been prosecuted originally." *Doyle*, 293 F.3d at 1361. In this case, the Board merely followed this Court's well-established precedent that whenever an examiner issues a restriction requirement, she is by definition requiring the applicant to claim every other distinct invention disclosed in the specification—whether originally claimed or not—in a timely-filed divisional application. By acquiescing to the restriction requirement, the applicant has agreed to do so. As this Court has explained, there is nothing unfair about applying the reissue statute in such a way that it holds the patentee to his word, *Doyle*, 293 F.3d at 1360, and prevents circumvention of the co-pendency requirement of 35 U.S.C. § 120, *Orita*, 550 F.2d at 1280-81.

In sum, reissue does not exist to correct Morrison's "error." As this Court has repeatedly explained, reissue simply is not "a panacea" to correct every prosecution mistake. *See MBO Labs., Inc. v. Becton, Dickinson & Co.*, 602 F.3d 1306, 1313 (Fed. Cir. 2010); *Serenkin*, 479 F.3d at 1362; *Doyle*, 293 F.3d at 1358; *Nupla Corp. v. IXL Mfg. Co.*, 114 F.3d 191, 195 (Fed. Cir. 1997); *Weiler*, 790 F.2d at 1582.

3. *Weiler* dictates that Morrison cannot claim in reissue distinct subject matter that was disclosed but not originally claimed in the '914 patent

Morrison asserts that his reissue claims represent a disclosed but "overlooked embodiment" of his invention, and that the claims are proper subject matter for a reissue application. Br. at 21. Not so. Even if he never presented the reissue claims in his original application, Morrison's failure to timely file a divisional application to the subject matter of those claims during the prosecution of the '914 patent does not constitute an error correctable by reissue. *Weiler*, 790 F.2d at 1579. As explained above, this conclusion is based on the rationale that failing to file a divisional application to distinct subject matter—whether originally claimed or not—that could not have been prosecuted in the original application due to an examiner's restriction requirement is not an "error" within the meaning of § 251.

Assuming that the reissue claims are distinct from the nonelected claims of Group III, the facts of *Weiler* are identical to those here. The examiner in *Weiler*, like the Examiner during prosecution of the '914 patent, issued a restriction requirement. 790 F.2d at 1578. The applicants in *Weiler*, like Morrison, deliberately chose to claim one invention without traverse, but failed to file any divisional applications to the non-claimed subject matter. *Id.* Finally, the applicants in *Weiler*, again like Morrison, filed a reissue application with

claims to subject matter not claimed at all in the original application. *Id.* at 1578-79. But this Court held that "an inventor's failure to claim particular subject matter was not the result of the 'error' required by § 251." *Id.* at 1583. Thus, even if Morrison is correct that the reissue claims are distinct from the original, nonelected claims of Group III (and would not have been grouped with those claims), this Court's holding in *Weiler* would still preclude him from obtaining the claims in reissue. *Id.* at 1582 ("If it were not error to forego divisional applications on subject matter to which claims had been made in the original application, it cannot on the present record have been error to forego divisional applications on subject matter to which claims had never been made.").

Morrison attempts to cast doubt over *Weiler* by mischaracterizing its holding, and by relying on subsequent cases (which could not have overruled *Weiler*) and commentary in Chisum. Br. at 21-24, 34. But these arguments lack merit.

First, contrary to Morrison's arguments otherwise, the "intent to claim" issue addressed in *Weiler* does not alter its holding that failure to file a divisional application does not constitute correctable error under § 251. The *Weiler* Court addressed "intent to claim" because lack of an "intent to claim" formed the basis of the Board's rejection of Weiler's claims. Specifically, the Board found no

error in Weiler's original patent because, in its view, Weiler had no "intent to claim" the subject matter of reissue claims 13 and 19. 790 F.2d at 1580. The Board based its holding on the fact that Weiler never presented those claims during prosecution of his original application, and the Solicitor's brief in the appeal pointed out that Weiler's specification provided only minimal disclosure for those claims. *Id.* at 1579-80. But in addressing the "intent to claim" issue, this Court characterized the Board's focus on Weiler's "intent to claim" "infelicitous," and "reflect[ing] non-statutory language." *Id.* at 1580, 1583. The *Weiler* Court explained that the "central question" before it was "not whether there is disclosure, but whether Weiler ha[d] established 'error' which can be remedied by reissue." *Id.* at 1582. The Court concluded that Weiler's alleged "error"—the failure to file divisional applications to the previously unclaimed subject matter—is not one the reissue statute serves to remedy. *Id.* The same is true for Morrison's "error" here.

Morrison attempts to color the clear holding of *Weiler* as dicta. Br. at 33. But this Court's extensive analysis of *Weiler* in its subsequent *Doyle* opinion refutes Morrison's position. *See Doyle*, 293 F.3d at 1362-63. As the *Doyle* Court explained, "*Weiler* applies only to an attempt to assert new claims in reissue that read on a separate invention and not on the subject matter of the issued claims." *Id.* at 1363. *Doyle* further explained that because Weiler's

reissue claims 13 and 19 were directed to a distinct invention, "they too would have been subject to the original restriction requirement and the applicant would not have been allowed to prosecute them with the original patent—he would have been required to assert them in a divisional." *Id.* at 1362. *Doyle* concludes:

> Under *In re Orita*, of course, there is no correctable error in failing to prosecute divisional applications on inventions of the nonelected groups identified by the examiner in the original restriction requirement. In *In re Weiler*, we held that *the result should be the same* with respect to claims 13 and 19, which also asserted *wholly distinct inventions from that covered by the issued patent.*

Id. at 1362 (emphases added).

All parties agree that Morrison's reissue claims, like the reissue claims in *Weiler*, are distinct from his issued claims. As *Doyle* explains, whether under *Orita* or under *Weiler*, no error lies in failing to file divisional applications to claims "wholly distinct from that covered by the issued patent." *Id.* Otherwise, "the copendency requirement would become meaningless, for should an applicant fail to file a divisional application while maintaining copendency as required by section 120, he could simply revert to section 251 in order to cure his mistake." *Orita*, 550 F.2d at 1281.

Second, Morrison's invocation of *In re Amos*, 953 F.2d 613 (Fed. Cir. 1991) lacks merit. Morrison relies on *Amos* for the proposition that "overlooked embodiments" may be claimed through reissue. Br. at 22-25. But the only issue

this Court addressed in *Amos* was whether an applicant's "intent to claim" may serve as an independent basis for denying a reissue application under § 251. *Amos*, 953 F.2d at 616. And this Court held, as it did in *Weiler*, that "intent to claim" is not dispositive of any inquiry under § 251, and so reversed the Board's decision. *Id.* at 619. In contrast to the facts of *Amos*, the Board in this case did not refuse Morrison's reissue application for an alleged lack of "intent to claim." The Board upheld the Examiner's rejection of the reissue claims for lack of an error under § 251—a rejection that the *Amos* Court notes is proper under the statute. *Id.* at 617. Moreover, instead of criticizing *Weiler* as Morrison appears to assert, Br. at 35, *Amos* positively cites to *Weiler*. 953 F.2d at 617 (citing 790 F.2d at 1581). Thus, *Amos* does not stand for the proposition that "overlooked embodiments" are always proper subject matter for reissuance simply because they are disclosed in an original application, and its holding is inapposite here.

Third, Morrison's speculation that "there is no essential or substantive difference between reissue claims that broaden the scope of what is covered in the original patent, and reissue claims that cover subject matter not claimed at all in the original patent," Br. at 34 (citing 4A Donald S. Chisum, *Chisum on Patents* § 15.03[2][b][iv] (2004)), ignores the important distinction underlying the *Orita-Weiler-Doyle* line of cases. In each case denying reissue, the applicants *acquiesced* to an examiner's restriction requirement. They *agreed* to

prosecute all subject matter distinct from the elected invention—whether classified in a nonelected group of claims or never claimed at all—in timely-filed divisional applications. In other words, the applicants chose *what* to prosecute and thus *what not* to prosecute. As a result, they were "estopped from obtaining by reissue claims which, because of a requirement for restriction in which they had acquiesced, they could not claim in their patent." *Orita*, 550 F.2d at 1280.

This scenario—in which an applicant chooses the invention to pursue—stands in stark contrast with that in which an examiner makes no restriction requirement during the original prosecution. In the latter scenario, a patentee can claim through reissue any invention otherwise meeting all other statutory requirements under the Patent Act, because that patentee has made *no agreement* respecting the subject matter that might have been claimed in the original application. *See In re Staats*, 671 F.3d 1350, 1355 (Fed. Cir. 2012) (holding that patentee could obtain claims in a continuation reissue application to embodiment not claimed within two-year time period); 37 C.F.R. § 1.176(b) (discussing restriction between subject matter of original patent claims and previously unclaimed subject matter in reissue application). And, as Morrison must admit, none of the cases on which he appears to rely for his "overlooked embodiments" argument involved restriction requirements. *See* Br. at 25 (acknowledging that

the facts of *Amos* do not include a restriction requirement); *see also In re Swartzel*, 60 F.3d 843, 1995 WL 389111, *2 (Fed. Cir. 1995) (unpublished) (declining to extend the *Orita* "divisional doctrine" where no restriction requirement issued and applicant misunderstood scope of claims).

Moreover, although it would not change the outcome here, Morrison has made no allegation that he miscommunicated with his prosecuting attorney or agent or that he or the prosecuting attorney or agent lacked a full appreciation of the scope of disclosed subject matter, which as Chisum acknowledges, typically supports reissue applications to broader subject matter. *Chisum on Patents* § 15.03[2][b][iv]; *see also In re Wilder*, 736 F.2d 1516, 1519 (Fed. Cir. 1984) (holding that attorney's failure to appreciate the full scope of the invention resulted in error in the issued patent correctable by reissue).

4. *Orita* prevents Morrison from obtaining in reissue subject matter not elected in the original prosecution of the '914 patent

This Court need not decide Morrison's "overlooked embodiments" argument, because as the Board found, the facts of this case are on all fours with those of *Orita*. As Morrison acknowledges, Br. at 25-26, a patentee's failure to timely file a divisional application to nonelected subject matter in response to a restriction requirement is not an error correctable by reissue. *Orita*, 550 F.2d at 1280-81. Morrison nonetheless asserts that the *Orita* doctrine does not apply

here because the reissue claims "were never the subject of a restriction requirement." Br. at 25. Morrison is mistaken.

As an initial matter, Morrison's attempt to impose an *ipsis verbis* test for applying the *Orita* doctrine should be rejected. *See* Br. at 25-26 (arguing that the *Orita* doctrine only applies to claims that were actually nonelected in the original application). Nothing in *Orita* requires that the claims sought in reissue recite identical words as the nonelected claims of the original prosecution in order to run afoul of its holding. *Orita* instead involves a determination of whether the reissue claims would have been grouped with the nonelected claims because they are directed to the same subject matter. In other words, "substantially identical" as used in *Orita* refers to those "reissue claims which, because of a requirement for restriction in which [applicants] had acquiesced, they could not claim in their patent." *Orita*, 550 F.2d at 1280.

And here, the Board found that Morrison could not have prosecuted his reissue claims in his original application because those claims would have been withdrawn for belonging to a nonelected group of claims—*i.e.*, Group III. The Board compared reissue claim 28 and original, nonelected claim 34 of Group III and found that, although they are directed to different statutory categories, the claims have substantially the same scope and structural features and therefore

would have been classified in the same inventive group. A9-11. The Board's finding is supported by the words of the claims themselves.

Specifically, both reissue claim 28 and original, nonelected claim 34 recite specific structural features of a particular tow assembly for airborne electromagnetic surveying. A10. Claim 28 recites "providing a plurality of hollow transmitter support frame sections." *Id.* Claim 34 correspondingly recites "a transmitter section . . . consisting of a plurality of interconnectable transmitter section frame members." A320. Although the specification does not use the term "hollow" as recited in claim 28, the specification describes the transmitter section **16** as consisting of a support frame **20** consisting of a plurality of "substantially tube sections **22**." A21 (col. 4, ll. 26-30). The specification further explains the various tube sections are interconnected. *Id.* (col. 4, ll. 30-32). Thus, claim 28 recites a plurality of hollow transmitter frame sections substantially identical to the interconnectable transmitter section frame members recited in claim 34.

Reissue claim 28 also recites "providing a transmitter coil for generating a primary magnetic field . . . having the transmitter coil disposed within the hollow transmitter support frame." A3. Morrison contends the Board omitted the "transmitter coil disposed within the hollow transmitter support frame" in its analysis. Br. 27-28. But nonelected claim 34 similarly recites "the transmitter

section houses an elastically suspended transmitter coil." A320. The specification discloses the transmitter coil **28** is strung along the bottom of support frame **20** by attaching the transmitter coil from multiple points along the support frame **20** by a suitable form of attachment. A22 (col. 5, ll. 15-19). The specification further points out that the transmitter coil **28** can be disposed inside the support frame. *Id.* (col. 5, ll. 19-20). Therefore, claim 28 recites a transmitter coil disposed within the hollow transmitter support frame substantially identical to the elastically suspended transmitter coil housed within the transmitter section of claim 34.

Reissue claim 28 then recites "providing connector cables and a tow cable . . . with the connector cables each having a first end connected to a respective location on a circumference of the transmitter support frame and a second end connected to the first end of the tow cable." A3; A200. Similarly, claim 34 states "[w]herein the tow assembly apparatus is connected to the aircraft by means of at least one cable means connected to the transmitter section at a plurality of points." A320. The specification discloses the rope **26** is used to suspend the support frame **20** at points along the circumference thereof and attached to a central tow cable. A22 (col. 5, ll. 5-13). Thus, reissue claim 28 and original, nonelected claim 34 both recite cables connecting the transmitter to a tow cable at various points.

Further, claim 28 recites "assembling the transmitter support frame sections and the transmitter coil and the cables to provide a tow assembly having a . . . frame that is circular or approximates a circle." A3. Similarly, claim 34 incorporates "a transmitter section having a central axis . . . and the transmitter section frame members define a polygonal profile." A320. The specification contemplates that the support frame **15** of the tow assembly has a polygonal shape approximating a circular shape. A21 (col. 4, ll. 51-55). Therefore, reissue claim 28 and original, nonelected claim 34 both embody a circularly-shaped transmitter.

Claim 28 additionally incorporates "serially connecting the transmitter support frame sections." Morrison again argues the Board intentionally omitted this feature in its analysis. Br. 27-28. But the specification describes the support frame **20** consisting of tube sections **22** whereby adding tube sections **22** together provides a support frame. *See* A21 (col. 4, ll. 42-47). Claim 34 analogously recites a "transmitter section . . . consisting of a plurality of interconnectable transmitter section frame members, such that the transmitter section can be assembled." A320.

Morrison points out that claim 34 recites structural limitations related to the receiver section of the tow assembly. Br. at 29. But the absence of these features from claim 28 does not mean that claim 28 would have been classified

in a different inventive group than claim 34. Two claims can be indistinct and grouped together even though they do not recite identical limitations. This makes sense, as no two claims recite the exact same limitations or are of the exact same scope in a single application. *See* 37 C.F.R. § 1.75(b) ("More than one claim may be presented provided they differ substantially from each other and are not unduly multiplied.").

Morrison also argues that reissue claim 28 would not have been grouped with original claim 34 because claim 34 is an apparatus claim while claim 28 is a method claim having "manipulative steps." Br. at 27-29. Not so. As a general matter, no rule requires an automatic restriction between apparatus and method claims. Indeed, the Examiner in Morrison's original application grouped both method and apparatus claims into Group I. A205. Similarly here, the Board found no substantial difference between claim 28 and claim 34 "even though they are directed to different statutory categories." A11. Moreover, claims related as a process of making and a product made are grouped together when the process cannot make a product materially different from the product claimed, or the product cannot be made by any other process. *See* MPEP § 806.05(f). Here, claim 28 recites a method of making a tow assembly having specific structural features that are recited in claim 34. Thus, reissue claim 28 and claim 34 would have been joined together in nonelected Group III. A9.

Morrison's additional argument that the Board's analysis was "speculative" lacks merit. Br. at 29. Many questions of patent law require backward-looking inquiries (*e.g.*, obviousness and damages), and the analysis is no different with respect to the *Orita* doctrine. The only way the USPTO can give effect to *Orita* (and *Weiler*) is to necessarily look back in time and answer the question of whether the reissue applicant *could have* prosecuted his claims in his original application. *Orita*, 550 F.2d at 1280. That inquiry is not based on pure speculation, but on consideration of the original restriction requirement and the distinctions and similarities (if any) between the claims. Here, the Board compared the subject matter of the representative reissue claim to a nonelected claim, and found no patentable distinction between the two. That is not "speculation," but fact-finding based on the words and scope of the claims themselves.

5. Morrison's "law of the case" argument does not help him

Finally, Morrison asserts that the Board ignored the "law of the case," which Morrison refers to as "the Examiner's findings justifying the original restriction requirement between the elected claims of Group I and the non-elected Groups II and III." Br. at 30-31. Morrison's assertion misses the point. The issue in this case is whether the '914 patent contains an error correctable in the reissue application on appeal. Again, the *Orita-Weiler-Doyle* case law

requires a determination of whether the reissue claims would have been grouped with the original, elected claims of Group I. That the original claims of each of Groups I, II, and III were distinct from one another does not necessarily answer the question of whether the presently-appealed reissue claims are distinct from the claims of Group I. And in answering that question, the Board did not ignore what Morrison calls the "law of the case," and neither does the Director. Indeed, the fact that two different examiners in two applications restricted the reissue claims from the patented claims only further confirms the Director's point—that the reissue claims are distinct from the claims of Group I and therefore could not have been prosecuted in Morrison's original application. And whether the facts of this case align more closely to *Weiler* or to *Doyle* makes no difference to the outcome. That Morrison could not have prosecuted his reissue claims in his original application is dispositive, because he is now estopped from obtaining those claims in reissue. *Watkinson*, 900 F.2d at 232.

V. CONCLUSION

For the foregoing reasons, the Board's conclusion that the '914 patent lacks an error correctable by reissue is correct. This Court should affirm the Board's decision.

Respectfully submitted,

February 18, 2014

/s/ Kristi L. R. Sawert

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CERTIFICATE OF SERVICE

I hereby certify that on February 18, 2014, I electronically filed the foregoing BRIEF FOR APPELLEE—DIRECTOR OF THE UNITED STATES PATENT AND TRADEMARK OFFICE using the Court's CM/ECF filing system. Counsel for appellant was electronically served by and through the Court's CM/ECF filing system per Fed. R. App. P. 25 and Fed. Cir. R. 25(a) and 25(b).

/s/ Kristi L. R. Sawert

Kristi L. R. Sawert
Associate Solicitor

RULE 32(a)(7)(C) CERTIFICATE OF COMPLIANCE

I certify pursuant to Fed. R. App. Proc. 32(a)(7) that the foregoing BRIEF FOR APPELLEE—DIRECTOR OF THE UNITED STATES PATENT AND TRADEMARK OFFICE complies with the type-volume limitation required by the Court's rule. The total number of words in the foregoing brief, excluding the table of contents and the table of authorities, is 8471 words as calculated using the Word® software program.

/s/ Kristi L. R. Sawert
Kristi L. R. Sawert
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Exhibit H2

2012-1689
(Serial No. 11/358,951)

UNITED STATES COURT OF APPEALS FOR THE FEDERAL CIRCUIT

IN RE BEKA SOLOMON

Appeal from the United States Patent and Trademark Office,
Patent Trial and Appeal Board.

BRIEF FOR APPELLEE—ACTING DIRECTOR OF THE UNITED STATES PATENT AND TRADEMARK OFFICE

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REPRESENTATIVE CLAIM

5. A method of inhibiting aggregation of soluble β-amyloid in a human subject, comprising

causing an effective amount of an anti-β-amyloid antibody, or an antigen-binding fragment thereof, to come into contact with the soluble β-amyloid,

said antibody or fragment being effective to bind β-amyloid and to inhibit aggregation of the soluble β-amyloid in said subject to an extent at least as great as that obtainable with antibody AMY-33, thereby inhibiting aggregation of the soluble β-amyloid,

wherein said anti-β-amyloid antibody is one that is obtainable using an immunogen consisting of a peptide consisting of residues 1-28 of β-amyloid, and said antigen binding fragment is an antigen binding fragment of said antibody,

and wherein said antibody or fragment is not conjugated with a detectable moiety.

A00003 (formatting and emphasis added).

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STATEMENT OF RELATED CASES

The Acting Director is not aware of any other appeal in connection with this case that has previously been before this Court, or that is currently pending in any other court. The Acting Director is also unaware of any related cases pending in this or any other court that will directly affect, or be directly affected by, this Court's decision in the pending appeal.

I. STATEMENT OF THE ISSUES

Beka Solomon is the inventor of U.S. Patent No. 5,688,651 ("the '651 patent"). Solomon originally claimed multiple inventions in the application leading to the '651 patent. Accordingly, the Examiner restricted the claims into three groups. Solomon elected the first group and prosecuted those claims to allowance, but failed to file divisional application(s) to the nonelected claims or to any other subject matter prior to issuance of the '651 patent. Solomon now attempts to prosecute subject matter distinct from her elected invention by filing a reissue application. The first issue before this Court is whether the Board properly determined that Solomon's failure to timely file a divisional application cannot be corrected by reissue. If the Court affirms the Board on this first issue, it need not consider the next issue.

Solomon claims a method of inhibiting aggregation of a protein—β-amyloid—in a human by causing an antibody specific for β-amyloid or fragment of that antibody to bind β-amyloid with an inhibition strength at least as great as "AMY-33," an antibody already known in the art. The specification fails to disclose any antibody or fragment thereof that meets that description. Nor does it describe sufficient structural features that are common to the genus of antibodies required to practice the claimed method. The second issue, should

the Court need to reach it, is whether substantial evidence supports the Board's finding that the claimed method is not adequately supported by the specification.

II. STATEMENT OF THE CASE

The '651 patent, entitled "Prevention of Protein Aggregation," issued on November 18, 1997 with four claims. A00041-54. On February 22, 2006, Solomon filed a reissue application[1] canceling the original patent claims and adding new claims. A00039-40; A00075-77. The Examiner finally rejected all of the pending claims 5, 7-9, 17, 19-21, and 31-38 under 35 U.S.C. § 251 for lack of defect or error in the original patent correctable by reissue, and under 35 U.S.C. § 103(a) as obvious over certain prior art references. A00915-91 (Examiner's Answer). The Examiner also rejected claims 5, 7-9, and 31-34 under 35 U.S.C. § 112, first paragraph, for improper introduction of new matter and for lack of written description support in the specification. *Id.* Solomon appealed to the Board of Patent Appeals and Interferences ("Board"),[2] which affirmed the Examiner's reissue and written description rejections, but reversed the prior art and new matter rejections. A00002-30. This appeal followed.

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[1] The present application is the child of reissue application no. 09/441,140, filed November 16, 1999, which is currently pending in the Office.

[2] Effective September 16, 2012, Congress renamed the "Board of Patent Appeals and Interferences" as the "Patent Trial and Appeal Board." 35 U.S.C. § 6(a) (2011).

III. STATEMENT OF THE FACTS

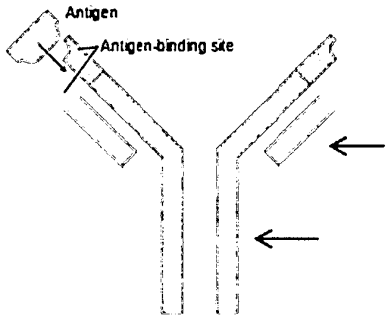
This appeal involves disputes centering on both the technology and the prosecution history. The Acting Director first describes the disclosure of the '651 patent, and then describes the prosecution before the Office that led up to the filing of the presently-appealed reissue application.

A. Solomon's '651 Patent: The Selection and Use of Antibodies or Antibody Fragments to Inhibit Protein Aggregation

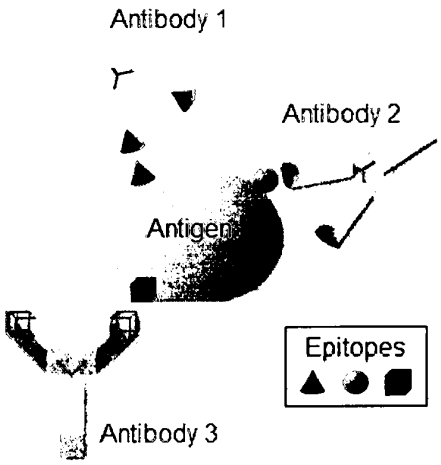
Following synthesis, proteins fold into a particular three-dimensional shape to fulfill their different functions. Occasionally, however, proteins fail to properly fold and subsequently accumulate and clump together. *See* A00041 (col. 1, ll. 12-22). This abnormal association of misfolded proteins is known in the art as protein aggregation, and is implicated in several disease states, including Down's syndrome and Alzheimer's disease. *Id.* (col. 1, ll. 42-46). The '651 patent proposes the use of "anti-aggregation molecules"—particularly antibodies and antibody fragments—to prevent protein aggregation and disassociate protein aggregates *in vivo*. *Id.* (col. 1, ll. 4-8).

Antibodies are Y-shaped proteins that play a significant role in the immune response. Antibodies work by binding an antigen, *e.g.*, a protein that evokes an immune response. As shown in the following diagram, the Y-shaped structure consists of two identical "light" polypeptide chains and two identical

"heavy" polypeptide chains. Each light chain connects with a heavy chain to create the "arms" of the Y-shaped molecule.



The upper arms of the antibody form a region called the "antigen-binding site." The antigen-binding site regions are highly variable, consisting of an amino acid sequence unique to each antibody. The specific region of the antigen to which the antigen-binding site binds is called an "epitope." A single antigen (shown in the diagram below) may have several different epitopes (illustrated as varying shapes), which consist of short amino acid sequences generally 5 to 8 residues in length. In nature, many different antibodies (shown in different colors) will bind to a single antigen, but those antibodies typically do not bind to the same epitope on that antigen.



For example, the antibody 1 of the diagram only binds to the "green" epitope,

whereas antibody 2 only binds to the "blue" epitope. In the laboratory, antibodies can be "raised against" (or created to bind to) particular antigens or even specific epitopes. Antibodies genetically engineered from the same clone—*i.e.*, monoclonal antibodies—are identical and therefore bind to the same epitope.

The '651 patent discloses a method of selecting antibodies or antibody fragments *in vitro* (in a test tube), and a method of using those antibodies or antibody fragments to inhibit protein aggregation and disassociate protein aggregates *in vivo*. A00042 (col. 3, ll. 40-55). The specification proposes several aggregating proteins as antibody targets, of which β-amyloid—a protein present in the brain "plaques" of Alzheimer's patients—is preferred. A00043 (col. 5, ll. 50-66).

The '651 patent shows that a commercially-available monoclonal antibody known as AMY-33 binds to and inhibits *in vitro* aggregation of β-amyloid. A00048 (col. 15, ll. 43-46); A00042 (col. 3, ll. 8-11). The '651 patent postulates that AMY-33 recognizes an epitope located somewhere within the first 28 amino acids (*i.e.*, amino acids 1-28) of β-amyloid, A00048 (col. 15, ll. 43-46), and "probably" residues 25-28, *id.* (col. 16, ll. 5-9). On the other hand, the '651 patent shows that another commercially-available monoclonal antibody known as 6F/3D has only partial or no appreciable effect on β-amyloid

aggregation *in vitro*. A00048 (col. 15, ll. 49-53). The '651 patent postulates that 6F/3D recognizes an epitope located somewhere within amino acids 8-17 of β-amyloid. *Id.*

B. Prosecution of the '651 Patent

The '651 patent originated as U.S. patent application no. 08/358,786, which was filed on December 16, 1994 with 24 claims. The Examiner issued a restriction requirement,[3] separating the claims into three groups for purposes of examination:

- Group I, claims 1-6, drawn to a method of selection for anti-aggregation molecules;
- Group II, claims 7-23, drawn to a method of treating a protein aggregation disease by administering an expression vector encoding the anti-aggregation molecule, a pharmaceutical composition, and expression vectors; and
- Group III, claim 24, drawn to a method of treating a protein aggregation disease by administering a monoclonal antibody.

A01352-54.

[3] Section 121 of the Patent Act grants the Director the authority to limit a patent to one invention. 35 U.S.C. § 121. In a so-called "restriction requirement," the examiner typically organizes the claims into groups (*i.e.*, independent or distinct inventions) and requires the applicant to elect one group for prosecution. 37 C.F.R. § 1.142(a). Claims to the invention(s) not elected are withdrawn from consideration, and if not later subject to a rejoinder process, are canceled prior to issuance. 37 C.F.R. § 1.142(b).

The Examiner explained that the different methods distinguish the invention of Group I from the inventions of Groups II and III. A01353. Specifically, the invention recited in the claims of Group I is directed to a method for using an *in vitro* assay to identify molecules that prevent protein aggregation, whereas the inventions recited in the claims of Groups II and III are directed to methods of treating a disease associated with protein aggregation by administering an aggregation-inhibiting nucleic acid molecule or antibody, respectively. *Id.* The Examiner also noted that the art recognizes each invention as having divergent subject matter and separate classification. *Id.*

In response to the restriction requirement, Solomon elected without traverse the claims of Group I, and "withdr[e]w claims 7-24 without prejudice." A01359; *see also* A01363 (noting the election without traverse of Group I). Eventually, the Examiner allowed claims 1, 3, 4, and 6 of Group I (renumbered 1, 2, 3, and 4, respectively), and with Solomon's permission, canceled the nonelected claims of Groups II and III. *See* A01374-77 (Notice of Allowability and Examincr's Amcndmcnt).

The '651 patent issued on November 18, 1997. A00053. Claims 1 and 3, the only independent claims, both recite a "method of selecting an anti-aggregation molecule having the chaperone-like activity of anti-aggregation." A00049 (col. 18, ll. 14-15 & 39-40). Solomon failed to file any divisional or

continuation application(s) to the nonelected subject matter or to any other non-claimed subject matter prior to issuance. *See, e.g.*, A00133-34 ("It is noted that there are no divisionals or continuations filed from the parent 08/358,786 application.").

C. Prosecution of the Reissue Application

On February 22, 2006, Solomon filed the reissue application underlying this appeal. A00039-40. In her first reissue declaration ("the 2006 declaration"), A00055-58, Solomon attested that the '651 patent was "wholly or partly inoperative or invalid by reason of the patentee claiming more or less than it had the right to claim in the patent," A00056. Specifically, Solomon declared that all parties involved in the prosecution of the original application focused their attentions on patenting the method of selecting an anti-aggregation molecule as embodied in issued claims 1-4 to the exclusion of other inventions disclosed in the application. A00056. Solomon further explained:

> It was only recently that the Applicant/Patentee and the Assignee realized that the concepts fully disclosed in the specification directed to pharmaceutical compositions for preventing or reducing aggregation of an aggregating protein or for disaggregating preaggregated aggregates of the aggregating protein and methods of disaggregating an aggregate of or preventing the aggregation of an aggregating protein by causing an anti-aggregation molecule to come into contact with the aggregate or with the soluble aggregating protein were not claimed *per se*, and further they could be and should be now so claimed.

A000056-57.

In a preliminary amendment accompanying the reissue application, Solomon canceled issued claims 1-4 of the '651 patent and submitted new claims 5-28. A00075-77. Independent claim 5 recited a method for preventing or reducing the aggregation of an aggregating protein by administering an anti-aggregation molecule, whereas the only other independent claim 17 recited a method for disassociating (*i.e.*, "disaggregating") a protein aggregate by administering an anti-aggregation molecule. A00075-76. Several dependent claims limited the aggregating protein to β-amyloid, carboxypeptidase A, and others. *See* A00075-76 (claims 10-16); A00077 (claims 22-28).

The Examiner issued a restriction requirement requiring Solomon to select one particular aggregating protein for examination. A00117-22. The Examiner explained that the aggregating proteins are unique in that they possess different amino acid sequences, are implicated in different diseases, and are inhibited by different anti-aggregating agents. A00120-21. In response, Solomon elected to prosecute the claimed methods as relating to β-amyloid. A00123.

In the first Office Action on the merits, the Examiner rejected the claims under 35 U.S.C. § 251 for lack of defect or crror in the original patent correctable by reissue. A00133-35. Comparing the proposed reissue claims with nonelected claim 24 of Solomon's original application, the Examiner

concluded that the reissue claims relate to subject matter not patentably distinct from the nonelected claim, as both are directed to the use of an antibody to treat a human suffering from a protein-aggregation disease. A00135. Relying on the Manual of Patent Examining Procedure ("MPEP") § 1412.01 and cases cited therein, the Examiner explained that Solomon's failure to timely file a divisional application to the nonelected subject matter prior to issuance of the '651 patent is not an error correctable by reissue. A00133-34.

Solomon eventually submitted a substitute declaration ("the 2008 declaration"). A00427-31. In the 2008 declaration, Solomon attested that she submitted a claim to a "generic method of treating a protein aggregation disease" during the original prosecution of the '651 patent, but chose not to prosecute that claim in a divisional application. A00428-29. Later, however, she and the assignee realized that no claims had ever been submitted "specifically directed to methods of disaggregating an aggregate of or inhibiting the aggregation of soluble β-amyloid" with anti-β-amyloid antibodies or fragments thereof. A00429. Thus, Solomon continued, "[i]t was realized that the failure to claim these concepts was an error without deceptive intent and that they could and should be now so claimed." *Id.*

The Examiner, however, maintained the rejection under § 251. The Examiner found no patentable distinction between a method for "reversing

aggregation" as claimed in original claim 24 and a method for "inhibiting aggregation" or "disaggregating an aggregate" as claimed in reissue claims 5 and 17. A00555. Indeed, the Examiner explained, the subject matter of the reissue claims falls within the scope of original claim 24. A00558-59. Finally, the Examiner noted that, had the reissue claims been presented in the original prosecution, those claims could not have been prosecuted in that application because they encompass subject matter distinct from the invention Solomon elected. A00556-58.

D. The Board's Decision

The Board affirmed the Examiner's rejection of all of the pending claims under 35 U.S.C. § 251 for lack of defect or error in the original patent correctable by reissue, and the rejection of claims 5, 7-9, and 31-34 under 35 U.S.C. § 112, first paragraph for failing to satisfy the written description requirement. A00030.

First, the Board found independent claim 5, as follows, representative:

> 5. A method of inhibiting aggregation of soluble β-amyloid in a human subject, comprising
>
> causing an effective amount of an anti-β-amyloid, or an antigen-binding fragment thereof, to come into contact with the soluble β-amyloid,
>
> *said antibody or fragment being effective to bind β-amyloid and to inhibit aggregation of the soluble β-amyloid in said subject to an extent at least as great as that obtainable with*

> *antibody AMY-33*, thereby inhibiting aggregation of the soluble β-amyloid,
>
> wherein said anti-β-amyloid antibody is one that is obtainable using an immunogen consisting of a peptide consisting of residues 1-28 of β-amyloid, and said antigen binding fragment is an antigen binding fragment of said antibody,
>
> and wherein said antibody or fragment is not conjugated with a detectable moiety.

A00003 (formatting and emphasis added).

The Board then addressed the rejection under § 251, and found that the evidence of record supported the Examiner's conclusion that the original patent does not contain an error. A00005-12. The Board agreed with the Examiner that Solomon's reissue claims are drawn to methods of treating protein aggregation diseases and therefore are substantially similar to the nonelected claims of Solomon's original application. A00008. Because Solomon failed to file a divisional application to the nonelected subject matter, the Board concluded that she is precluded from pursuing those claims under the *Orita* doctrine. A00009 (citing *In re Orita*, 550 F.2d 1277 (CCPA 1977)). The Board explained that allowing Solomon to pursue claims to a nonelected invention in reissue would render the co-pendency requirement of 35 U.S.C. § 120[4]

[4] Section 120 provides that any continuation or divisional application of an originally filed application must be filed "before the patenting or abandonment of or termination of proceedings on the first application or on an

"'meaningless,'" a result the *Orita* Court found undesirable. A00011-12 (quoting *Orita*, 550 F.2d at 1281).

The Board also found unpersuasive Solomon's reliance on this Court's decision in *In re Doyle*, 293 F.3d 1355 (Fed. Cir. 2002), noting that the reissue claim presented in that case was broader than all of the original claims and therefore encompassed the elected invention. A00010. But here, the Board explained, the reissue claims would have either fallen within nonelected Group III or another nonelected group had the claims been presented in the original application. A00010-11. And in any event, the newly introduced claims do not encompass the elected invention. A00011 (stating that the reissue claims "d[o] not encompass nor relate to the elected Group I which was prosecuted to issuance").

With respect to the rejection under section 112, first paragraph, written description, the Board agreed with the Examiner that the specification failed to adequately describe a genus of antibodies or fragments of those antibodies that "bind[] β-amyloid and inhibit[] aggregation of soluble β-amyloid to an extent at least as great as that obtainable with antibody AMY-33." A00026. The Board found no examples of any antibodies (or fragments thereof) in the specification

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application similarly entitled to the benefit of the filing date of the first application" in order to receive the benefit of the original application's filing date. 35 U.S.C. § 120 (2011).

that meet that description. A00029. Accordingly, the Board concluded that Solomon's claims "'merely recite a description of the problem to be solved while claiming all solutions to it and . . . cover any compound later actually invented and determined to fall within the claim's functional boundaries—leaving it to the pharmaceutical industry to complete an unfinished invention.'" *Id.* (quoting *Ariad Pharms., Inc. v. Eli Lilly and Co.*, 598 F.3d 1336, 1353 (Fed. Cir. 2010) (*en banc*)).

IV. SUMMARY OF THE ARGUMENT

The *Orita-Weiler-Doyle* line of cases prohibits a patentee from using a reissue proceeding to obtain claims that the patentee could not have prosecuted in his or her original application. These cases are based on the rationale that, in acquiescing to an examiner's restriction requirement, the applicant has chosen to pursue claims only to the elected invention. In other words, the applicant has agreed to independently prosecute any invention distinct from that elected in divisional application(s). This Court accordingly does not treat a patentee's failure to file those divisional application(s) as an "error" correctable under the reissue statute. Otherwise, the co-pendency requirement of 35 U.S.C. § 120 would be eviscerated.

Solomon attempts to do exactly what the *Orita-Weiler-Doyle* line of cases rejects. In response to the Examiner's restriction requirement, Solomon elected

without traverse claims to a method of selecting an anti-aggregation molecule *in vitro* and prosecuted them to allowance. Those claims were distinct from the nonelected claims directed to a method of treating protein aggregation in a human by administering an "anti-aggregation" antibody. Solomon failed, however, during the pendency of her application to file divisional applications to the nonelected claims or to any other distinct subject matter she disclosed.

Solomon's attempt to blunt the impact of *Orita-Weiler-Doyle* fails. Her assertion that her "error" lies not in failing to file divisional application(s), but in failing to present the reissue claims during prosecution of the '651 patent reflects an attempt to get an improper do-over: to prosecute an invention that is distinct from one she expressly chose to pursue in her original application. Indeed, because Solomon claimed exactly what she had a right to claim in her original application—*i.e.*, the elected *in vitro* method claims—the Board correctly determined that the '651 patent lacks an error correctable under the reissue statute.

Should this Court need to reach the issue, the Board also correctly determined that some of Solomon's claims fail to comply with the written description requirement. The rejected claims encompass a broad genus of antibodies (or fragments thereof) that bind β-amyloid with an inhibition strength "at least as great as" a prior art antibody known as AMY-33. But the

specification fails to describe any antibody or fragment thereof falling within the claimed genus, or sufficient structural features common to that genus. For these reasons, the Board's rejection should be affirmed.

V. ARGUMENT

A. Standard of Review

Solomon has the burden to show that the Board committed reversible error. *In re Watts*, 354 F.3d 1362, 1369 (Fed. Cir. 2004). Whether the claims of a reissue application are unpatentable under 35 U.S.C. § 251 is a question of law based on underlying findings of fact. *In re Serenkin*, 479 F.3d 1359, 1361 (Fed. Cir. 2007). Whether a claim comports with the written description standard of 35 U.S.C. § 112(a)[5] is a question of fact. *Ariad*, 598 F.3d at 1351.

This Court reviews the Board's legal conclusions *de novo* but affirms the Board's factual findings that are supported by substantial evidence. *In re Jolley*, 308 F.3d 1317, 1320 (Fed. Cir. 2002); *see also* 5 U.S.C. § 706(2)(E). Substantial evidence is "such relevant evidence as a reasonable mind might accept as adequate to support" the conclusion reached. *Consol. Edison Co. v. NLRB*, 305 U.S. 197, 229 (1938). It is "something less than the weight of the evidence but more than a mere scintilla of evidence." *In re Kotzab*, 217 F.3d

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[5] In the Leahy-Smith America Invents Act ("AIA"), Congress added a paragraph letter to the first paragraph of § 112, without otherwise substantively changing its content. 35 U.S.C. § 112(a) (2011).

1365, 1369 (Fed. Cir. 2000). "[W]here two different, inconsistent conclusions may reasonably be drawn from the evidence in record, an agency's decision to favor one conclusion over the other is the epitome of a decision that must be sustained upon review for substantial evidence." *Jolley*, 308 F.3d at 1329.

B. The Board Correctly Concluded That Solomon's Patent Lacks an Error Correctable by Reissue

Section 251 of the 1952 Patent Act provides for the reissue of a patent that "through error without any deceptive intention, [is] deemed wholly or partly inoperative or invalid . . . by reason of the patentee claiming more or less than he had a right to claim in the patent." 35 U.S.C. § 251 (2006), *amended by* 35 U.S.C. § 251(a) (2011) (adding paragraph letters and deleting "without any deceptive intention"). Fundamental principles of equity and fairness guide the application of the reissue statute. But "[b]y its terms section 251 restricts reissue to situations in which an error occurred." *Doyle*, 291 F.3d at 1358. And not just any "error" qualifies under the statute. *E.g.*, *In re Weiler*, 790 F.2d 1576, 1579 (Fed. Cir. 1986) ("[N]ot every event or circumstance that might be labeled 'error' is correctable by reissue.").

This Court and its predecessor court have generally refused to call "error" the deliberate choices a patent applicant makes (albeit later regretted) to obtain allowance. *See e.g.*, *In re Yamazaki*, 702 F.3d 1327, 1333 (Fed. Cir. 2012) (holding that patentee's failure to prevent a patent from issuing with terminal

disclaimer not an error correctable by reissue); *In re Mostafazadeh*, 643 F.3d 1353, 1358 (Fed. Cir. 2011) (reiterating that intentional surrender of subject matter during original prosecution to overcome prior art not an error); *Serenkin*, 479 F.3d at 1363 (holding that a "conscious decision" to accept a later filing date for application not a correctable error); *In re Mead*, 581 F.2d 251, 257 (CCPA 1978) (holding that patentee's "conscious choice" not to file a continuing application not correctable error). The *Orita*, *Weiler*, and *Doyle* decisions, relevant to this appeal and discussed in detail below, stand in accord.

1. The *Orita-Weiler-Doyle* line of cases prohibits a patentee from using a reissue proceeding to obtain claims that the patentee could not have prosecuted in his or her original application

Orita, *Weiler*, and *Doyle* hold that when an applicant makes a deliberate choice in response to a restriction requirement to prosecute a single invention and fails to file divisional application(s) to the remaining, nonelected inventions—whether originally claimed or not—error under § 251 does not exist. These cases are based on the rationale that once an applicant acquiesces to a restriction requirement, any invention distinct from the one elected and prosecuted to allowance can only be pursued in a timely-filed divisional application.

In *Orita*, the CCPA addressed the issue of whether an applicant's failure to file a divisional application to nonelected claims constituted an "error" under

§ 251. 550 F.2d at 1280. During the prosecution of Orita's original application, the examiner issued a restriction requirement separating the claims into two groups. *Id.* at 1278. The applicants elected the first group and canceled the nonelected claims of the second. *Id.* After issuance, applicants filed a reissue application to claims "substantially identical to the originally non-elected claims," and stated in a declaration that they had claimed less than they had a right to claim because "they had forgotten to timely file a divisional application covering the originally non-elected subject matter." *Id.* at 1279.

The court upheld the Board's denial of the reissue, holding that applicants' acquiescence to the examiner's restriction requirement did not constitute an "error" under § 251. The court explained that while "appellants undoubtedly erred by failing to file a timely divisional application," the resulting patent was error-free: "Patentees claimed exactly what they had a right to claim in the patent, no more nor less, and appellants' failure to timely re-file does not change this fact." *Id.* at 1280.

The court also noted that allowing reissue claims substantially identical to those not elected would render the co-pendency requirement of § 120 "meaningless." The court continued:

> for should an applicant fail to file a divisional application while maintaining copendency as required by section 120, he could simply revert to section 251 in order to cure his mistake. Section 251 is not a panacea designed to cure every mistake which might be

> committed by an applicant or his attorney, and the case at bar exemplifies a mistake which this section cannot cure.

Id. at 1281.

The CCPA's decision gave rise to what is now commonly referred to as "the *Orita* doctrine." It finds its roots in two pre-1952 Patent Act cases: *In re Cornell*, 150 F.2d 702 (CCPA 1945) and *In re Smyser*, 135 F.2d 747 (CCPA 1943). In both of these cases, the CCPA held that the reissue statute did not authorize claiming of subject matter in a reissue that could not have been included in the original application as a result of applicants' acquiescence in the examiner's requirement for "division." *Cornell*, 150 F.2d at 704; *Smyser*, 135 F.2d at 751.

Following *Orita*, which dealt with *claimed* subject matter, this Court held in *Weiler* that error under § 251 does not lie in failing to file divisional applications to *non-claimed* subject matter distinct from the subject matter that the applicant elected. *Weiler*, 790 F.2d at 1580. The examiner in *Weiler* restricted the original claims into three groups. Weiler chose the first group without traverse and prosecuted those claims to allowance, but failed to file divisional applications directed to the nonelected subject matter or to any other disclosed subject matter. *Id.* at 1578. After issuance, Weiler filed a reissue application with claims to subject matter disclosed but never claimed in the original application. *Id.* at 1578-79. This Court upheld the Board's rejection of

the reissue application for lack of error. In doing so, this Court noted that the subject matter of the claims on appeal was "clearly independent of and distinct" from that of both the elected and nonelected claims, and accordingly, "Weiler would thus have had no right to insert and present [the reissue claims] in the original application after the examiner's requirement for restriction." *Id.* at 1581. Following the logic of *Orita*, this Court held that by acquiescing in an examiner's restriction requirement, "it cannot on the present record have been error to forego divisional applications on subject matter to which claims had never been made." *Id.* at 1582.

While *Orita* and *Weiler* addressed specific claims to distinct inventions, *Doyle* addressed a "linking" claim, "a claim broad enough to read on—or link—two or more groups of claims subject to a restriction requirement." 293 F.3d at 1358. This Court characterized the issue in *Doyle* as "whether failure to present a so-called linking claim . . . is an error correctable by reissue." *Id.* Doyle's original application included both compound and method claims. *Id.* at 1356. In response to the examiner's nine-way restriction requirement, Doyle elected the claimed invention of using a genus of catalysts to insert carbenes into certain chemical bonds, and canceled the remaining claims. *Id.* Doyle did not file any divisional applications to the nonelected claims. But following issuance of the patent, Doyle filed a reissue application with claims directed to a method of

using a genus of catalysts to insert any prochiral molecule (*i.e.*, not just carbene) into the chemical bonds. *Id.* at 1357. Doyle conceded that the new claims, while broader in scope than the elected invention, read on some of the nonelected claims. *Id.*

The Board upheld the rejection of the claims based on lack of error correctable by reissue. *Id.* at 1358. This Court reversed. In doing so, the Court first noted that "[t]he so-called *Orita* doctrine . . . precludes a reissue applicant from obtaining substantially identical claims to those of nonelected groups identified in an examiner's restriction requirement when such claims could not have been prosecuted in the application from which they were restricted." *Id.* at 1359. The Court then explained that *Weiler* prevents a reissue applicant from pursuing claims to "a completely separate invention from the issued claims or any of the nonelected claims." *Id.* at 1362. Such claims, the *Doyle* Court explained, if presented during the original prosecution, "would have been subject to the original restriction requirement and the applicant would not have been allowed to prosecute them with the original patent—he would have been required to assert them in a divisional." *Id.*

The *Doyle* Court held, however, that neither *Orita* nor *Weiler* applied to Doyle because his new claims "are genus claims, whereas the nonelected claims are species that fall within the new genus claims. In other words, the reissue

claims are substantially broader than the claims of the nonelected groups." *Id.* at 1360. Unlike Orita and Weiler, Doyle could have—and indeed *should have*—prosecuted the broader linking claims (linking the elected and nonelected claims) in the original application. *Id.* And importantly, because Doyle did not agree to prosecute the broader linking claims in a divisional, the estoppel rationale underlying *Orita* and *Weiler* did not apply. *Id.*

The *Orita* and *Weiler* cases stand for the "established principle that applicants are 'estopped from obtaining by reissue claims which, because of a requirement for restriction in which they had acquiesced, they could not claim in their patent.'" *In re Watkinson*, 900 F.2d 230, 232 (Fed. Cir. 1990) (quoting *Orita*, 550 F.2d at 1280). *Doyle* comports with that principle, as Doyle could have prosecuted his reissue claims in his original application. That he failed to do so constituted the error of claiming less than Doyle had a right to claim under § 251. In contrast, the applicants in *Orita* and *Weiler* could not have pursued their reissue claims in their original applications because those claims were distinct from the elected invention. Again, those applicants agreed to limit their original application to the elected invention, and therefore were estopped from submitting other, distinct subject matter in reissue that they failed to timely submit in a divisional application during the pendency of their original applications.

2. The '651 patent lacks an error because the now-claimed subject matter could not have been prosecuted in Solomon's original application

In this case, the Board correctly found that Solomon is estopped from prosecuting via reissue claims directed to an invention distinct from her elected—and ultimately patented—claimed invention. A00008-09; *see also* A00943 (stating that "the present claims are directed to a patentably distinct invention from the elected group of the patent"). The Examiner restricted the claims of the original application into three independent groups (inventions). A01352-54. The invention recited in the claims of Group I was directed to a method for using an *in vitro* assay to select molecules that prevent protein aggregation, whereas the inventions recited in the claims of Groups II and III were directed to methods of treating a disease associated with protein aggregation by administering an aggregation-inhibiting nucleic acid molecule or antibody, respectively. A01353; *see also* A00008 (citing FF1-2). Solomon elected Group I without traverse, thereby acquiescing to the Examiner's determination that the *only* invention that the '651 patent could claim was an *in vitro* method for selecting anti-protein aggregation molecules. A00006 (FF2). Put differently, Solomon agreed to prosecute all subject matter distinct from the claimed molecule selection method in timely-filed divisional applications.

Instead of filing divisional applications prior to issuance of the '651 patent, however, Solomon now attempts to claim that subject matter through reissue. But this Court's precedent of almost 70 years forecloses her from doing so. And whether Solomon deliberately abandoned the now-claimed subject matter following the restriction requirement, or simply failed to originally claim it at all (presented as alternative arguments in sections 3 and 4, below) makes no difference in the end. The reissue claims are distinct from Solomon's elected invention, and therefore could not have been prosecuted in her original application. *Doyle*, 293 F.3d at 1361; *Watkinson*, 900 F.2d at 232; *Weiler*, 790 F.2d at 1582, *Orita*, 550 F.2d at 1280; *Cornell*, 150 F.2d at 704; *Smyser*, 135 F.2d at 751.

Specifically, issued claim 1 of the '651 patent recites a method of selecting an anti-aggregation molecule having specific properties, through five specific *in vitro* reaction steps (*i.e.*, denaturing, mixing, incubating, selecting, and testing). A00049. Reissue claim 5, on the other hand, is directed to a method of inhibiting aggregation of β-amyloid *in vivo* by administering certain antibodies. A00003. In other words, claim 5 treats a disease in a human by administering a specific therapeutic, whereas issued claim 1 tests and identifies certain molecules in a laboratory setting. Thus, the subject matter Solomon now attempts to claim does not share the same purpose, method steps, or outcome as

the invention she elected during the original prosecution of the '651 patent. *See* A00011 (stating that "newly introduced claims 5-28 . . . did not encompass nor relate to methods of selecting anti-aggregation molecules of the elected Group I which was prosecuted to issuance"); *see also* MPEP § 802.01(I) (stating that two inventions are distinct for purposes of restriction when they are unconnected in design, operation, or effect).

In sum, reissue does not exist to correct Solomon's "error." As this Court has repeatedly explained, reissue simply is not "a panacea" to correct every prosecution mistake. *See MBO Labs., Inc. v. Becton, Dickinson & Co.*, 602 F.3d 1306, 1313 (Fed. Cir. 2010); *Serenkin*, 479 F.3d at 1362; *Doyle*, 293 F.3d at 1358; *Nupla Corp. v. IXL Mfg. Co.*, 114 F.3d 191, 195 (Fed. Cir. 1997); *Weiler*, 790 F.2d at 1582.

3. The *Orita* doctrine prevents Solomon from obtaining in reissue subject matter not elected in the original prosecution of the '651 patent

As Solomon acknowledges, Br. at 19-20, a patentee's failure to timely file a divisional application to nonelected subject matter in response to a restriction requirement is not an error correctable by reissue. *Orita*, 550 F.2d at 1280-81. Solomon nonetheless asserts that the *Orita* doctrine does not apply to the instant facts because "[t]he present claims are not the same as, or substantially identical

to, the claim in the original patent application that was cancelled subsequent to a restriction requirement." Br. at 27. Solomon is mistaken.

As an initial matter, Solomon's attempt to impose an *ipsis verbis* test for applying the *Orita* doctrine, Br. at 27-29, should be rejected. Nothing in *Orita* requires that the claims sought in reissue recite identical words as the nonelected claims of the original prosecution in order to run afoul of its holding. *Orita* instead requires a determination of whether the reissue claims would have been grouped with the nonelected claims because they are directed to the same subject matter.

And here, although worded differently, both reissue claim 5 and original, nonelected claim 24 are directed to the use of an antibody to treat a human suffering from a protein-aggregation disease. In other words, both claim 5 and original claim 24 concern the same host (a human), the same method step (administering an antibody to the human), and the same biological mechanism (causing an antibody to come into contact with an aggregating protein). Thus, as the Board correctly found, reissue claim 5 would have been categorized in the same inventive group as original claim 24. A00008.

Solomon's argument that claim 5 is not directed to a method of treating a protein aggregation disease rings hollow; as the Examiner aptly observed, but for the disease, no reason exists to administer the antibodies to the human in the

first place. A00944. Indeed, the specification makes clear that once an appropriate antibody is selected (through the *in vitro* method of issued claim 1), the "invention provides for its use therapeutically to prevent or reduce protein aggregation in vivo." A00043 (col. 6, ll. 1-5). The skilled artisan would therefore recognize that inhibiting aggregation of soluble β-amyloid as recited in reissue claim 5 constitutes the same therapeutic use originally recited in nonelected claim 24. A00944.

Solomon also argues that claim 5 is "quite different" from original, nonelected claim 24 because claim 24 states that the antibody "reverses" aggregation, whereas claim 5 states that the antibody "inhibits" aggregation. Br. at 28. But the original Examiner distinguished claim 24 from the only other human treatment claim presented during the original prosecution (*i.e.*, original claim 23) on the basis of the therapeutic administered (monoclonal antibody versus nucleic acid), and not on the biological effect of the antibody itself (*i.e.*, whether it "reverses" or "inhibits" aggregation). Moreover, Solomon fails to show that such a distinction even exists. A01353. As the current Examiner explained, the specification contemplates that a monoclonal antibody that binds to an aggregated protein and causes disaggregation would also necessarily bind to a soluble protein to prevent aggregation. A00953-54 (citing A00043 (col. 4, ll. 62-66 & col. 5, ll. 23-29)). Solomon presents no evidence rebutting the

Examiner's finding that an antibody that "inhibits" aggregation also "reverses" aggregation. *In re Geisler*, 116 F.3d 1465, 1470 (Fed. Cir. 1997) (attorney argument no substitute for evidence).

Solomon's argument that the *Orita* doctrine does not apply because claim 5 is narrower than original claim 24 (and therefore not of "substantially similar scope" as allegedly required by *Doyle*), Br. at 28-29, misses the mark. This Court noted that the reissue claims asserted in *Doyle* were "significantly broader" than both the elected and nonelected claims, and therefore could have been prosecuted in the original patent because they read on the *elected* group of claims. 293 F.3d at 1361. Here, however, not only is reissue claim 5 *narrower* than nonelected claim 24, it also bears no relationship to the elected group of claims. In other words, reissue claim 5—unlike the reissue claim presented in *Doyle*—is not a linking claim that could have been prosecuted in the application that matured into the '651 patent. A00010. Moreover, if the *Orita* doctrine forecloses obtaining a broader genus claim (like original, nonelected claim 24) as Solomon admits, then permitting a narrower species claim (like representative claim 5) via reissue makes little sense. Thus *Doyle*, contrary to Solomon's arguments, does not support the notion that narrowing the subject matter of original claim 24 to a specific species of protein provides an escape route from the *Orita* doctrine.

Finally, Solomon's reference to the restriction requirement issued in the present reissue application bears no relevance to the issue of whether the '651 patent contains an error correctable by reissue. As the Examiner pointed out, that each of the proteins listed in the specification are *distinct from one another* does not mean that a therapeutic method targeting one of those proteins is distinct from a broader claim to the therapeutic method not limited to a particular protein, like that recited in original claim 24. A00944-45.

4. Alternatively, this Court's decision in *Weiler* dictates that Solomon cannot claim in reissue distinct subject matter that was disclosed but not originally claimed in the '651 patent

Even if the reissue claims are directed to a different invention from original, nonelected claim 24 as Solomon asserts, the result is the same. After affirmatively electing an invention to prosecute, Solomon's failure to timely file a divisional application to distinct subject matter disclosed but never claimed during the prosecution of the '651 patent does not constitute an error correctable by reissue. *Weiler*, 790 F.2d at 1579. As explained above, this holding is based on the rationale that failing to file a divisional application to any subject matter—whether claimed or not—that could not have been prosecuted in the original application due to an examiner's restriction requirement is not an "error" within the meaning of § 251.

The facts of *Weiler* are identical to those here. The examiner in *Weiler*, like the Examiner during prosecution of the '651 patent, issued a restriction requirement. 790 F.2d at 1578. The applicants in *Weiler*, like Solomon, deliberately chose to claim one invention without traverse, but failed to file any divisional applications to the non-claimed subject matter. *Id.* Finally, the applicants in *Weiler*, again like Solomon, filed a reissue application with claims to subject matter not claimed at all in the original application, and asserted that the reissue claims "should have been made in the original application." *Id.* at 1578-79. But this Court held that "an inventor's failure to claim particular subject matter was not the result of the 'error' required by § 251." *Id.* at 1583. Thus, even if Solomon is correct that the reissue claims are distinct from original, nonelected claim 24 (and would not have been grouped with that claim), Br. at 24, this Court's holding in *Weiler* would still preclude her from obtaining the claims in reissue.

Solomon attempts to cast doubt over *Weiler* by relying on subsequent cases, which could not have overruled *Weiler*, and commentary in Chisum. Br. at 21-24. But these arguments lack merit. *First*, contrary to Solomon's arguments otherwise, the holdings of *Doyle* and *Landmark Screens, LLC v. Morgan, Lewis, & Bockius, LLP*, 676 F.3d 1354 (Fed. Cir. 2012), are consistent with that of *Weiler*. In both *Doyle* and *Landmark*, the patentee presented a claim

in reissue that *could* have been prosecuted in the original patent because it encompassed within its scope the elected claims. Thus, neither patentee in those cases—unlike the patentees in *Orita* and *Weiler*—was estopped from presenting broader claims in reissue.[6]

Solomon's invocation of *In re Amos*, 953 F.2d 613 (Fed. Cir. 1991), Br. at 23-24, fares no better. In *Amos*, this Court reiterated that an applicant's "objective intent to claim" does not serve as an independent basis for denying a reissue application under § 251, and so reversed the Board's decision. *Id.* at 618-19. In this case, the Board did not refuse Solomon's reissue application for an alleged lack of an "objective intent to claim" as Solomon appears to imply. Instead, the Board upheld the Examiner's rejection of the reissue claims for lack of an error under § 251—a rejection that the *Amos* Court notes is proper under the statute. *Id.* at 617. Moreover, instead of "distinguishing" *Weiler* as Solomon asserts, *Amos* positively cites to *Weiler*. 953 F.2d at 617. The *Weiler* Court also observed that an "objective intent to claim" rejection lacks any

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[6] Solomon's second block quote from *Landmark*, Br. at 22, is misleading in that it implies—via omitted text—that this Court found that Landmark's reissue application met the requirements of § 251 because the claims of the reissue application were broader than the *nonelected* claims of Landmark's original application. In fact, the district court made this finding. 676 F.3d at 1365. This Court explained that "[w]hen the reissue claims are broader than the *issued* claims," § 251 may be satisfied by the patentee asserting that he claimed less than he had a right to claim in the patent. *Id.* at 1364 (emphasis added).

statutory basis in § 251. 790 F.2d at 1581. And given that the issue in *Amos* was whether the "original patent" language of § 251 is conclusively determined through a written description analysis, and not whether the patentee had demonstrated "error" under § 251, this Court called the *Amos* parties' attention to *Weiler* "inexplicable." 953 F.2d at 618 n.3. *Amos* is similarly inapposite here.

Second, Chisum's speculation that "there is no essential or substantive difference between reissue claims that broaden the scope of what is covered in the original patent claims and reissue claims that cover subject matter not claimed at all in the original patent," *see* Br. at 23, ignores the important distinction underlying the *Orita-Weiler-Doyle* line of cases. In each case denying reissue, the applicants had *acquiesced* to an examiner's restriction requirement. They *agreed* to prosecute all subject matter distinct from the elected invention—whether classified in a nonelected group of claims or never claimed at all—in timely-filed separate applications. In other words, the applicants chose *what* to prosecute and thus *what not* to prosecute. As a result, they were "estopped from obtaining by reissue claims which, because of a requirement for restriction in which they had acquiesced, they could not claim in their patent." *Orita*, 550 F.2d at 1280.

This scenario—in which an applicant chooses the invention to pursue—stands in stark contrast with that in which an examiner makes no restriction requirement during the original prosecution. In the latter scenario, a patentee can claim through reissue any invention otherwise meeting all other statutory requirements under the Patent Act, because that patentee has made *no agreement* respecting the subject matter that might have been claimed in the original application. *See In re Staats*, 671 F.3d 1350, 1355 (Fed. Cir. 2012) (holding that patentee could obtain claims in reissue application to embodiment not claimed within two-year time period). Moreover, although it would not change the outcome here, Solomon has made no allegation that she miscommunicated with her prosecuting attorney or agent or that she or the prosecuting attorney or agent lacked a full appreciation of the scope of disclosed subject matter, which as Chisum acknowledges, typically supports reissue applications to broader subject matter. 4A Donald S. Chisum, *Chisum on Patents* § 15.03[2][b][iv] (2004); *see also, e.g.*, *Hester Indus., Inc. v. Stein, Inc.*, 142 F.3d 1472, 1479-80 (Fed. Cir. 1998).

Solomon also asserts that this Court wrongly decided *Weiler* because, in coming to its decision, thc Court ignored an agency procedural rule,

37 C.F.R. § 1.145. Br. at 24-25.[7] Solomon relies on this rule for the notion that she could have "inserted and presented" her reissue claims in her original application, and accordingly her "error" lies not in failing to file a divisional application to those claims, but in failing to present those claims during prosecution of the original application. Br. at 27. Solomon misses the point.

Section 251 requires error *in the issued patent*. *See, e.g.*, *Doyle*, 293 F.3d at 1361. The thrust of the *Orita-Weiler-Doyle* line of cases is that when a patentee has claimed exactly that to which they are entitled in a patent because they have agreed to claim only the elected invention—no error exists *in that patent*. *Orita*, 550 F.2d at 1280. And here, Solomon claimed exactly what she had a right to claim—the elected method claims of selecting an anti-aggregation molecule *in vitro*. Thus, *even if* Solomon had presented the reissue claims during the original prosecution of the '651 patent *and* the Examiner had entered that amendment under Rule 145, the result is still a dead end. After having been presented, those new claims would have been withdrawn as directed to nonelected subject matter, *see* MPEP § 821.03, and consequently would have been lost through Solomon's failure to file a timely divisional application, *Doyle*, 293 F.3d at 1362.

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[7] Because *Weiler* is binding precedent, this argument is better suited to the *en banc* Court. *Newell Cos. v. Kenney Mfg. Co.*, 864 F.2d 757, 765 (Fed. Cir. 1988).

Moreover, Solomon's argument that the Examiner might not have issued a second restriction requirement per MPEP § 803, Br. at 25, cannot be squared with the original prosecution of the '651 patent. At the very least, the reissue claims and the original, nonelected claims share the common feature of being directed to the administration of a therapeutic in a human (*i.e.*, an *in vivo* use), whereas the elected invention recites a method of selecting antibodies in a laboratory (*i.e.*, an *in vitro* use). Thus, the original Examiner would have properly restricted the reissue claims from the elected *in vitro* method claims of Group I for the same reasons that she separated the *in vivo* human treatment claims of Groups II and III from the elected *in vitro* method claims in the first place. *See* A01353.

Solomon's mention of the prohibition against double patenting rejections, Br. at 25-26, is a non sequitur. Once the USPTO restricts the claims of an application and therefore forces an applicant to file a second, divisional application to the nonelected claims, 35 U.S.C. § 121 prevents the Office from rejecting the claims of that divisional application over the original, restricted application. That makes sense, as the Office cannot issue a double patenting rejection when the Office itself forced the applicant into filing the second application. But here, no divisional applications were filed; thus, whether or not § 251 would have barred a double patenting rejection simply has no

applicability. "In other words, this tortured hypothetical does not correspond to the record in this case," and accordingly fails to change the outcome. *Perricone v. Medicis Pharm. Corp.*, 432 F.3d 1368, 1375 (Fed. Cir. 2005).

In sum, no matter how Solomon spins the "error" she made during the original prosecution, her *issued patent* lacks an error correctable by reissue "because the matter sought on reissue could not have been prosecuted originally." *Doyle*, 293 F.3d at 1361. This Court should therefore uphold the Board's denial of Solomon's reissue application.

5. Solomon's remaining arguments lack merit

Solomon argues that the Board's reference to specific language in her 2006 declaration without reference to the specific language in her 2008 declaration is reversible error. Br. at 15. It is not. As an initial matter, the Board committed no error because Solomon essentially made the same statements in both the 2006 declaration and the 2008 declaration. Specifically, Solomon stated in the 2006 declaration that the reissue claims cover concepts "not claimed *per se*," A00057, whereas in the 2008 declaration she stated that "no claims had ever been submitted" to those concepts, A00429. Thus, each declaration characterizes her "error" as not claiming the subject matter of the reissue claims during the original prosecution, which is consistent with Solomon's argument before this Court.

But even if, *arguendo*, the declarations make materially different statements as to the origin of Solomon's "error," the Board's consideration of the 2006 declaration does not compel reversal of its decision. Under the "harmless error" rule, "the appellant must not only show the existence of error, but also show that the error was in fact harmful because it affected the decision below." *Watts*, 354 F.3d at 1369. And here, no matter the specific words the Board quoted, Solomon's issued patent simply contains no "error" correctable under the reissue statute. *Supra* at 17-37; *see also* A00011.

Finally, the Board did not "establish" a *per se* rule as Solomon contends. Br. at 20-21. The Board merely followed this Court's well-established precedent that whenever an examiner issues a restriction requirement, she is by definition requiring the applicant to claim every other distinct invention disclosed in the specification—whether originally claimed or not—in a timely-filed divisional application. And by acquiescing to the restriction requirement, the applicant has agreed to do so. As this Court has explained, there is nothing unfair about applying the reissue statute in such a way that it holds the patentee to his word, *Doyle*, 293 F.3d at 1360, and prevents circumvention of the co-pendency requirement of 35 U.S.C. § 120, *Orita*, 550 F.2d at 1280-81.

C. Substantial Evidence Supports the Board's Finding That Claims 5, 7-9, and 31-34 Lack Adequate Written Description in the Specification

Section 112(a) requires that the specification contain a written description of the invention. 35 U.S.C. 112(a) (2011). "[T]he hallmark of written description is disclosure." *Ariad*, 598 F.3d at 1351. In other words, the specification must "convey[] to those skilled in the art that the inventor had possession of" and "actually invented" the claimed subject matter. *Id.* "A 'mere wish or plan' for obtaining the claimed invention is not adequate written description." *Centocor Ortho Biotech, Inc. v. Abbott Labs.*, 636 F.3d 1341, 1348 (Fed. Cir. 2011) (quoting *Regents of the Univ. of Cal. v. Eli Lilly & Co.*, 119 F.3d 1559, 1566 (Fed. Cir. 1997)).

1. Solomon's disclosure fails to meet any of the written description tests for a genus of antibodies

Representative claim 5 is broad, claiming a genus of anti-β-amyloid antibodies (or fragments thereof) that are capable of both binding to β-amyloid and inhibiting aggregation of β-amyloid "to an extent at least as great as" prior art monoclonal antibody AMY-33. A00034. Typically, for a claim to a genus of antibodies, the written description requirement is satisfied by the disclosure of "a representative number of species falling within the scope of the genus," or an

established "correlation between structure and function." *Ariad*, 598 F.3d at 1350.[8] Solomon's disclosure satisfies neither of these tests.

First, the specification fails to disclose *any* antibody or antibody fragment that binds to β-amyloid and inhibits β-amyloid aggregation at least as well as AMY-33. A00029. The specification only discloses two relevant antibodies, AMY-33 and 6F/3D, neither of which falls within the scope of the claim of representative claim 5. A00048 (Example 2). Claim 5 does not encompass 6F/3D because that antibody does not function as well as AMY-33. *Id.* (col. 15, ll. 49-53) (disclosing that 6F/3D had partial or no appreciable effect on β-amyloid aggregation *in vitro* compared to AMY-33). Claim 5 also does not encompass AMY-33 because that antibody *is* the comparative molecule recited in the claim, *i.e.*, the antibody to which all antibodies or antibody fragments must be compared. Thus, contrary to Solomon's assertions otherwise, Br. at 32, 33, & 39, AMY-33 does not serve as an example of a species falling within the scope of the genus of antibodies encompassed by the claim.

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[8] The third test commonly used to show possession of chemical molecules, *i.e.*, the disclosure of "structural features common to the members of the genus so that one of skill in the art can visualize or recognize the members of the genus," *Ariad*, 598 F.3d at 1350 (quotation omitted), is not commonly used in the antibody art. Persons of skill in this art generally do not consider disclosure of the complete amino acid sequence of an antibody necessary to show possession. *See Written Description Training Materials Rev. 1, March 25, 2008* at 46 (available at http://www.uspto.gov/web/menu/written. pdf) (hereinafter "*PTO Guidelines*").

While this Court has declined to set forth a bright-line rule regarding the number of species sufficient to describe a genus, *Ariad*, 598 F.3d at 1351, the "representative number of species" test is not met where, as here, the number is zero. *See, e.g.*, *Centocor*, 636 F.3d at 1350-51; *Ariad*, 598 F.3d at 1356-58 (finding lack of written description for claimed method of reducing NF-κB activity where specification failed to show any molecule that actually reduced NF-κB activity); *Univ. of Rochester v. G.D. Searle & Co.*, 358 F.3d 916, 927-928 (Fed. Cir. 2004) (finding lack of written description for claimed method of selectively inhibiting COX-2 enzyme where specification failed to describe any specific compound that could selectively inhibit COX-2).

Second, the specification fails to disclose any correlation between structure and function sufficient to show that Solomon actually invented the claimed genus of antibodies or antibody fragments. The function claimed is binding to β-amyloid and inhibiting β-amyloid aggregation at least as well as AMY-33. The specification fails to provide any structure of an antibody or antibody fragment capable of performing these functions at least as well as AMY-33. Moreover, while the specification provides some structure of the antigen by expressing preference for an antibody that binds to an epitope located somewhere within the first 28 amino acids of β-amyloid, *see, e.g.*, A00043 (col. 6, ll. 23-26), the specification does not definitively disclose *which* epitope(s)

within those residues the antibody or antibody fragment must bind in order to have the function of inhibiting β-amyloid aggregation at least as well as AMY-33. Such a disclosure is critical here, as the specification warns that an appropriate antibody must be "directed to the *appropriate epitope* on the β-amyloid molecule" in order to inhibit aggregation while still allowing normal functioning of the protein. A00042 (col. 3, ll. 24-27) (emphasis added); *see also Ariad*, 598 F.3d at 1351 (sufficiency of disclosure must be determined on a case-by-case basis).

The absence of disclosure is also particularly problematic here because the specification clearly conveys to the skilled artisan that not every antibody or antibody fragment raised against amino acid residues 1-28 will actually have the claimed function of inhibiting β-amyloid aggregation. As shown in the specification, monoclonal antibody 6F/3D had only partial or no appreciable effect on β-amyloid aggregation *in vitro*, even though it ostensibly binds to an epitope found within amino acids 8-17 of β-amyloid. A00048 (col. 15, ll. 49-53). Moreover, during prosecution, Solomon called "the bold statement that all antibodies that recognize an epitope between 1-28 of Aβ will prevent aggregation of β-amyloid or will cause aggregated β-amyloid to disaggregate, [] unsupportable and untrue." A00512.

Accordingly, Solomon's specification fails to show adequate possession of the claimed genus of antibodies through either the disclosure of a representative number of species or the disclosure of a function-structure correlation. Solomon's arguments to the contrary fail.

2. Solomon's rebuttal arguments fail to show that she had possession of the large genus of antibodies and antibody fragments encompassed by the scope of representative claim 5

Solomon asserts that recitation of the claimed antibodies' "properties" sufficiently describes a genus of antibodies and antibody fragments having the claimed functions. Br. at 32-33. Not so. The "properties" to which Solomon refers are the wished-for functions of binding to and inhibiting β-amyloid aggregation as well as AMY-33. *Halliburton Energy Servs., Inc. v. M-I LLC*, 514 F.3d 1244, 1255 (Fed. Cir. 2008) (explaining that functional claim language defines an element by "what it does rather than what it is") (quotation omitted); *see also* A00980. And "functional characteristics" satisfy the written description requirement only where they are "coupled with a known or disclosed correlation between function and structure." *Enzo Biochem, Inc. v. Gen-Probe Inc.*, 323 F.3d 956, 964 (Fed. Cir. 2002) (quotation omitted).

As explained immediately above, the specification provides no correlation between the claimed function of binding to and inhibiting β-amyloid aggregation and the structure of an antibody or antibody fragment capable of performing

these functions at least as well as AMY-33. And in addition to failing to provide even one example of an antibody or antibody fragment that possesses the claimed functions, the specification also "teaches nothing about the structure, epitope characterization, binding affinity, specificity, or pharmacological properties common" to the antibodies of the claimed genus. *In re Alonso*, 545 F.3d 1015, 1021-22 (Fed. Cir. 2008). Accordingly, Solomon's reliance on the functional abilities of the claimed antibodies fails.

Solomon also argues that her specification requires little detail because finding members of the claimed genus of antibodies would be a "simple matter," because skill in the antibody art is high and the field is mature. Br. at 33 (citing *Capon v. Eshhar*, 418 F.3d 1349, 1357-58 (Fed. Cir. 2005)). Solomon relies on Example 13 of the agency's *PTO Guidelines* as evidence for that notion. Br. at 33-37. But Example 13 is inapposite to Solomon's claim.

As explained in the *PTO Guidelines*, production of antibodies that bind to a well-characterized antigen, such as β-amyloid here, is conventional. *PTO Guidelines* at 46. But Solomon's claim is not limited to an antibody that merely binds β-amyloid. A00028-29. The antibody or antibody fragment must *also* inhibit β-amyloid aggregation, and do so *at least as well* as AMY-33. As this Court explained in *Centocor*, claiming antibodies with specific properties beyond mere binding ability—*i.e.*, a subgenus of binding antibodies—requires

more disclosure than just the antigen. 636 F.3d at 1352 ("Claiming antibodies with specific properties, e.g., an antibody that binds to human TNF-α with A2 specificity, can result in a claim that does not meet written description even if the human TNF-α protein is disclosed . . . ."); *see also* A00028.

Solomon's related argument that *Centocor* is inapplicable to this case, Br. at 36-38, also lacks merit. Solomon correctly notes that Centocor's claims lacked sufficient written description because, at the time of Centocor's application, it was not technologically possible to make the fully-humanized antibodies that Centocor claimed. *Centocor*, 636 F.3d at 1352. But this Court's explanation of why Centocor's arguments failed applies directly to this case. Specifically, Centocor—like Solomon here—claimed a large genus of antibodies but failed to disclose any single antibody falling within the scope of its claims. *Id.* at 1348. And Centocor—like Solomon here—indirectly relied on the *PTO Guidelines* to argue that no examples are necessary to meet the written description requirement for a genus of antibodies claimed by their binding affinity for a certain protein and ability to inhibit certain biological reactions. *Id.* at 1349 (describing Centocor's argument that the claimed antibodies were adequately described by their binding affinity to TNF-α and ability to competitively inhibit binding of the A2 mouse antibody to TNF-α).

This Court disagreed, explaining that Example 13 of the *PTO Guidelines* only applies where "the applicant is disclosing a novel protein and then claiming both the protein and an antibody that binds to it." *Id.* at 1351. This Court then held that both Example 13 as well as its own precedent stand for the proposition that an applicant may claim an antibody to a particular antigen without disclosing an example of that antibody only where (1) the applicant discovers and fully characterizes a novel antigen to which the antibody binds, and (2) the creation of those antibodies is routine. *Id.* at 1352; *see also Noelle v. Lederman*, 355 F.3d 1343, 1349 (Fed. Cir. 2004) (finding that disclosure of novel, fully-characterized mouse antigen showed possession of a genus of mouse antibodies, but did not similarly show possession of a genus of human antibodies because the human antigen was unknown).

Here, as in *Centocor*, there is no dispute that the antigen—*i.e.*, β-amyloid—was known in the art prior to Solomon's filing date. *See, e.g.*, A00041 (col. 1, ll. 47-50). Further, while creation of some antibodies simply binding to β-amyloid *in vitro* was conventional, selection of the subgenus of antibodies that both bind to β-amyloid and inhibit β-amyloid aggregation in a human was not equally conventional, as the specification itself demonstrates, *see* A00048 (col. 15, ll. 49-53) (showing that 6F/3D had only partial or no appreciable effect on β-amyloid aggregation *in vitro*, even though it ostensibly

binds to an epitope found within amino acids 8-17 of β-amyloid), and as Solomon has admitted, A00512. *See also* A00982 (Examiner's reasoning that the predictability of producing antibodies having the desired ability to inhibit β-amyloid aggregation is low based on the fact that only half of the antibodies tested in the specification exhibited the desired functions). Thus, as in *Centocor*, Example 13 of the *PTO Guidelines* does not allow Solomon to claim a genus of antibodies by merely disclosing an antigen.

Finally, Solomon implies that she should receive a patent because she was the first to discover that antibodies can be used to inhibit soluble β-amyloid aggregation. Br. at 38. Solomon's contribution to the sciences is not the dispute. As this Court explained in *Ariad*, "[p]atents are not awarded for academic theories, no matter how groundbreaking or necessary to the later patentable inventions of others." 598 F.3d at 1353. Here, Solomon's specification does not identify "the practical implications of [her] research," *i.e.*, the antibodies and antibody fragments that can be administered to a human to bind to β-amyloid and inhibit β-amyloid aggregation at least as well as AMY-33. *Id.* Rather, as the Board explained, Solomon's disclosure provides a plan for obtaining the claimed antibodies, "'leaving it to the pharmaceutical industry to complete an unfinished invention.'" A00029 (quoting *Ariad*, 598 F.3d at 1353).

Solomon's specification fails to show that she invented, or had possession of, a genus of antibodies or antibody fragments falling within the broad scope of representative claim 5. Accordingly, the Board's written description rejection is supported by substantial evidence and should be affirmed should the Court need to reach this issue.

VI. CONCLUSION

For the foregoing reasons, the Board's conclusion that the '651 patent lacks an error correctable by reissue is correct, and its finding that claims 5, 7-9, and 31-34 lack sufficient written description under § 112, first paragraph, is supported by substantial evidence. This Court should affirm the Board's decision.

Respectfully submitted,

March 15, 2013

/s/ Kristi L. R. Sawert

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Attorneys for the Acting Director of the United States Patent and Trademark Office

CERTIFICATE OF SERVICE

I hereby certify that on March 15, 2013, I electronically filed the foregoing BRIEF FOR APPELLEE—ACTING DIRECTOR OF THE UNITED STATES PATENT AND TRADEMARK OFFICE using the Court's CM/ECF filing system. Counsel for appellant was electronically served by and through the Court's CM/ECF filing system per Fed. R. App. P. 25 and Fed. Cir. R. 25(a) and 25(b).

/s/ Kristi L. R. Sawert
Kristi L. R. Sawert
Associate Solicitor

RULE 32(a)(7)(C) CERTIFICATE OF COMPLIANCE

I certify pursuant to Fed. R. App. Proc. 32(a)(7) that the foregoing BRIEF FOR APPELLEE—ACTING DIRECTOR OF THE UNITED STATES PATENT AND TRADEMARK OFFICE complies with the type-volume limitation required by the Court's rule. The total number of words in the foregoing brief, excluding the table of contents and the table of authorities, is 10,812 words as calculated using the Word® software program.

/s/ Kristi L. R. Sawert
Kristi L. R. Sawert
Associate Solicitor

Docket No.: 135576.010627/REI/CON
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Daniel J. Wendt et al.

U.S. Serial No.: 15/646,822 Confirmation No.: 7727

Filed: July 11, 2017 Art Unit: 3991

For: *VARIANTS OF C-TYPE NATRIURETIC PEPTIDE* Examiner: Johnny F. RAILEY

SUBMITTED VIA EFS-Web
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

TRANSMITTAL ACCOMPANYING SUBMISSION OF SUPPLEMENTAL APPLICATION DATA SHEET (ADS)

This transmittal accompanies the submission of a Supplemental Application Data Sheet (ADS) in the above-identified patent application.

This Supplement ADS is submitted pursuant to guidance kindly provided by Examiner Jean Witz during a telephone call on March 7, 2018. The Supplemental ADS presents the information indicating that the subject application is a Continuation Reissue Application of U.S. Serial No. 14/604,262 (now patented) in the 'Domestic Priority Information' section in a manner that more readily allows this information to be entered and processed by the USPTO Electronic Filing System.

The issuance of a corrected Filing Receipt reflecting the corrected information provided in the accompanying Supplemental ADS is respectfully requested in this application.

AUTHORIZATION

No fee is believed to be due in connection with this submission. However, the Commissioner is hereby authorized to charge any fee(s) that may be required to Deposit Account No. 50-2678, Reference: 135576.010627/REI/CON.

Respectfully submitted,
Greenberg Traurig, LLP

Dated: March 7, 2018

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Supplemental
Application Data Sheet

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Correspondence Information

Correspondence Customer Number::	~~90849~~ 35893

Application Information

Application Type::	~~Reissue~~ Continuation Reissue
Subject Matter::	Utility
CD-ROM or CD-R?::	None
Sequence submission?::	Yes
Computer Readable Form (CRF)?::	Yes
Title::	Variants of C-Type Natriuretic Peptide
Attorney Docket Number::	~~30610/44556C1-R2~~ 135576.010627/REI/CON
Request for Early Publication?::	No
Request for Non-Publication?::	No
Total Drawing Sheets::	57
Small Entity?::	No
Petition included?::	No
Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37	No

CFR 5.2::

This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013::	No

Representative Information

Representative Customer Number:: ~~90849~~ 35893

Domestic Priority Information

Application::	Continuity Type::	Parent Application::	Parent Filing Date::	Status::
~~This Application~~	~~Continuation Reissue~~	~~14/604,262~~	~~1/23/2015~~	~~Pending~~
This Application	Continuation of	14/604,262	01/23/2015	Patented
and This Application	Reissue of	14/604,262	01/23/2015	Patented
14/604,262	Reissue of	13/466,672	5/08/2012	Patented
13/466,672	Continuation of	12/784,117	5/20/2010	Patented
12/784,117	An application claiming the benefit under 35 USC 119(e)	61/254,563	10/23/2009	Expired
12/784,117	An application claiming the benefit under 35 USC 119(e)	61/180,112	5/20/2009	Expired

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Authorization or Opt-Out of Authorization to Permit Access

When this Application Data Sheet is properly signed and filed with the application, applicant has provided written authority to permit a participating foreign intellectual property (IP) office access to the instant application-as-filed (see paragraph A in subsection 1 below) and the European Patent Office (EPO) access to any search results from the instant application (see paragraph B in subsection 1 below).

Should applicant choose not to provide an authorization identified in subsection 1 below, applicant **must opt-out** of the authorization by checking the corresponding box A or B or both in subsection 2 below.

NOTE: This section of the Application Data Sheet is **ONLY** reviewed and processed with the **INITIAL** filing of an application. After the initial filing of an application, an Application Data Sheet cannot be used to provide or rescind authorization for access by a foreign IP office(s). Instead, Form PTO/SB/39 or PTO/SB/69 must be used as appropriate.

1. Authorization to Permit Access by a Foreign Intellectual Property Office(s)

A. Priority Document Exchange (PDX) - Unless box A in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the State Intellectual Property Office of the People's Republic of China (SIPO), the World Intellectual Property Organization (WIPO), and any other foreign intellectual property office participating with the USPTO in a bilateral or multilateral priority document exchange agreement in which a foreign application claiming priority to the instant patent application is filed, access to: (1) the instant patent application-as-filed and its related bibliographic data, (2) any foreign or domestic application to which priority or benefit is claimed by the instant application and its related bibliographic data, and (3) the date of filing of this Authorization. See 37 CFR 1.14(h) (1).

B. Search Results from U.S. Application to EPO - Unless box B in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the EPO access to the bibliographic data and search results from the instant patent application when a European patent application claiming priority to the instant patent application is filed. See 37 CFR 1.14(h)(2).

The applicant is reminded that the EPO's Rule 141(1) EPC (European Patent Convention) requires applicants to submit a copy of search results from the instant application without delay in a European patent application that claims priority to the instant application.

2. Opt-Out of Authorizations to Permit Access by a Foreign Intellectual Property Office(s)

☐ A. Applicant **DOES NOT** authorize the USPTO to permit a participating foreign IP office access to the instant application-as-filed. If this box is checked, the USPTO will not be providing a participating foreign IP office with any documents and information identified in subsection 1A above.

☐ B. Applicant **DOES NOT** authorize the USPTO to transmit to the EPO any search results from the instant patent application. If this box is checked, the USPTO will not be providing the EPO with search results from the instant application.

NOTE: Once the application has published or is otherwise publicly available, the USPTO may provide access to the application in accordance with 37 CFR 1.14.

Signature:

NOTE: This Application Data Sheet must be signed in accordance with 37 CFR 1.33(b). **However, if this Application Data Sheet is submitted with the INITIAL filing of the application and either box A or B is not checked in subsection 2 of the "Authorization or Opt-Out of Authorization to Permit Access" section, then this form must also be signed in accordance with 37 CFR 1.14(c).**

This Application Data Sheet **must** be signed by a patent practitioner if one or more of the applicants is a **juristic entity** (e.g., corporation or association). If the applicant is two or more joint inventors, this form must be signed by a patent practitioner, **all** joint inventors who are the applicant, or one or more joint inventor-applicants who have been given power of attorney (e.g., see USPTO Form PTO/AIA/81) on behalf of **all** joint inventor-applicants.

See 37 CFR 1.4(d) for the manner of making signatures and certifications.

Signature	/Leslie Serunian/ ~~/Katherine L. Neville/~~	Date (YYYY-MM-DD)	2018-03-07 ~~2017-07-11~~
Name	Leslie Serunian ~~Katherine L. Neville~~	Registration Number	35,353 ~~53,379~~

Electronic Acknowledgement Receipt

EFS ID:	31985670
Application Number:	15646822
International Application Number:	
Confirmation Number:	7727
Title of Invention:	Variants of C-Type Natriuretic Peptide
First Named Inventor/Applicant Name:	Daniel J. Wendt
Customer Number:	35893
Filer:	Leslie A. Serunian
Filer Authorized By:	
Attorney Docket Number:	30610/44556C1-R2
Receipt Date:	07-MAR-2018
Filing Date:	11-JUL-2017
Time Stamp:	16:08:24
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Transmittal Letter	135576_010627REICON_Transmittal.pdf	108882 22b0447d1a89730cd0e676ece02d6f7c93c75ecb	no	2
Warnings:					

Information:					
2	Application Data Sheet	135576_010627REICONSUPPLEMENTALADS.pdf	82931 9f9041621c599971aab38947ce5e46931ce80546	no	6
Warnings:					
Information:					
This is not an USPTO supplied ADS fillable form					
			Total Files Size (in bytes):	191813	

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.
National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.
New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Docket No.: 135576.010627/REI/CON
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Daniel J. Wendt et al.

U.S. Serial No.: 15/646,822 | Confirmation No.: 7727

Filed: July 11, 2017 | Art Unit: 3991

For: *VARIANTS OF C-TYPE NATRIURETIC PEPTIDE* | Examiner: Johnny F. RAILEY

SUBMITTED VIA EFS-Web
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

MARCH 7, 2018 INTERVIEW SUMMARY BY APPLICANTS

This Interview Summary is submitted by Applicants in the above-identified Continuation Reissue application to confirm matters discussed with Examiner Jean Witz, Group Art Unit 3991, and Applicants' undersigned representative during a telephone interview with the Examiner on March 7, 2018.

During the telephone interview, Examiner Witz confirmed that the Notice of Incomplete Reply (Reissue), *Filing Date Granted*, having a mailing date of January 17, 2018 ("Notice"), was sent in error from the U.S. Patent and Trademark Office (USPTO) and is currently nullified. Accordingly, Examiner Witz confirmed that no response to the January 17, 2018 Notice is required to be filed by Applicants and that the application remains in good standing in the USPTO.

In addition, Examiner Witz indicated that Applicants should submit a Supplemental Application Data Sheet (ADS) in the subject application to present the designation of this application as a Continuation Reissue application in the Supplemental ADS in a format that can be processed by the USPTO so as to properly designate the application as a Continuation Reissue application in a corrected, updated Official Filing Receipt to be issued in this application by the USPTO. Examiner Witz confirmed that upon the filing of a Supplemental ADS in this

application, the USPTO would issue a corrected, updated Official Filing Receipt in which this application is properly designated as a Continuation Reissue application. In accordance with the Examiner's notification, Applicants submitted a Supplemental ADS in the subject application on March 7, 2018. Applicants therefore request that a corrected, updated Official Filing Receipt be issued in the subject application at the earliest convenience of the USPTO.

Examiner Witz performed a review of the File Wrapper of the subject application and confirmed that the Power of Attorney and Inventors' Reissue Declaration documents and related documents that were previously submitted in the subject application were entered into the application. The Examiner indicated that no further declaration documentation was required in the subject application as of the March 7, 2018 date.

Applicants appreciate the time spent by Examiner Witz in reviewing the file wrapper of the subject application and confirming by telephone with Applicants' representative that no response to the January 17, 2018 Notice is required by Applicants; that the USPTO will issue a corrected, updated Official Filing Receipt in the application; and that all documentation required to date in the subject application has been submitted by Applicants, as noted *supra*.

No fee is due for the filing of this Interview Summary by Applicants. However, the Commissioner is hereby authorized to charge any fee(s) that may be required or outstanding in the subject application to Deposit Account No. 50-2678, Reference: 135576.010627/REI/CON.

Respectfully submitted,
Greenberg Traurig, LLP

Dated: March 12, 2018

By: /Leslie Serunian/
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Electronic Acknowledgement Receipt

EFS ID:	32018271
Application Number:	15646822
International Application Number:	
Confirmation Number:	7727
Title of Invention:	Variants of C-Type Natriuretic Peptide
First Named Inventor/Applicant Name:	Daniel J. Wendt
Customer Number:	35893
Filer:	Leslie A. Serunian
Filer Authorized By:	
Attorney Docket Number:	30610/44556C1-R2
Receipt Date:	12-MAR-2018
Filing Date:	11-JUL-2017
Time Stamp:	10:48:46
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Applicant summary of interview with examiner	135576_010627_IntSummarybyAppl.pdf	115854 f5678bf073d6a995d09551764263c289b6ad76bb	no	2

Warnings:

Information:	
Total Files Size (in bytes):	115854

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/646,822	07/11/2017	Daniel J. Wendt	135576.010627/REI/CON	7727

35893 7590 03/16/2018

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EXAMINER	
RAILEY, JOHNNY F	

ART UNIT	PAPER NUMBER
3991	

NOTIFICATION DATE	DELIVERY MODE
03/16/2018	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

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Examiner-Initiated Interview Summary	Application No.	Applicant(s)	
	15/646,822	WENDT ET AL.	
	Examiner	Art Unit	
	JOHNNY F. RAILEY II	3991	

All participants (applicant, applicant's representative, PTO personnel):

(1) *JOHNNY F. RAILEY II*.

(2) *JEAN WITZ*.

(3) *LESLIE SERUNIAN*.

(4) _____.

Date of Interview: *07 March 2018*.

Type: ☒ Telephonic ☐ Video Conference
☐ Personal [copy given to: ☐ applicant ☐ applicant's representative]

Exhibit shown or demonstration conducted: ☐ Yes ☒ No.
If Yes, brief description: _____.

Issues Discussed ☐101 ☐112 ☐102 ☐103 ☒Others
(For each of the checked box(es) above, please describe below the issue and detailed description of the discussion)

Claim(s) discussed: *none*.

Identification of prior art discussed: *none*.

Substance of Interview
(For each issue discussed, provide a detailed description and indicate if agreement was reached. Some topics may include: identification or clarification of a reference or a portion thereof, claim interpretation, proposed amendments, arguments of any applied references etc...)

The Notice of Incomplete Reply mailed on 1/17/2018 was mailed in error. The declaration filed on 1/5/2018 is proper. However, a corrective ADS is still required, as the ADS filed on 7/11/2017 lists this application as a "continuation reissue" when the continuation status should be listed separately from the reissue status .

Applicant recordation instructions: It is not necessary for applicant to provide a separate record of the substance of interview.

Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

☐ Attachment

	/Jean C.Witz/ Supervisory Patent Reexamination Specialist CRU 3991

US00RE46707E

(19) **United States**

(12) **Reissued Patent**

Wendt et al.

(10) **Patent Number: US RE46,707 E**

(45) **Date of Reissued Patent: Feb. 13, 2018**

(54) **VARIANTS OF C-TYPE NATRIURETIC PEPTIDE**

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(51) **Int. Cl.**

A61K 38/22	(2006.01)
A61K 38/00	(2006.01)
A61K 38/16	(2006.01)
C12P 21/04	(2006.01)
C07K 14/58	(2006.01)
A61K 47/48	(2006.01)

(52) **U.S. Cl.**

CPC ***C07K 14/58*** (2013.01); ***A61K 47/48215*** (2013.01); *A61K 38/00* (2013.01); *G01N 2333/58* (2013.01); *G01N 2800/10* (2013.01); *G01N 2800/105* (2013.01); *G01N 2800/385* (2013.01); *G01N 2800/52* (2013.01)

(58) **Field of Classification Search**

CPC C07K 15/58; A61K 47/48215

USPC 514/12.4

See application file for complete search history.

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(57) **ABSTRACT**

The present disclosure provides variants of C-type natriuretic peptide (CNP), pharmaceutical compositions comprising CNP variants, and methods of making CNP variants. The CNP variants are useful as therapeutic agents for the treatment of diseases responsive to CNP, including but not limited to bone-related disorders, such as skeletal dysplasias (e.g., achondroplasia), and vascular smooth muscle disorders (e.g., restenosis and arteriosclerosis).

14 Claims, 61 Drawing Sheets

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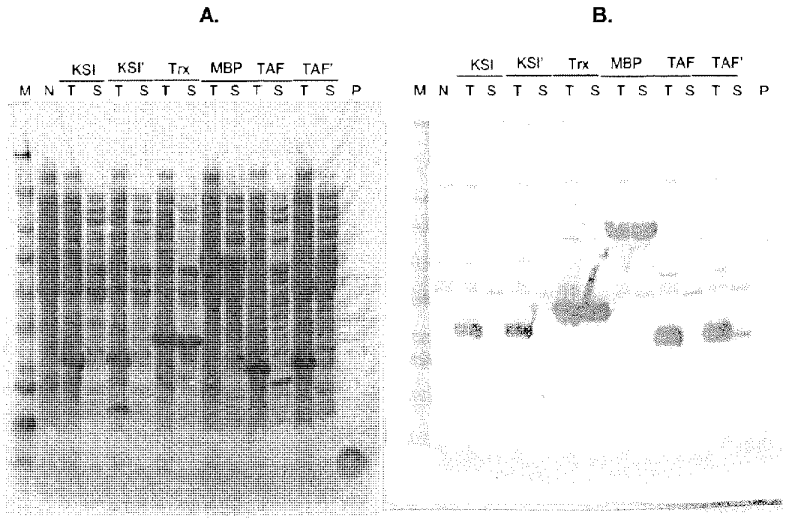
FIGURE 1



Coomassie blue stain

Western blot with anti-CNP antibody

FIGURE 2

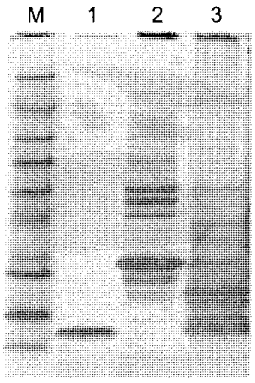
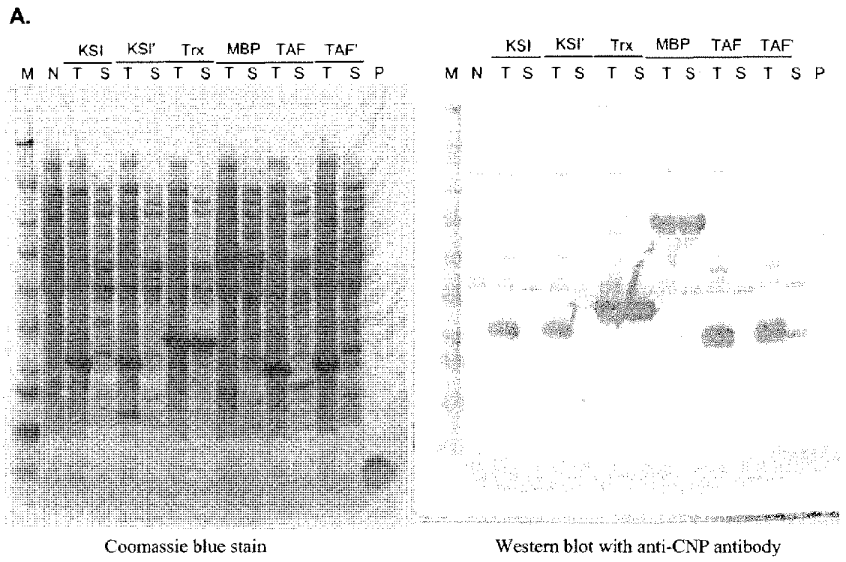


FIGURE 3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M N T S T S T S T S T S T S P
KSI KSI' Trx MBP TAF TAF'
M N T S T S T S T S T S T S P
Coomassie blue stain
Western blot with anti-CNP antibody
B.
BMP-Pro-CNP37 TAF-Pro-CNP37
M C Tu T S P Tu T S P
BMP-Pro-CNP37 TAF-Pro-CNP37
M C Tu T S P Tu T S P
Coomassie blue stain
Western blot with anti-CNP antibody

FIGURE 3 continued

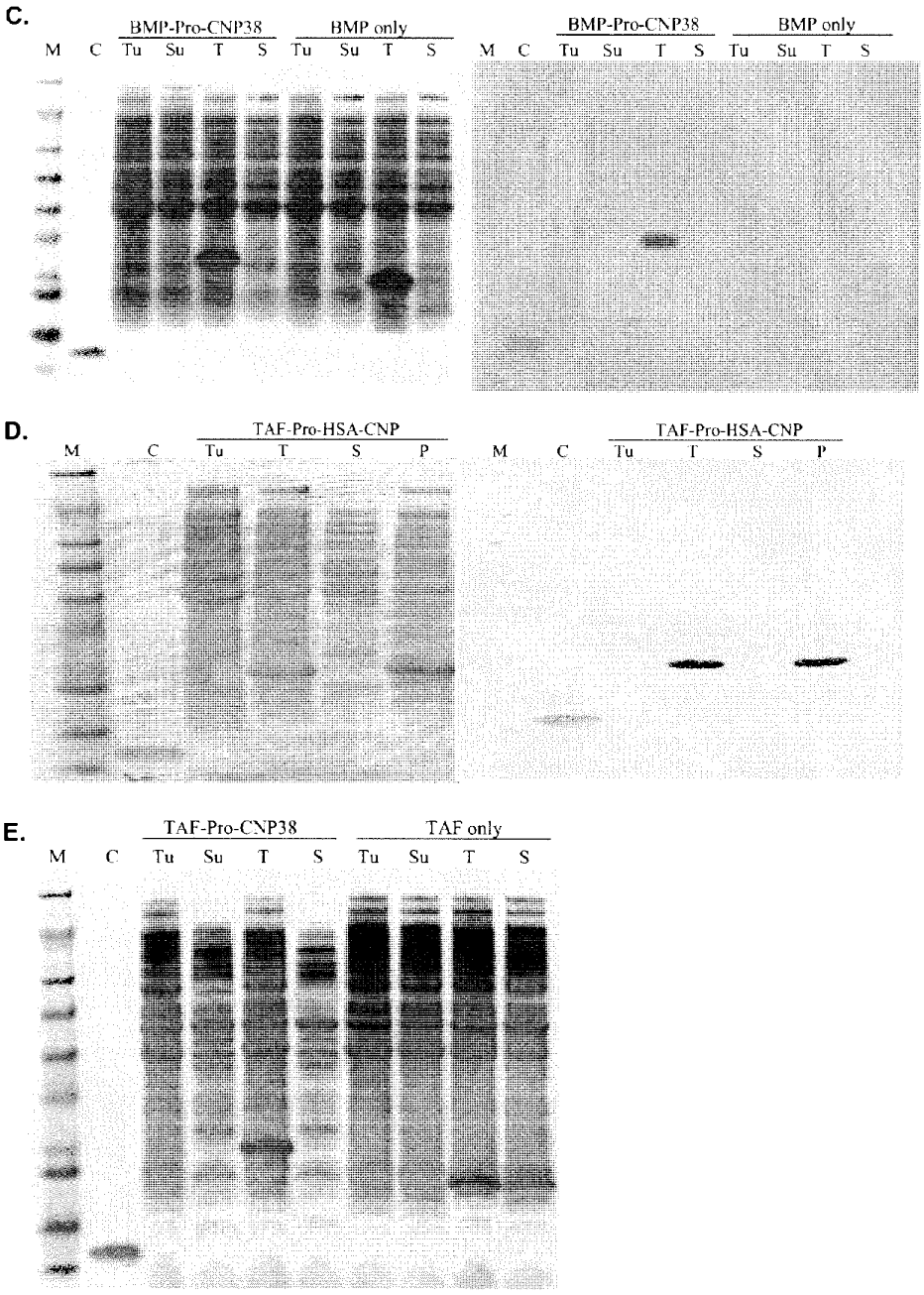


FIGURE 4

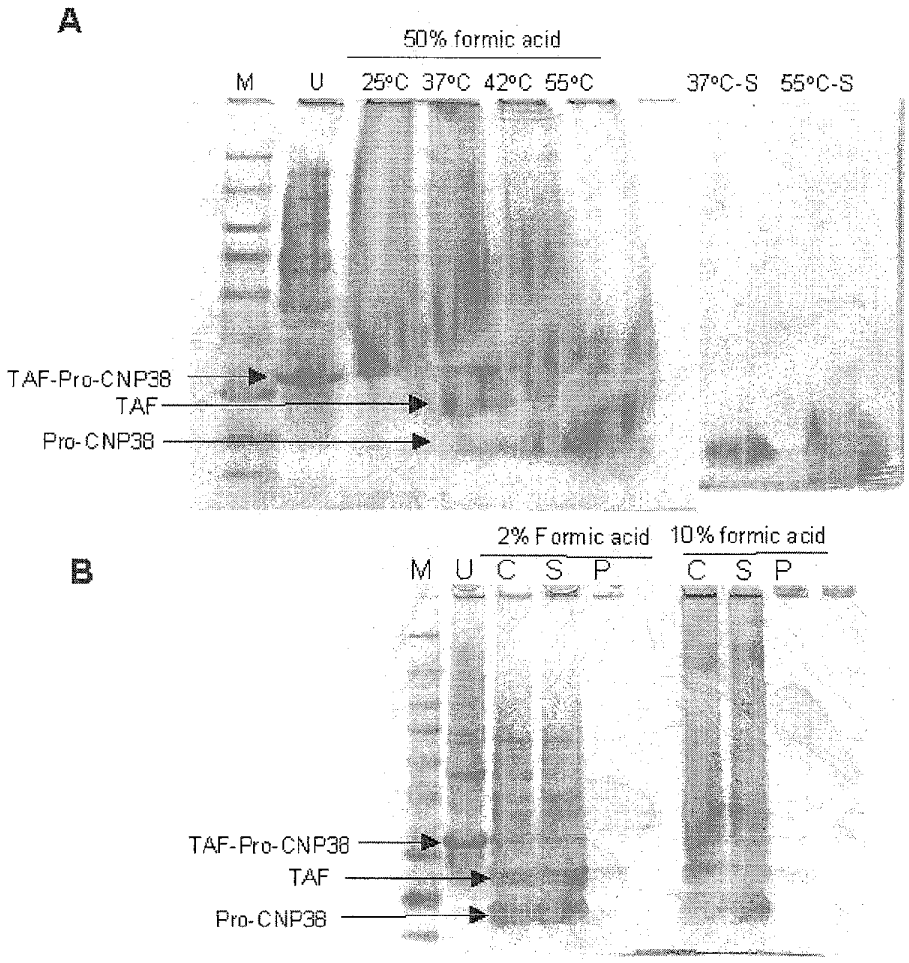
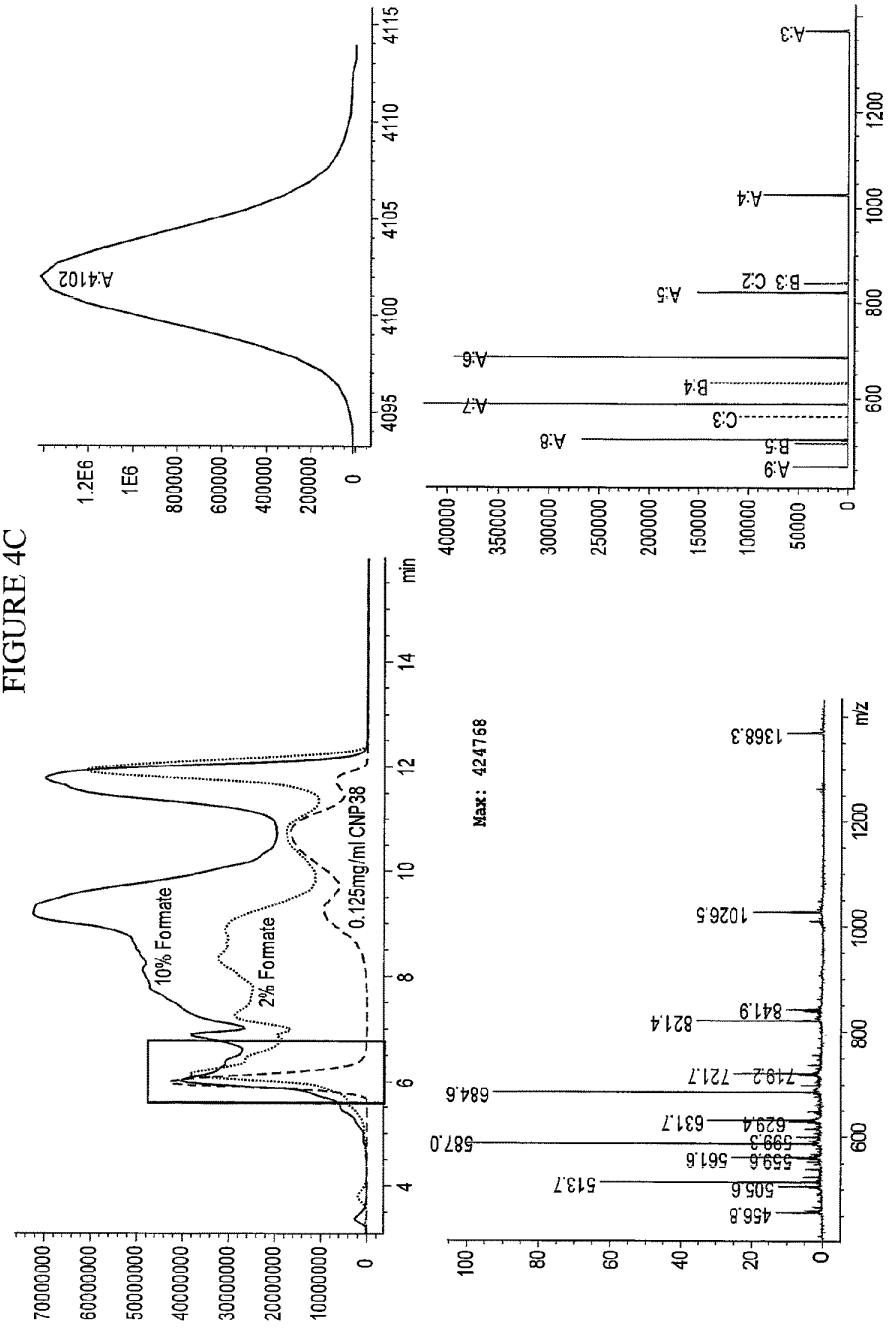

A
50% formic acid
M
U
25°C
37°C
42°C
55°C
37°C-S
55°C-S
TAF-Pro-CNP38
TAF
Pro-CNP38
B
2% Formic acid
10% formic acid
M
U
C
S
P
C
S
P
TAF-Pro-CNP38
TAF
Pro-CNP38

FIGURE 4C


10% Formate
2% Formate
0.125mg/ml CNP38
min
A:4102
Max: 424768
m/z
A:3
A:4
B:3 C:2
A:5
A:6
A:7
B:4
C:3
A:8
B:5
A:9
1368.3
1026.5
841.9
821.4
719.2
721.7
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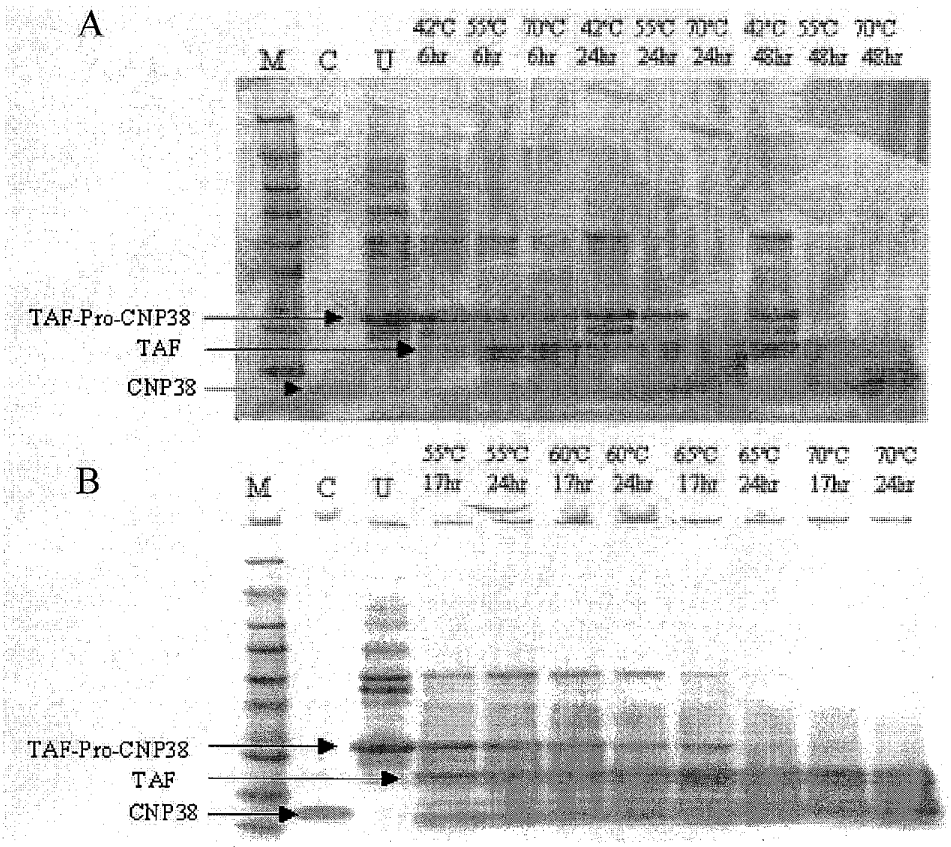
513.7
505.6
456.8

FIGURE 5


A
M
C
U
42°C 6hr
55°C 6hr
70°C 6hr
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70°C 24hr
42°C 48hr
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70°C 48hr
TAF-Pro-CNP38
TAF
CNP38
B
M
C
U
55°C 17hr
55°C 24hr
60°C 17hr
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TAF
CNP38

FIGURE 5C

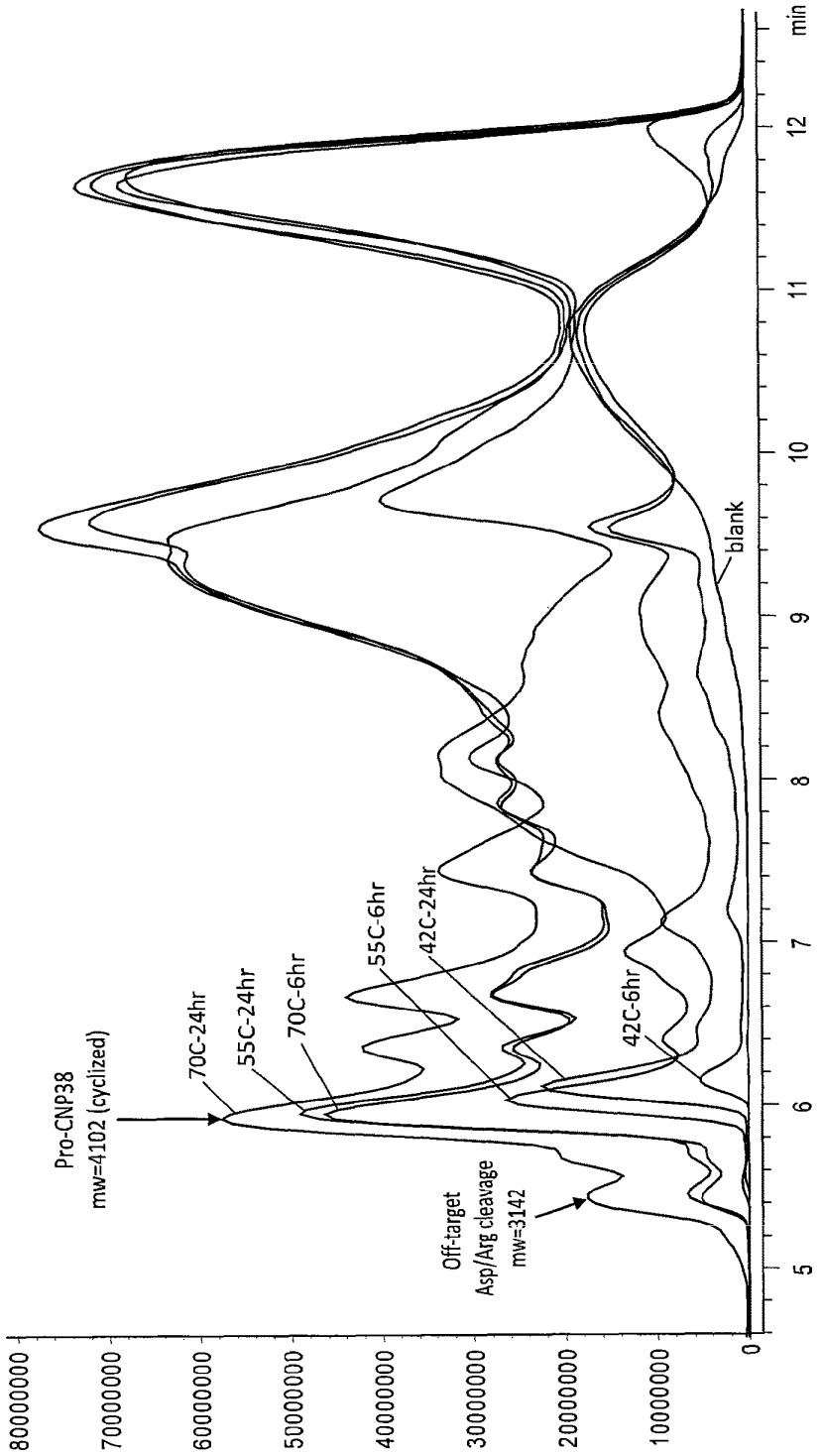

Pro-CNP38
mw=4102 (cyclized)
70C-24hr
55C-24hr
70C-6hr
55C-6hr
42C-24hr
42C-6hr
blank
Off-target
Asp/Arg cleavage
mw=3142
80000000
70000000
60000000
50000000
40000000
30000000
20000000
10000000
0
5
6
7
8
9
10
11
12
min

FIGURE 6

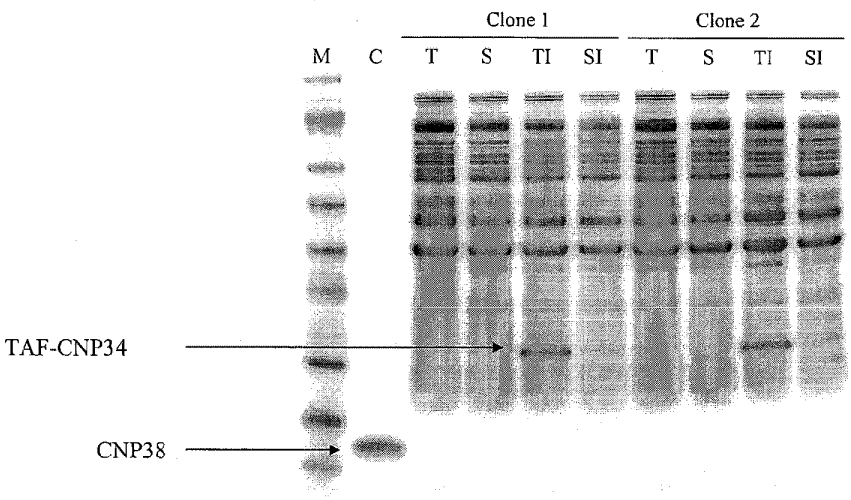


FIGURE 7

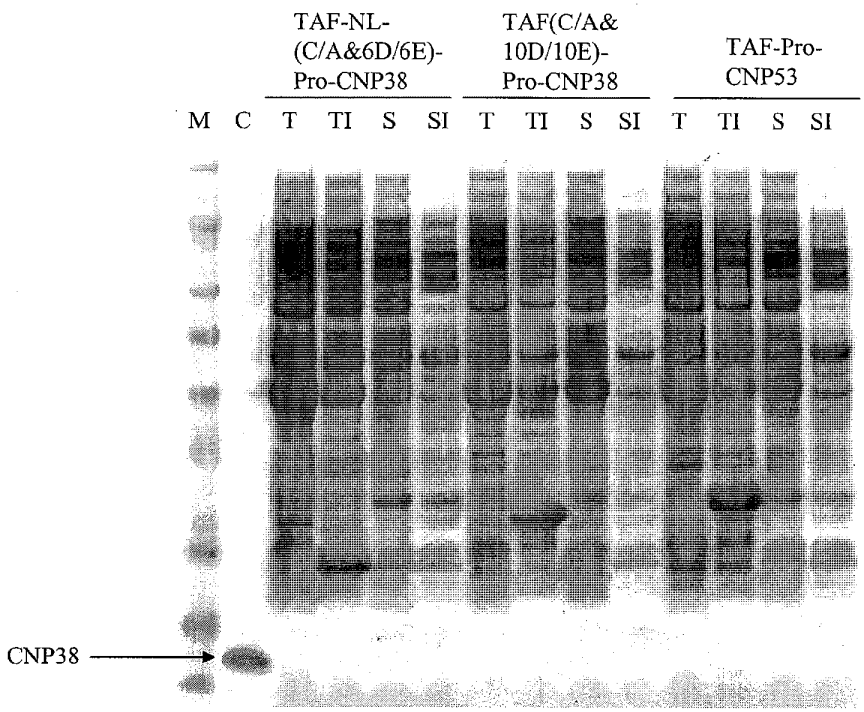


FIGURE 8

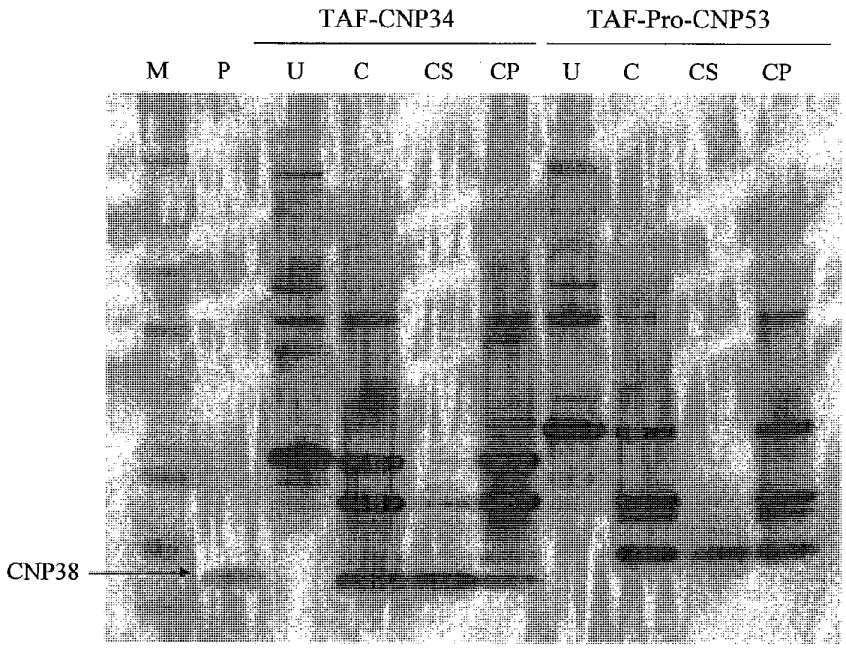


FIGURE 9

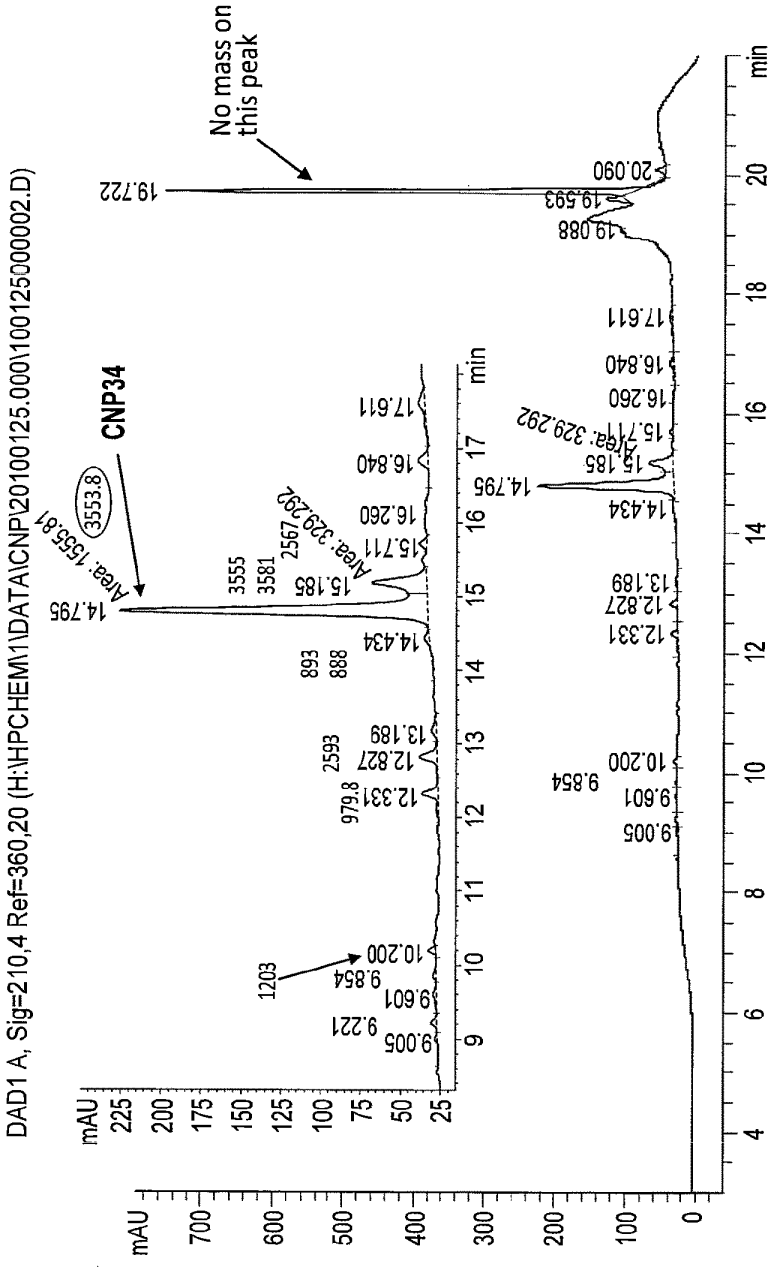

Calc. CNP34 monoisotopic mass = 3553.88
DAD1 A, Sig=210,4 Ref=360,20 (H:\HPCHEM\1\DATA\CNP\20100125.000\100125000002.D)
No mass on this peak
CNP34
3553.8
Area: 1555.81
Area: 329.292
mAU
min

FIGURE 10

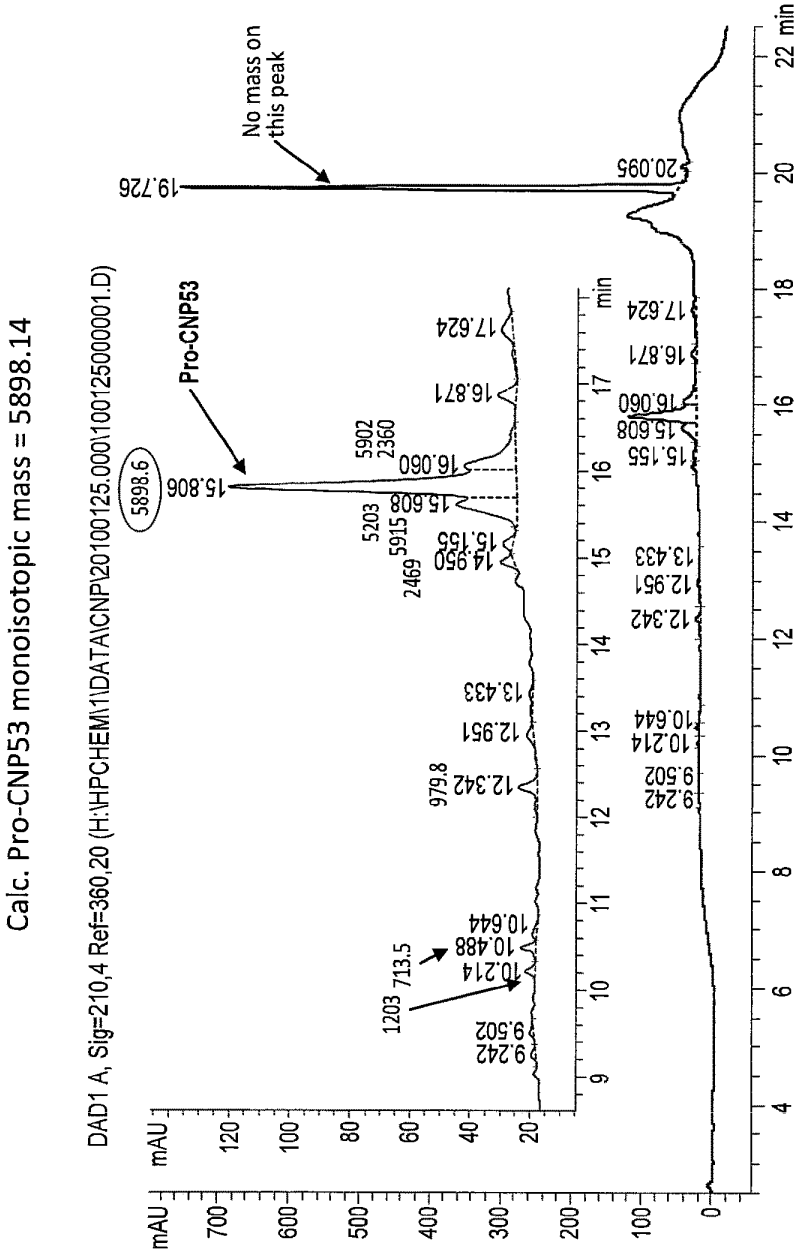

Calc. Pro-CNP53 monoisotopic mass = 5898.14
DAD1 A, Sig=210,4 Ref=360,20 (H:\HPCHEM\1\DATA\CNP\20100125.000\100125000001.D)
Pro-CNP53
No mass on this peak
5898.6
15.806
19.726
20.095
17.624
16.871
16.060
15.608
15.155
14.950
13.433
12.951
12.342
10.644
10.488
10.214
9.502
9.242
5902
2360
5203
5915
2469
979.8
1203
713.5
mAU
min

FIGURE 11

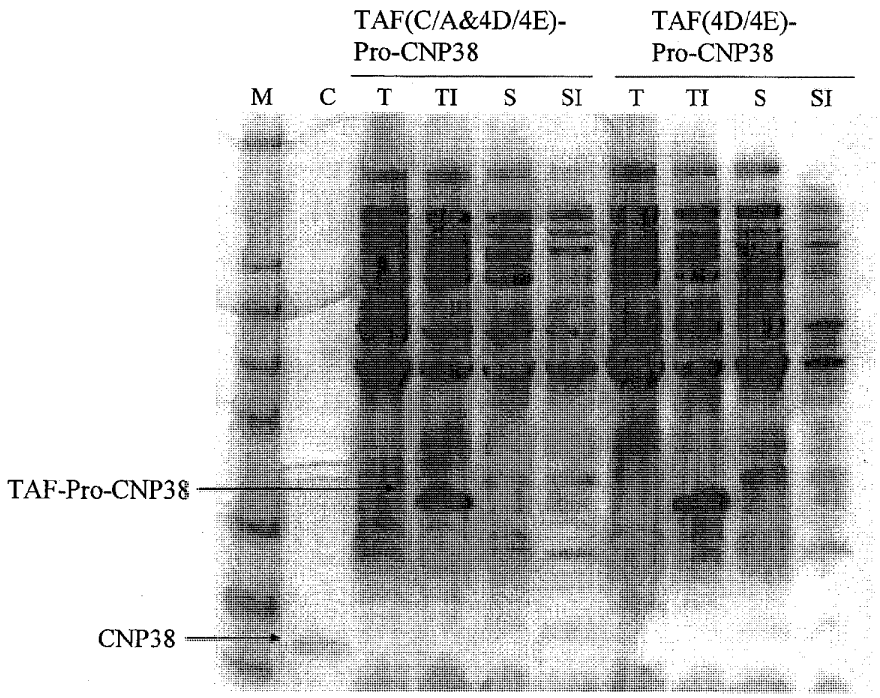

TAF(C/A&4D/4E)-
Pro-CNP38
TAF(4D/4E)-
Pro-CNP38
M
C
T
TI
S
SI
T
TI
S
SI
TAF-Pro-CNP38
CNP38

FIGURE 12

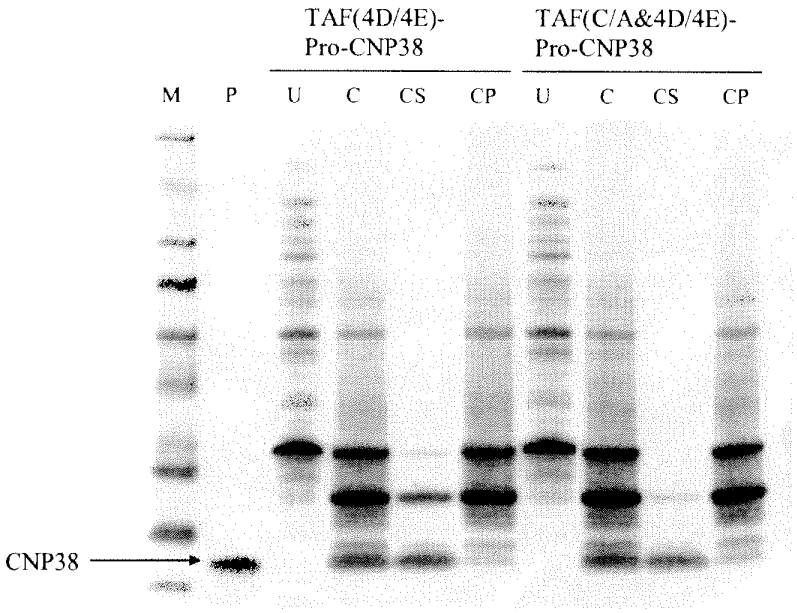


FIGURE 13

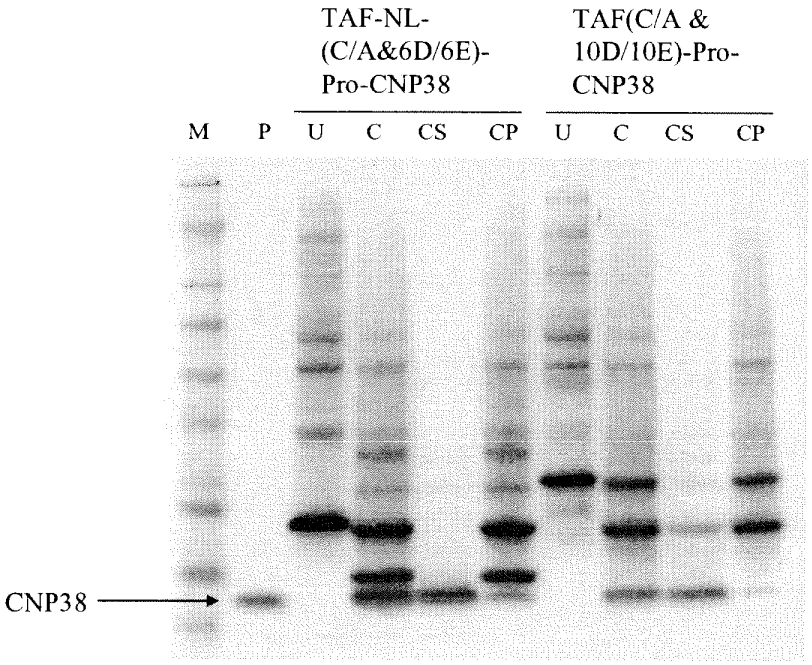


FIGURE 14

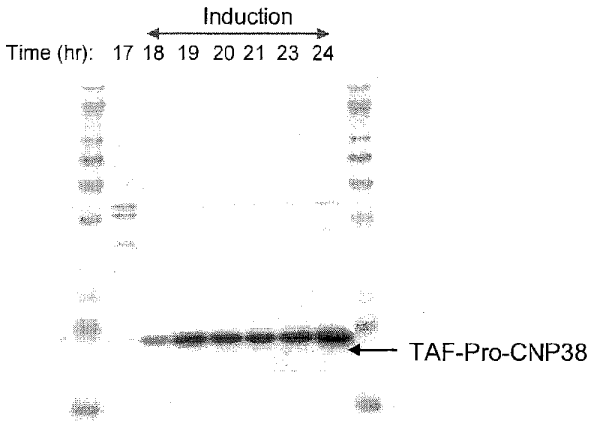


FIGURE 15

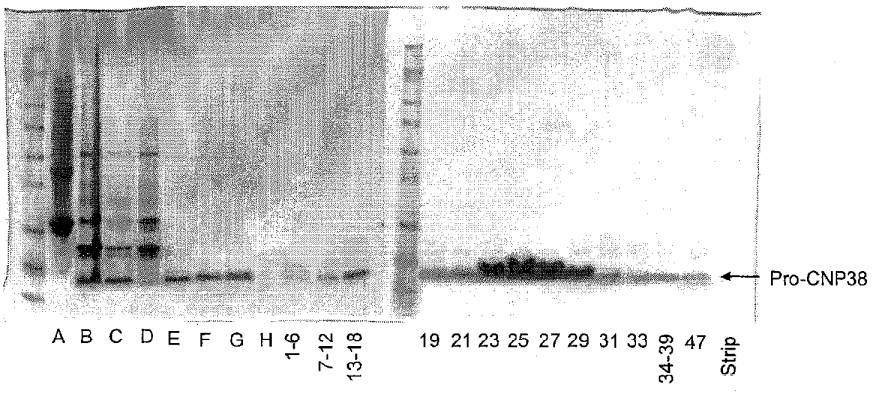


FIGURE 16

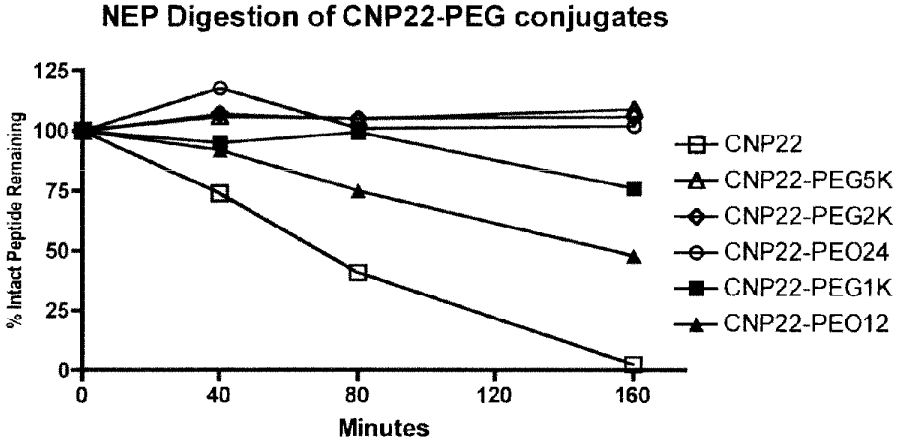


FIGURE 17

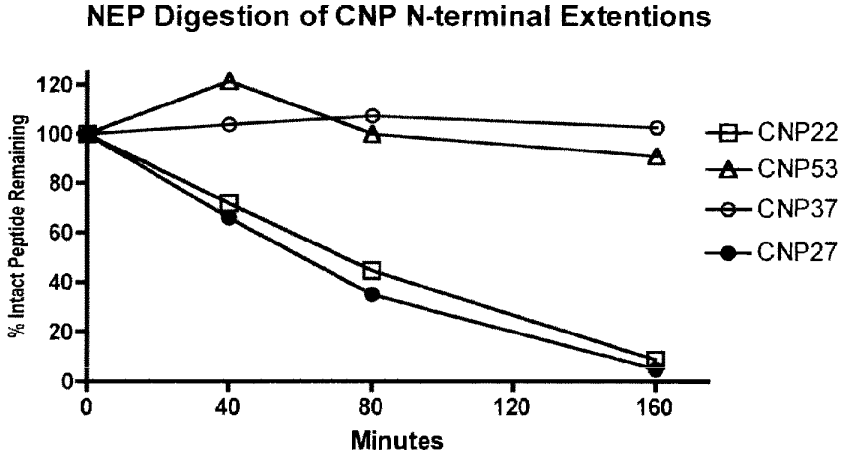


FIGURE 18

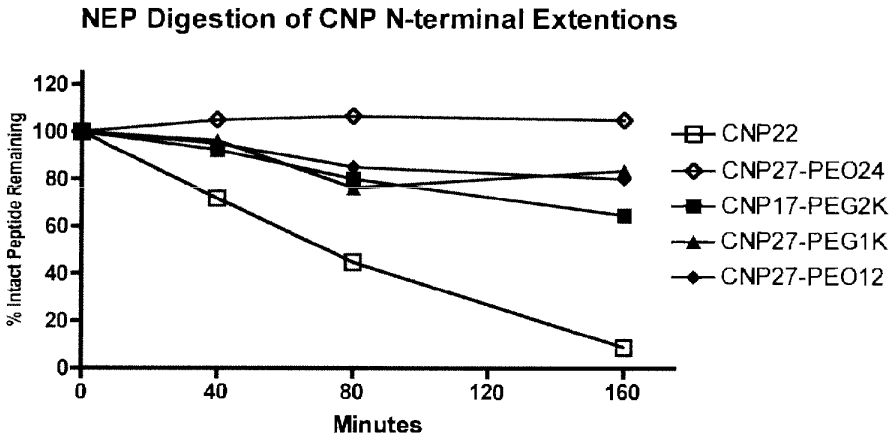


FIGURE 19

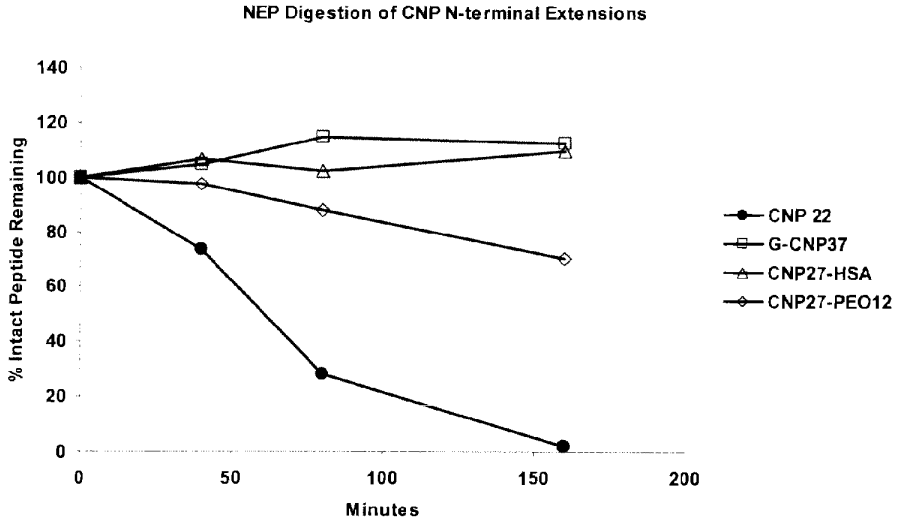


FIGURE 20

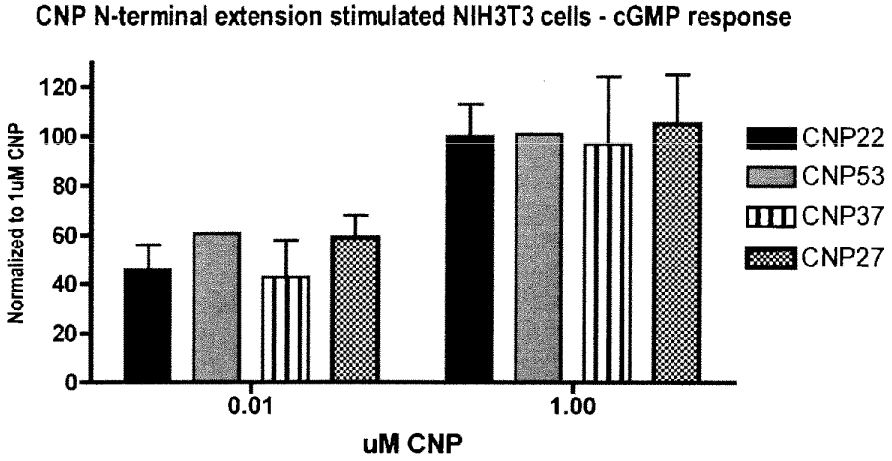


FIGURE 21

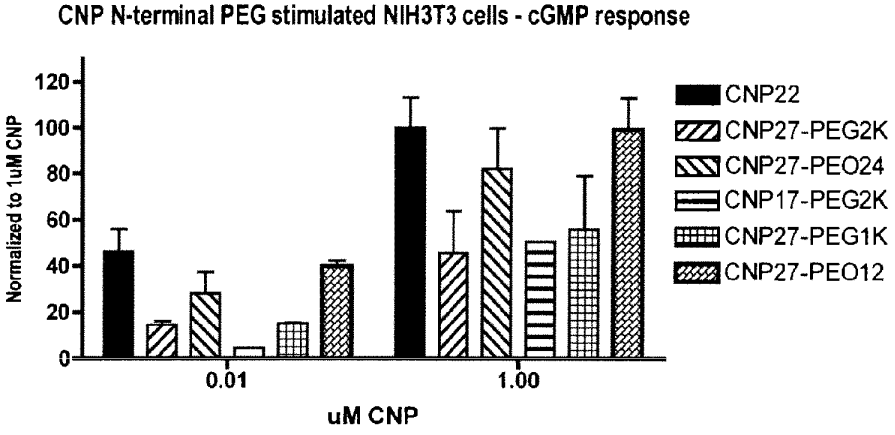


FIGURE 22

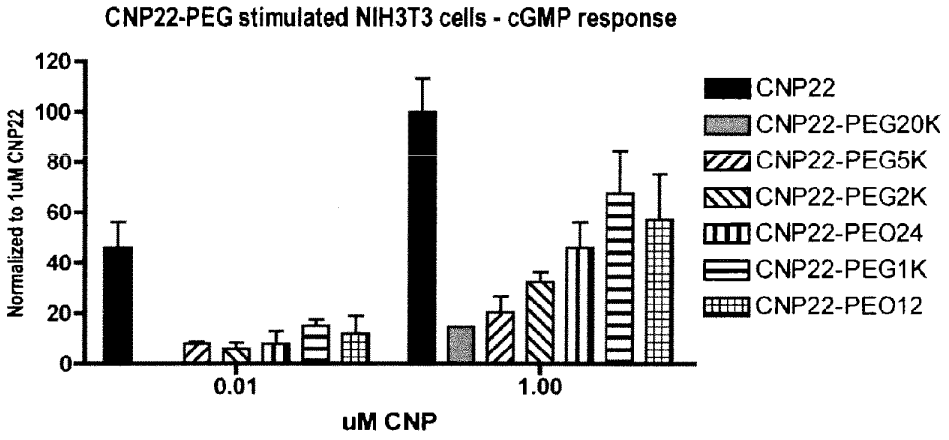


FIGURE 23

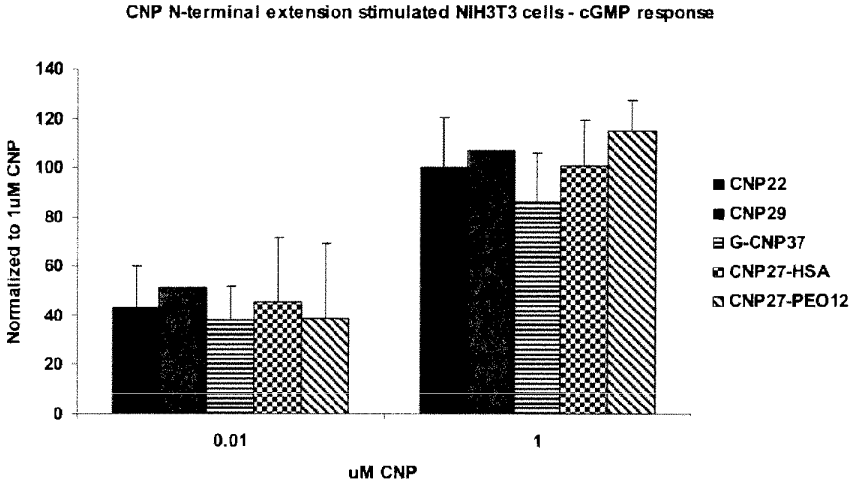
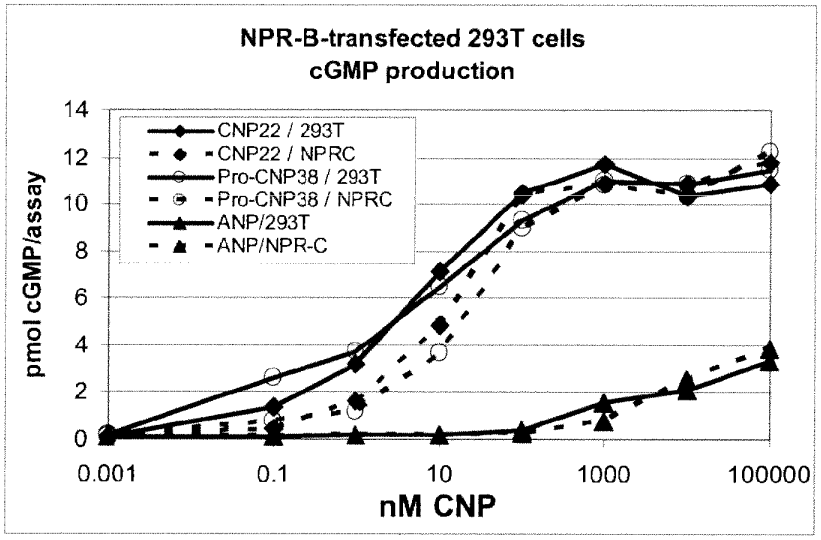


FIGURE 24

A.


NPR-B-transfected 293T cells
cGMP production
pmol cGMP/assay
nM CNP
CNP22 / 293T
CNP22 / NPRC
Pro-CNP38 / 293T
Pro-CNP38 / NPRC
ANP/293T
ANP/NPR-C
0
2
4
6
8
10
12
14
0.001
0.1
10
1000
100000

B.

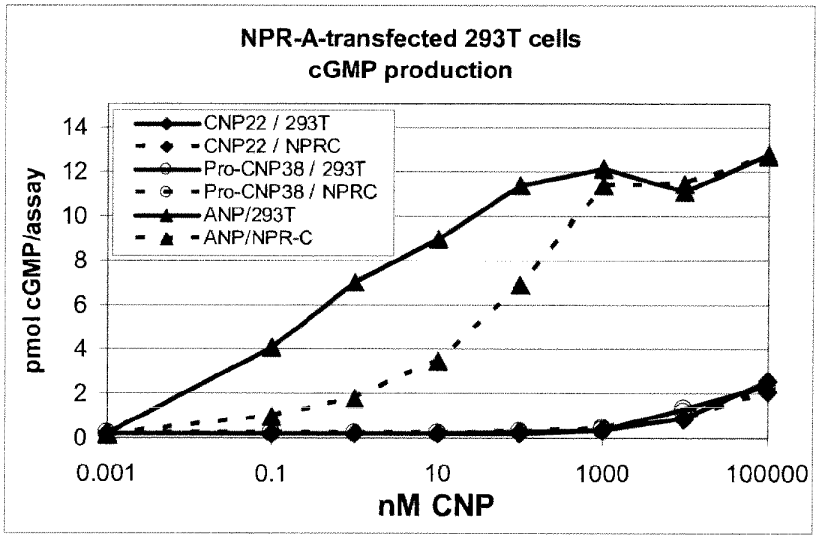
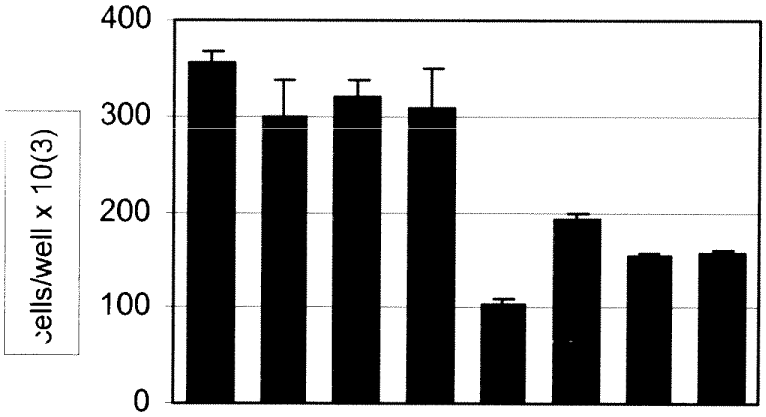

NPR-A-transfected 293T cells
cGMP production
pmol cGMP/assay
nM CNP
CNP22 / 293T
CNP22 / NPRC
Pro-CNP38 / 293T
Pro-CNP38 / NPRC
ANP/293T
ANP/NPR-C
0
2
4
6
8
10
12
14
0.001
0.1
10
1000
100000

FIGURE 25



Bars from left to right:

1	control (no CNP22, no FGF2)
2	CNP22 (0.2 uM) continuous
3	CNP22 (0.2 uM) 1 hr once daily
4	CNP22 (0.2 uM) 2 hr once daily
5	FGF2 (5 ng/mL) continuous
6	FGF2 + CNP22 (0.2 uM) continuous
7	FGF2 + CNP22 (0.2 uM) 1 hr daily
8	FGF2 + CNP22 (0.2 uM) 2 hr daily

FIGURE 26

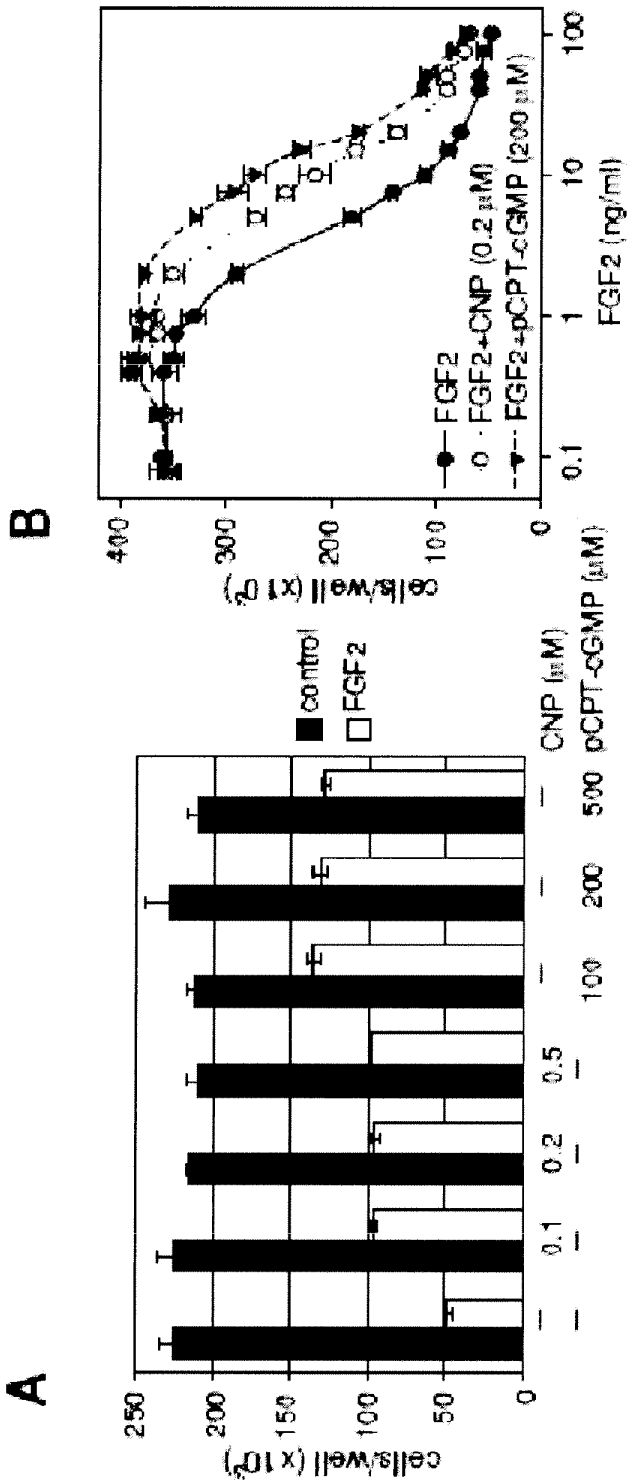

A
B
cells/well (x 10⁵)
control
FGF2
CNP (μM)
pCPT-cGMP (μM)
FGF2 (ng/ml)
FGF2
FGF2+CNP (0.2 μM)
FGF2+pCPT-cGMP (200 μM)

FIGURE 27

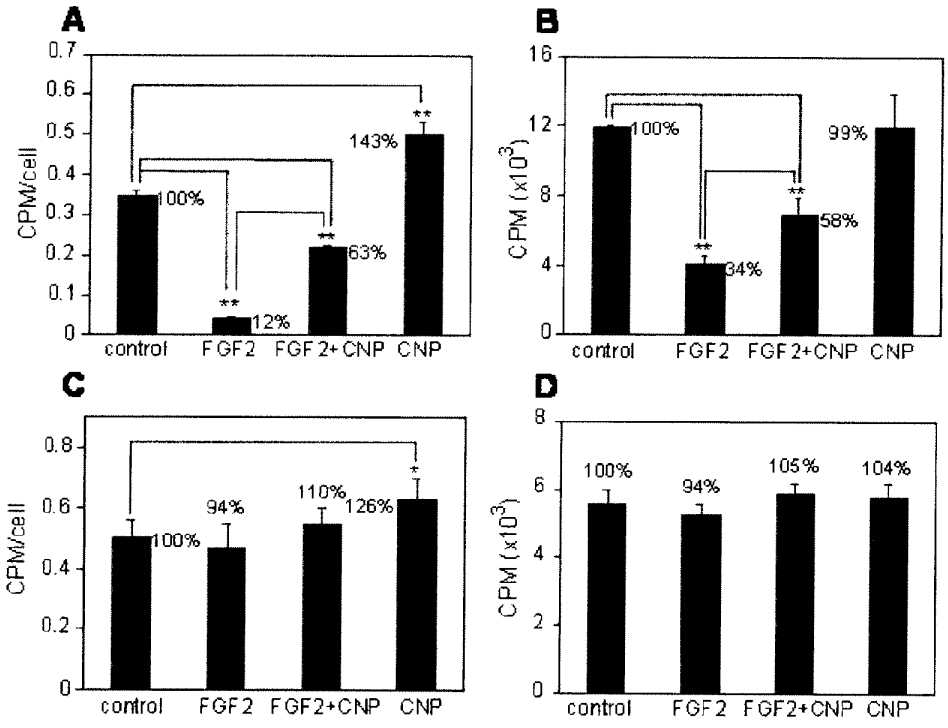

A
CPM/cell
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
100%
12%
63%
143%
**
control
FGF2
FGF2+CNP
CNP
B
CPM (x10^3)
16
12
8
4
0
100%
34%
58%
99%
C
CPM/cell
0.8
0.6
0.4
0.2
0
100%
94%
110%
126%
*
D
CPM (x10^3)
8
6
4
2
0
100%
94%
105%
104%

FIGURE 28

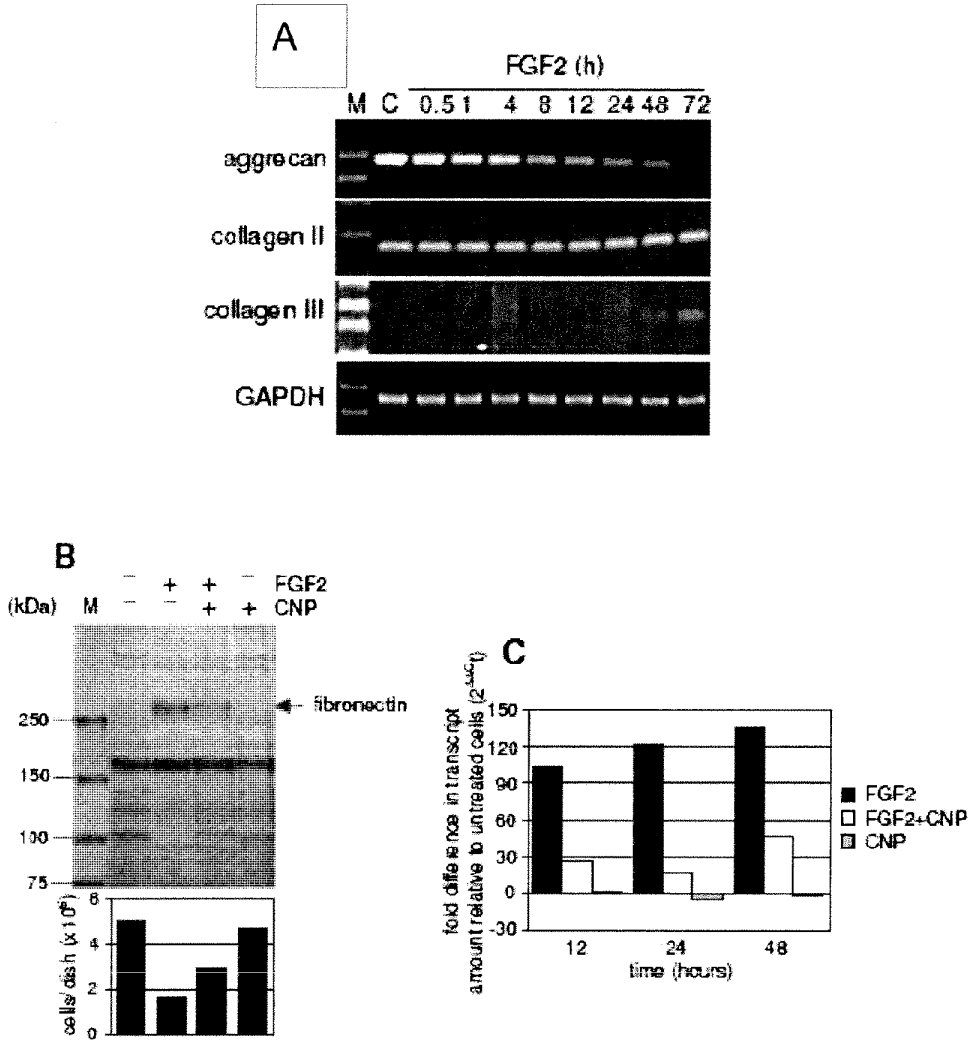

A
FGF2 (h)
M C 0.5 1 4 8 12 24 48 72
aggrecan
collagen II
collagen III
GAPDH
B
(kDa) M
FGF2
CNP
fibronectin
250
150
100
75
cells/ dish (x 10⁶)
C
fold difference in transcript amount relative to untreated cells
time (hours)
12
24
48
FGF2
FGF2+CNP
CNP

FIGURE 29

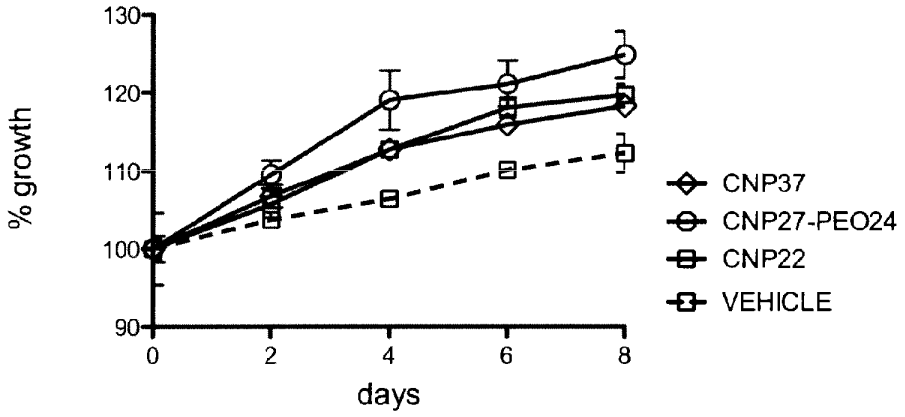


FIGURE 30

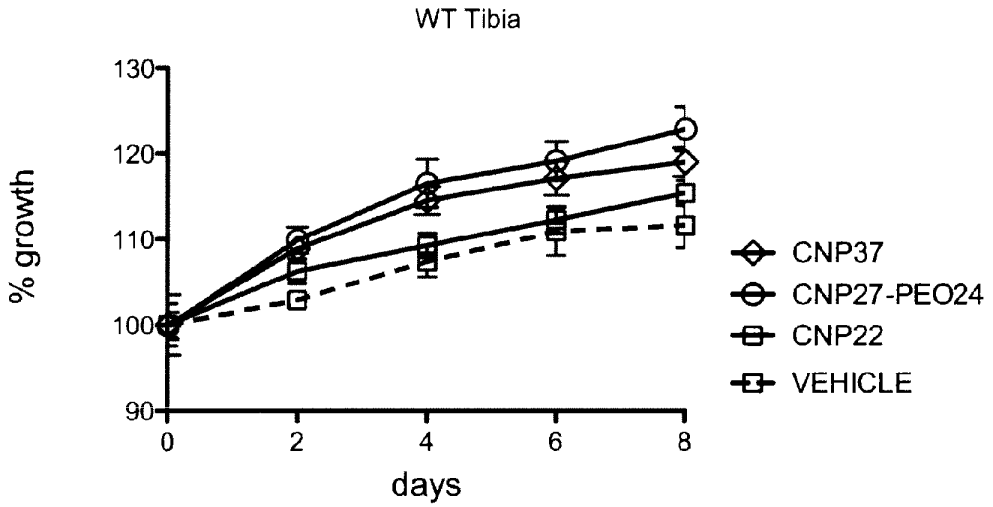


FIGURE 31

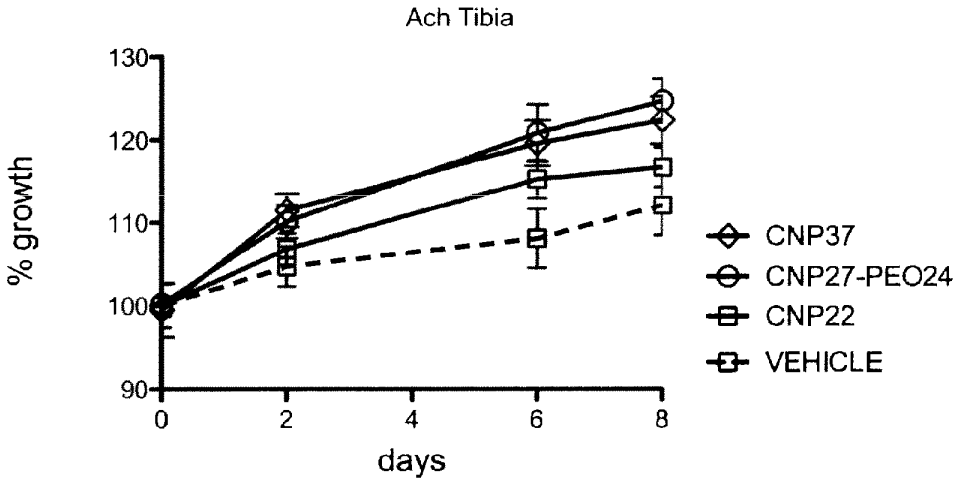


FIGURE 32

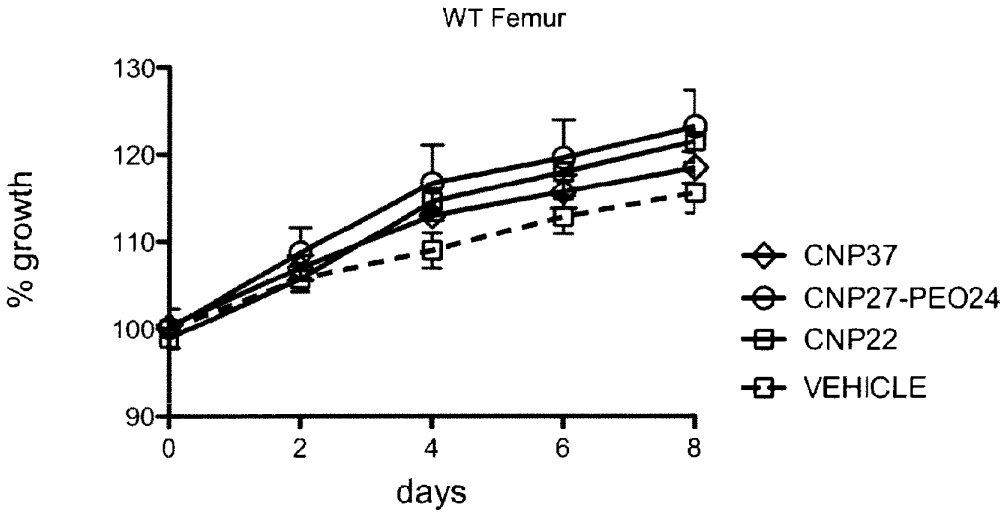


FIGURE 33

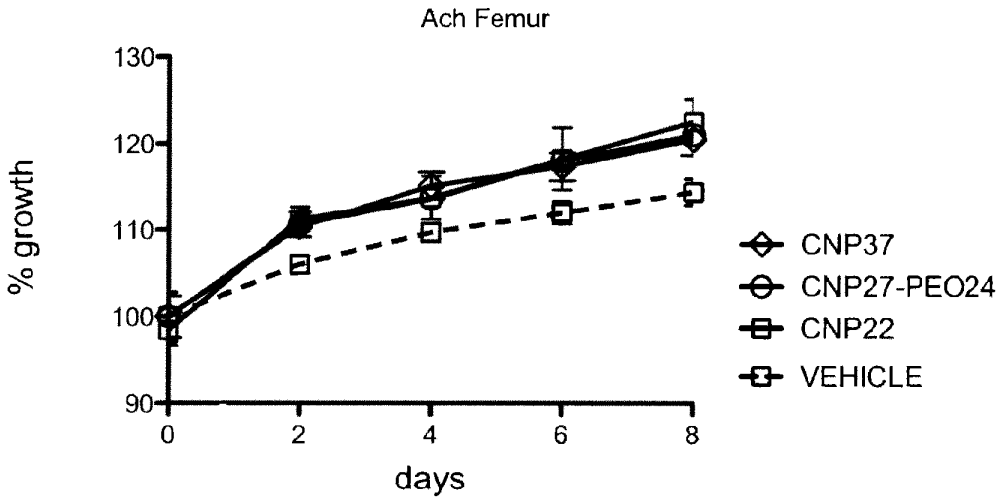

Ach Femur
% growth
130
120
110
100
90
0
2
4
6
8
days
CNP37
CNP27-PEO24
CNP22
VEHICLE

FIGURE 34

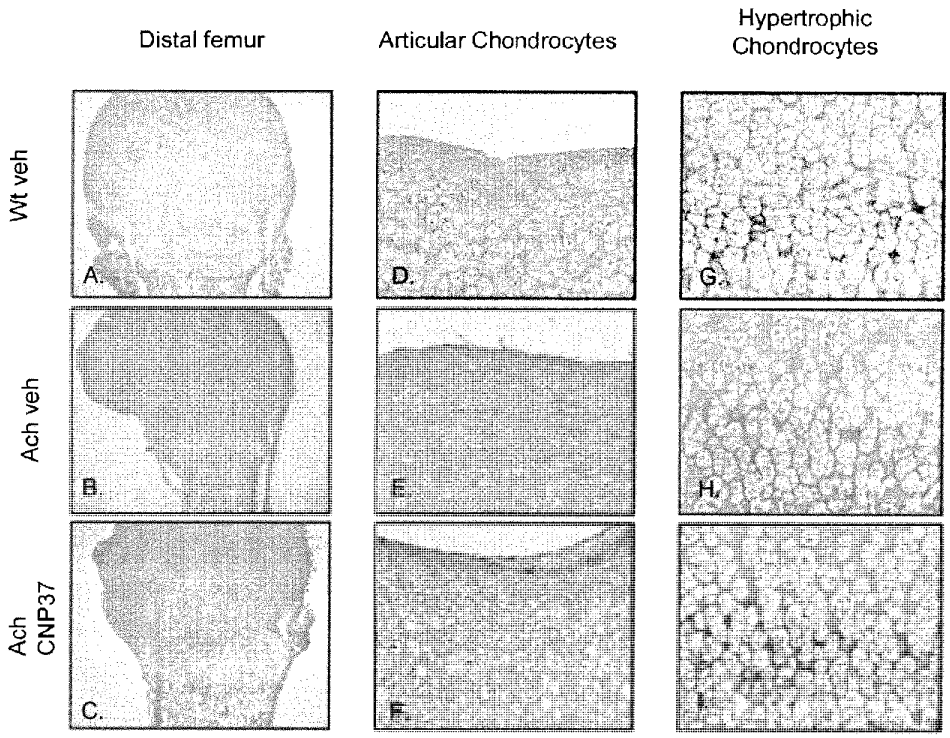

Distal femur
Articular Chondrocytes
Hypertrophic Chondrocytes
Wt veh
Ach veh
Ach CNP37
A.
B.
C.
D.
E.
F.
G.
H.
I.

FIGURE 35

Cellularity of Proliferating Columns

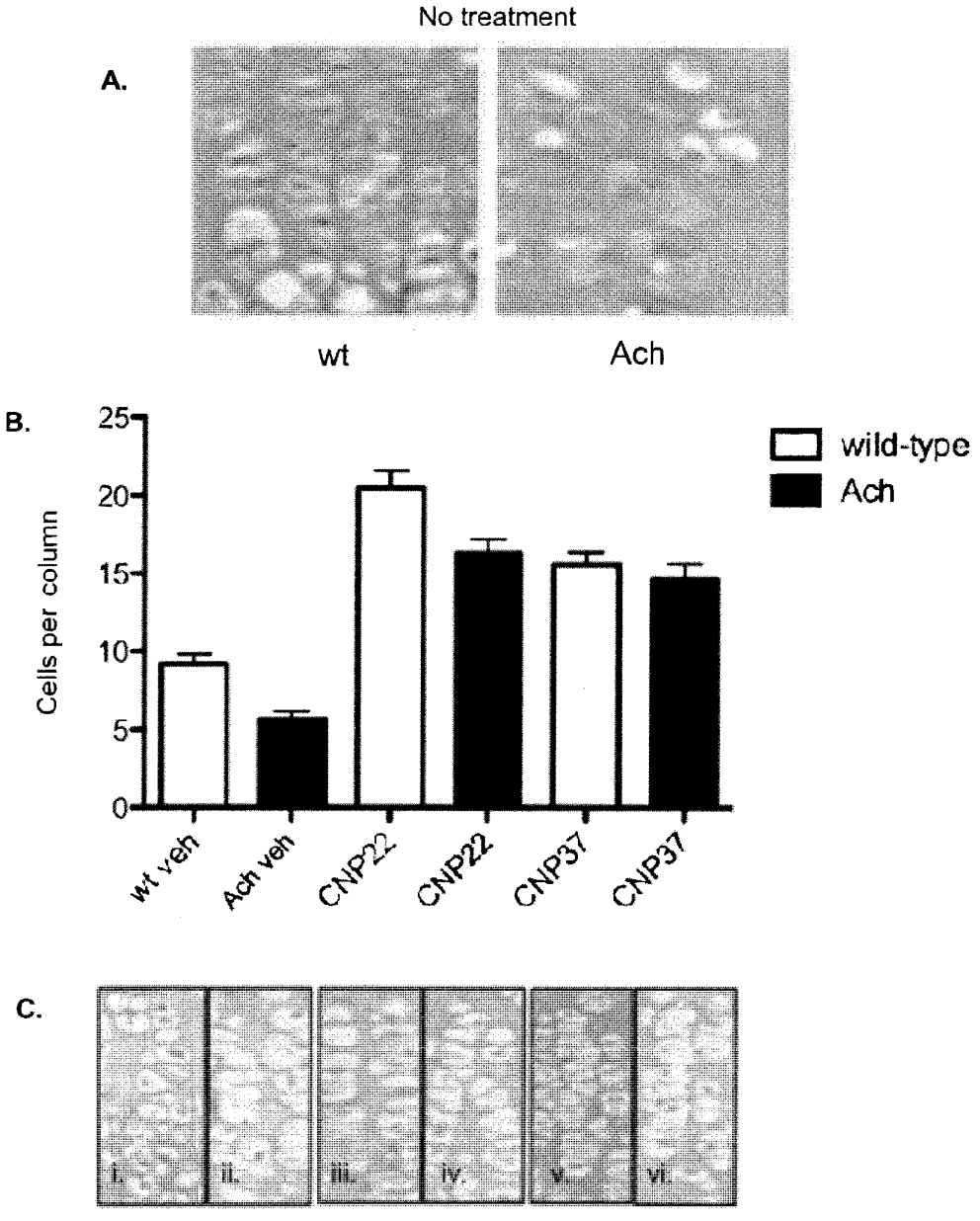


FIGURE 36

Chondrocyte Hypertrophy

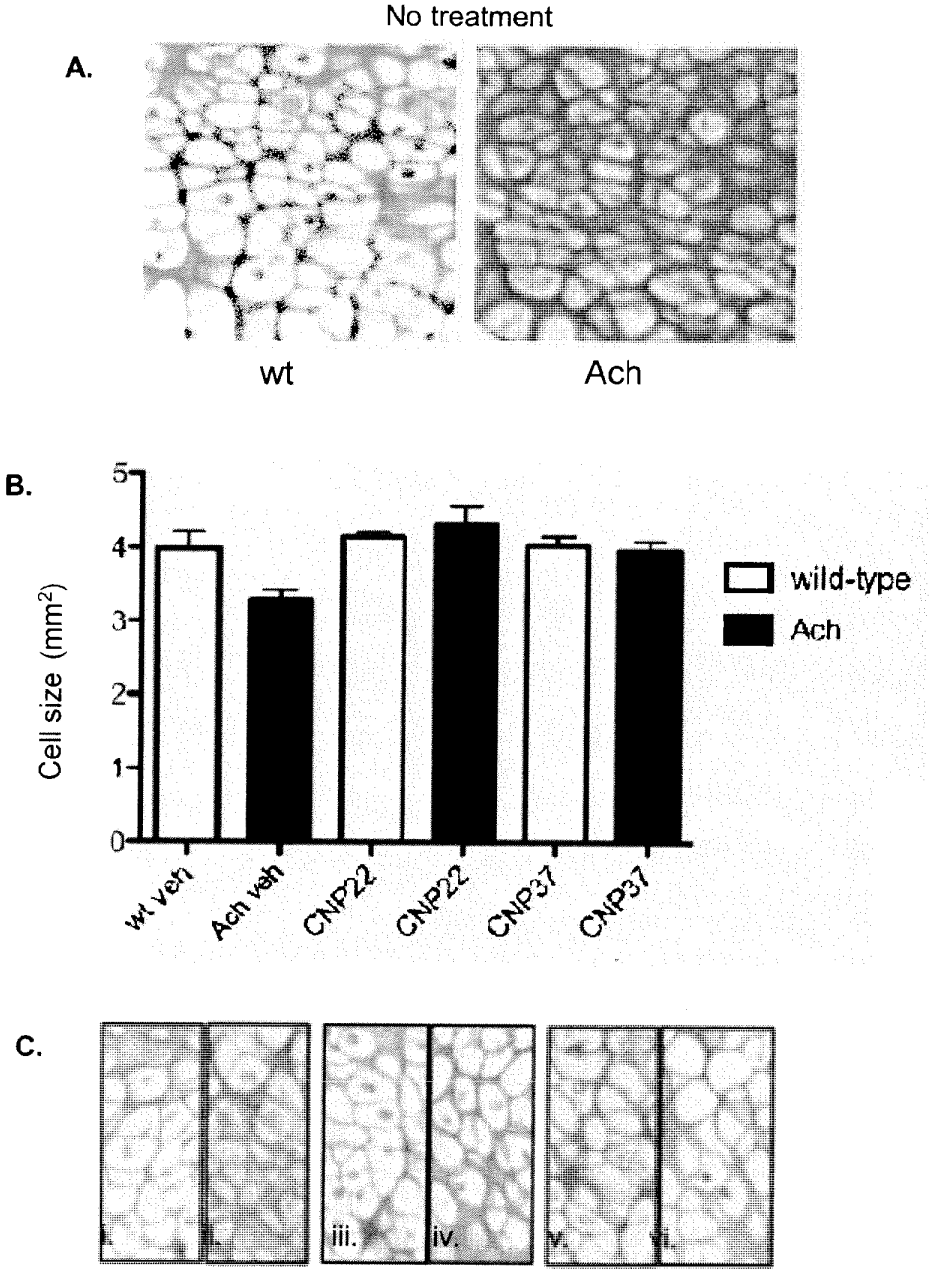


FIGURE 37

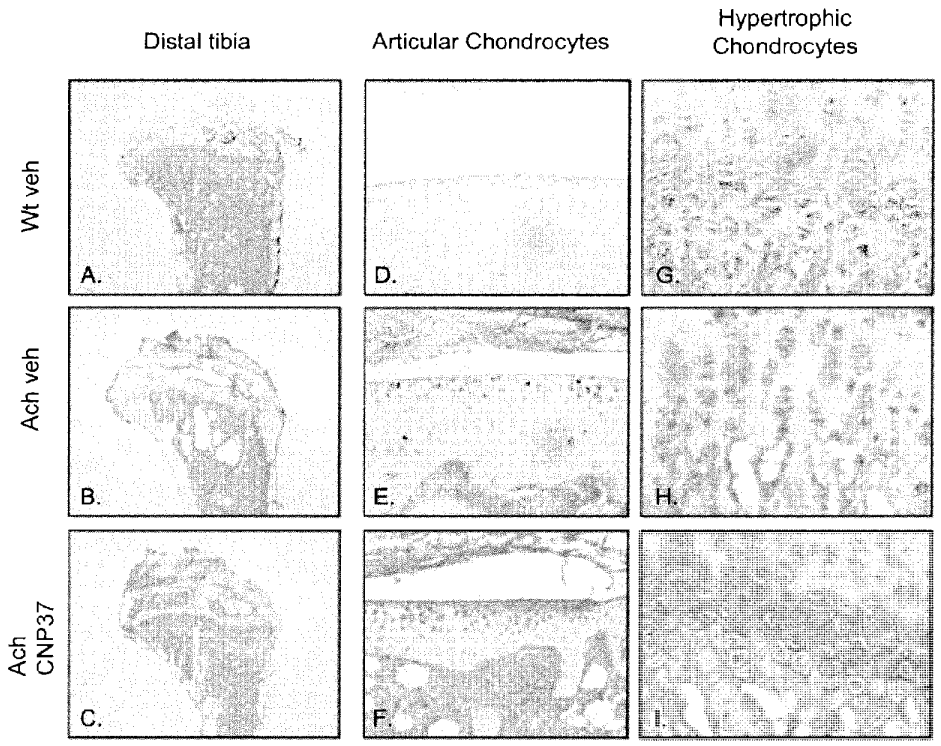
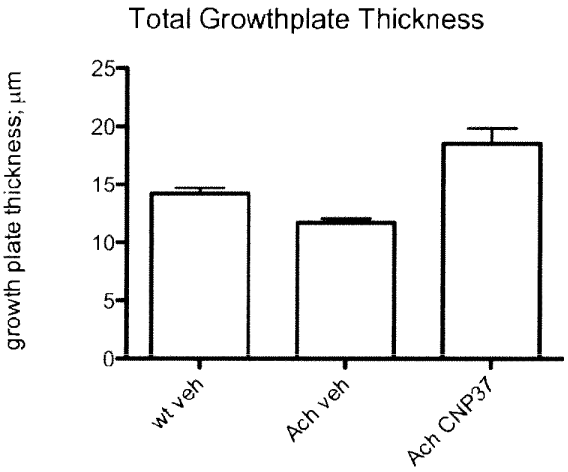
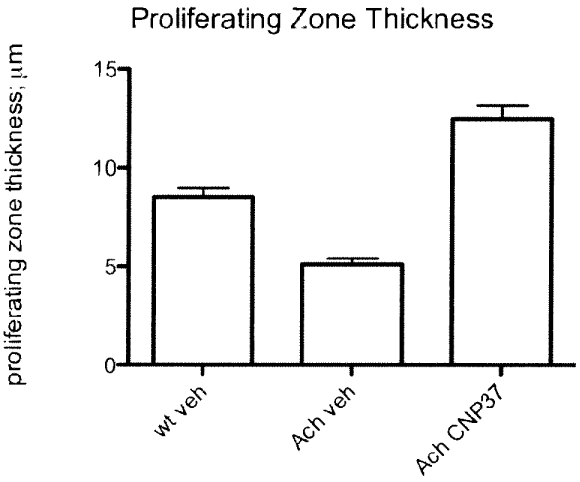

Distal tibia
Articular Chondrocytes
Hypertrophic Chondrocytes
Wt veh
Ach veh
Ach CNP37
A.
B.
C.
D.
E.
F.
G.
H.
I.

FIGURE 38

A.



B.



C.

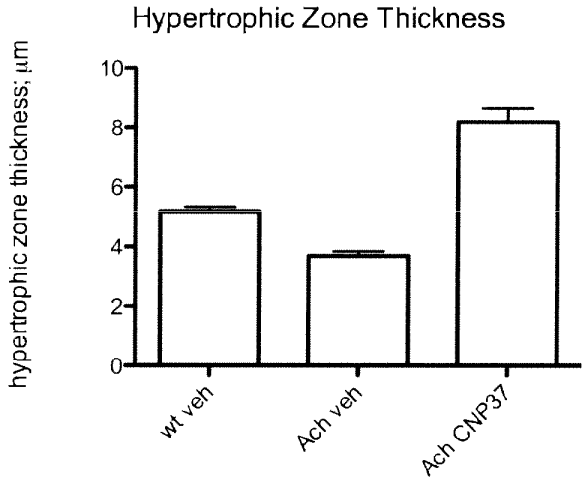


FIGURE 39

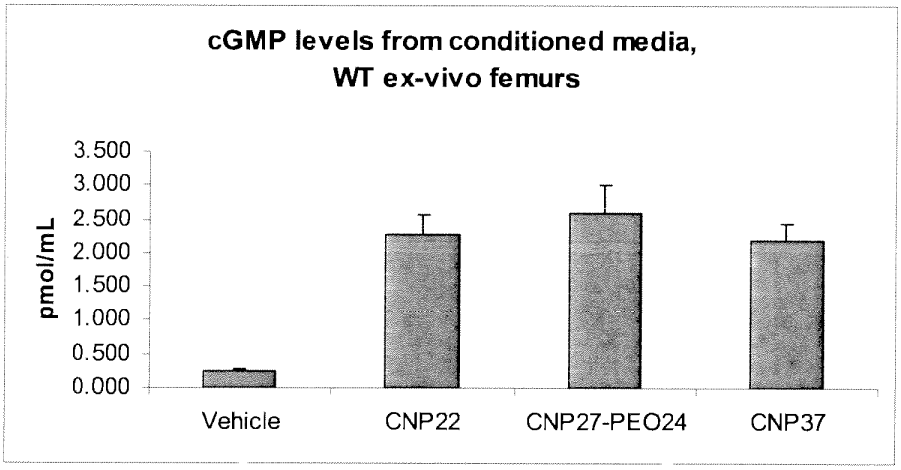


FIGURE 40

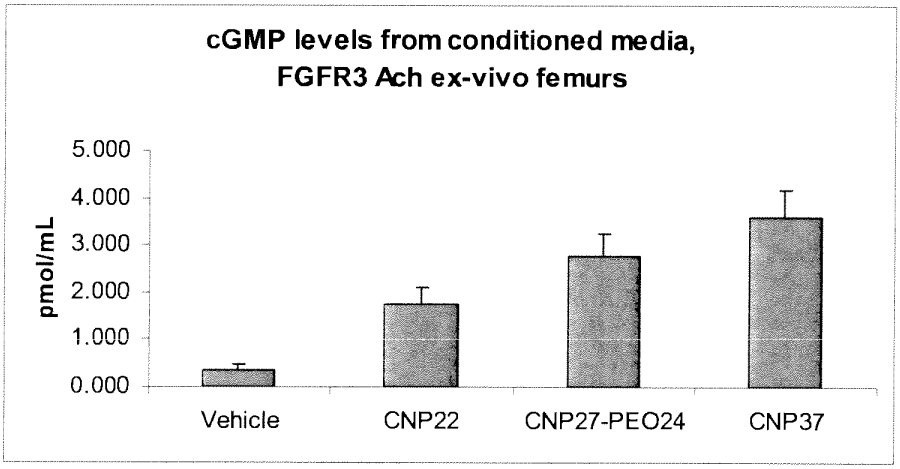


FIGURE 41

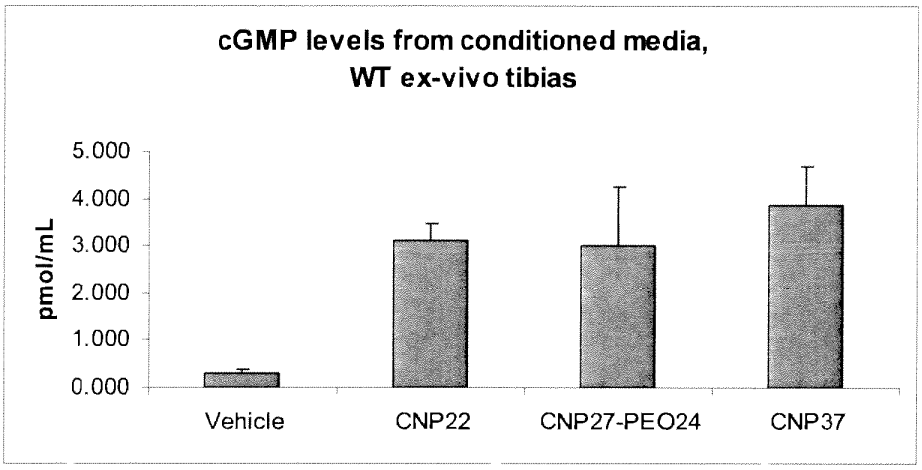


FIGURE 42

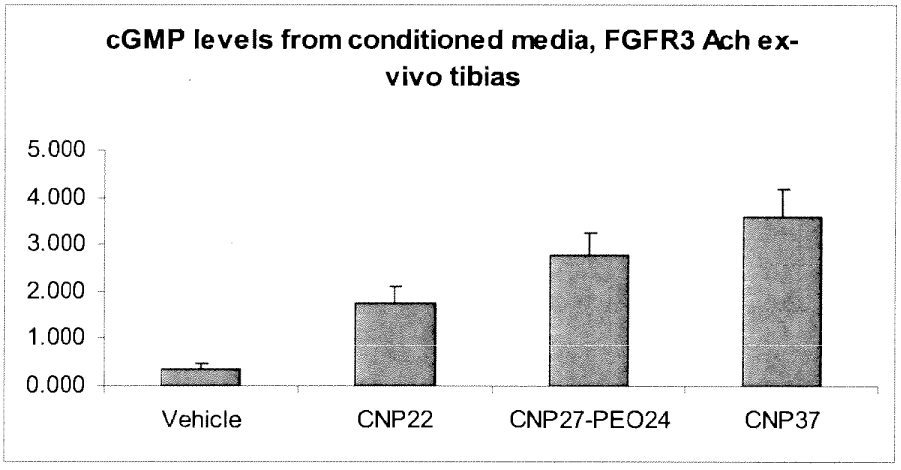


FIGURE 43

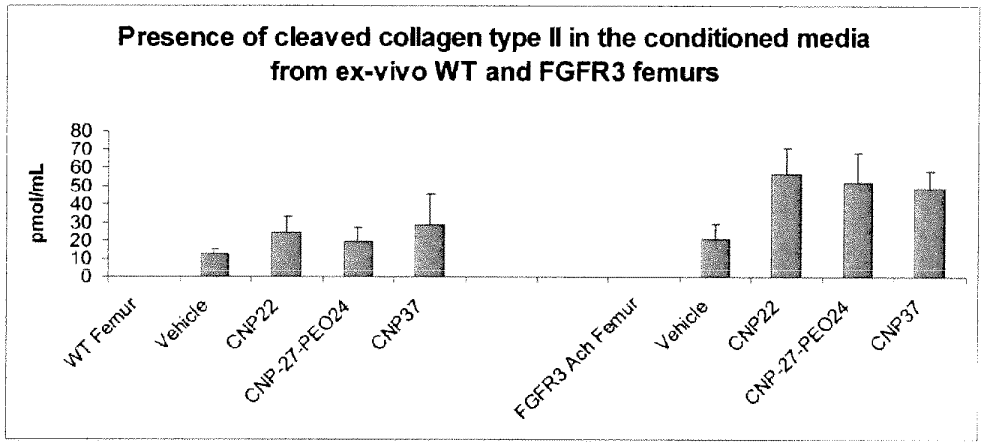


FIGURE 44

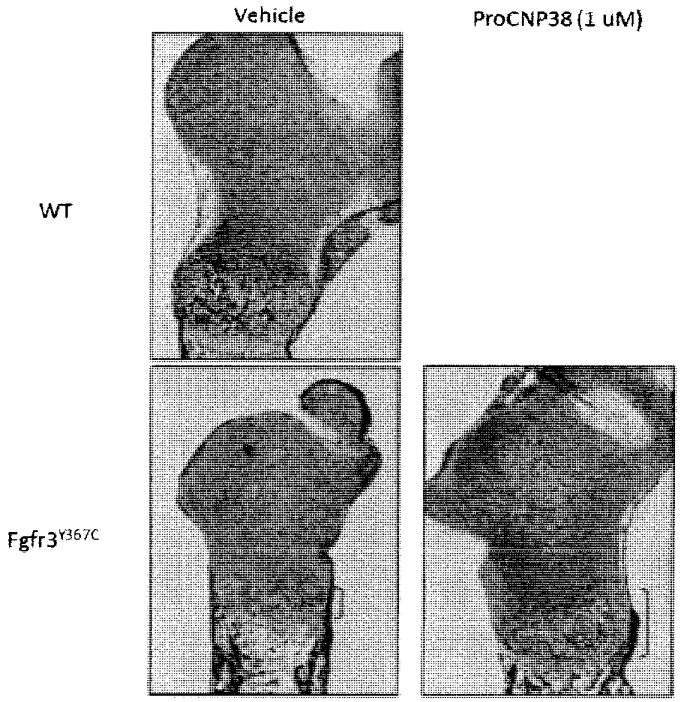


FIGURE 45

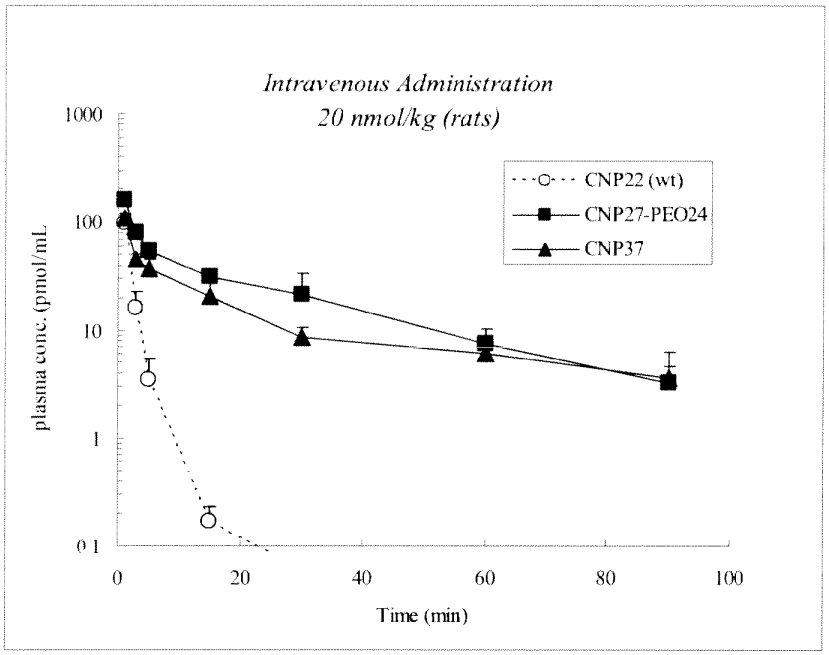


FIGURE 46

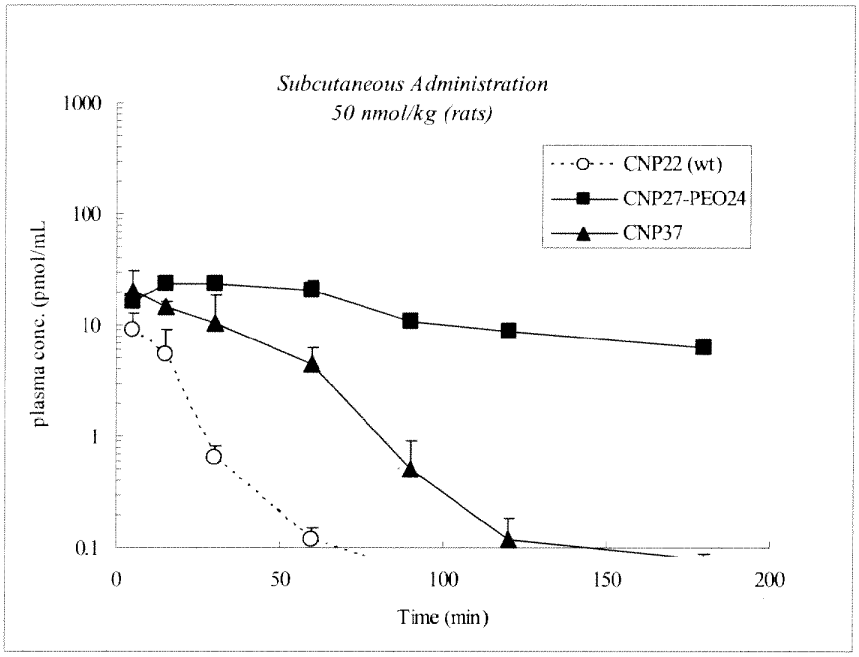


FIGURE 47

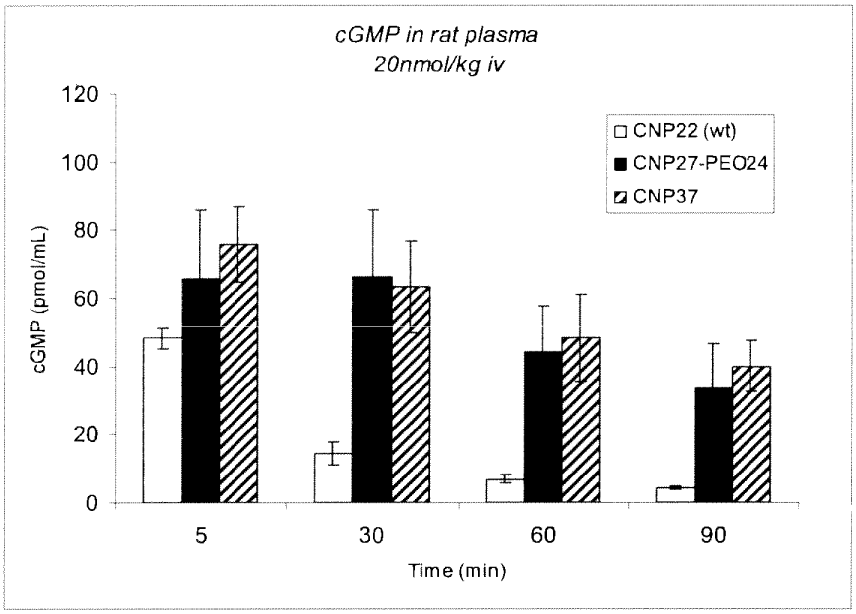


FIGURE 48

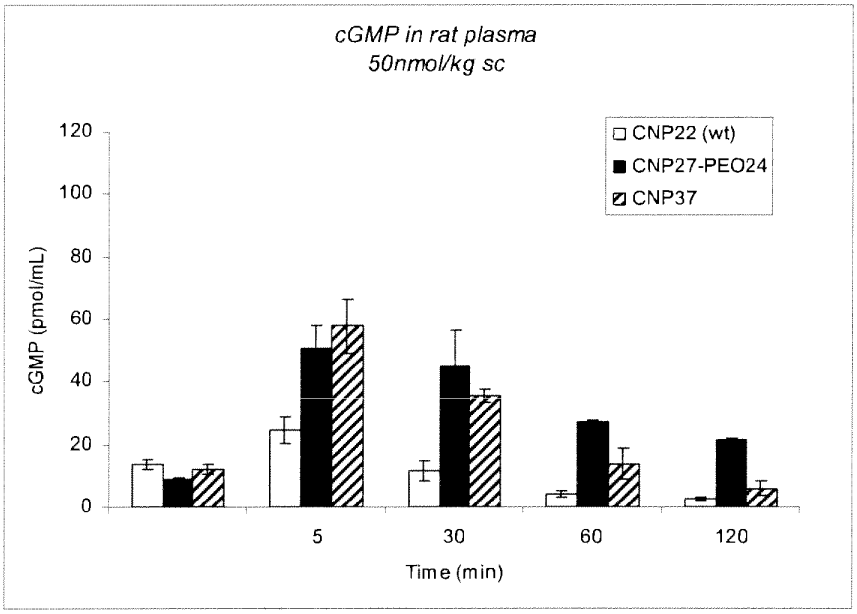


FIGURE 49

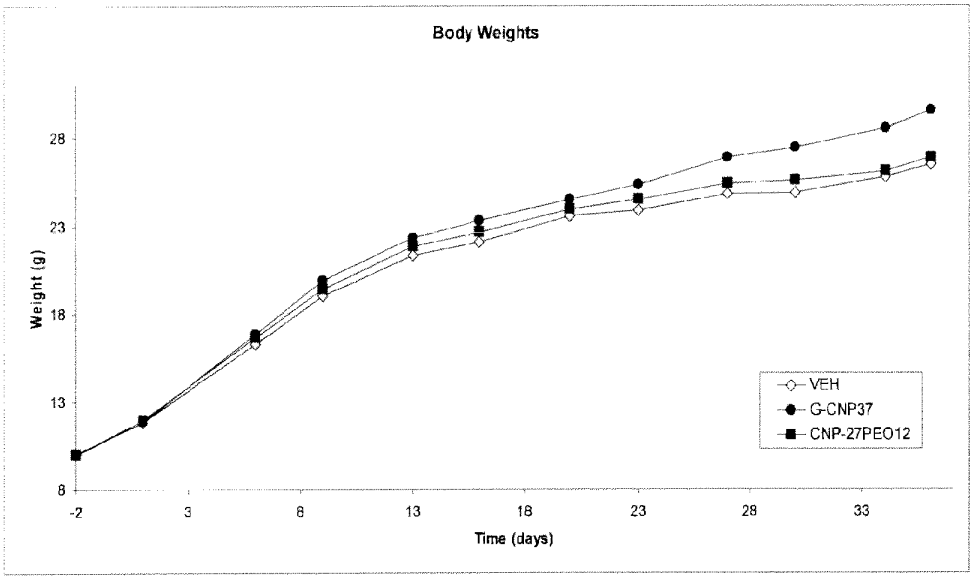


FIGURE 50

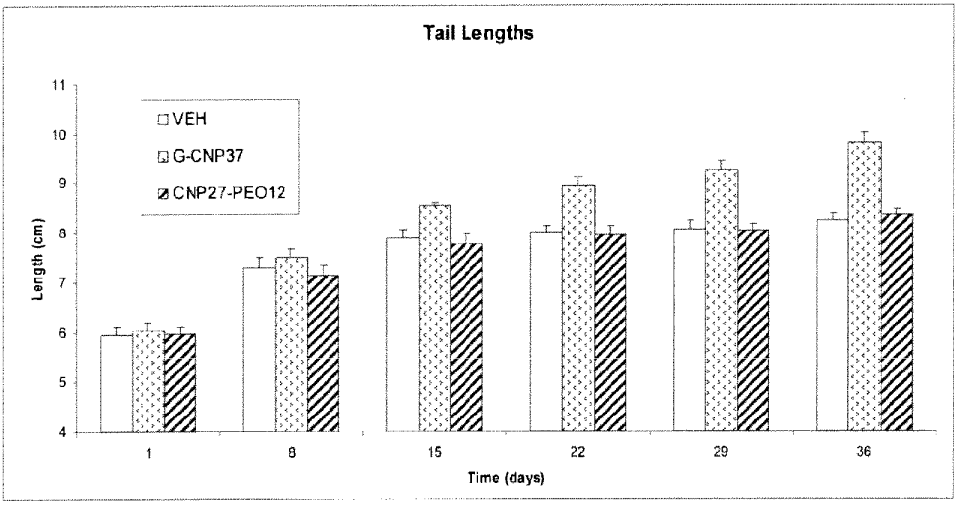


FIGURE 51

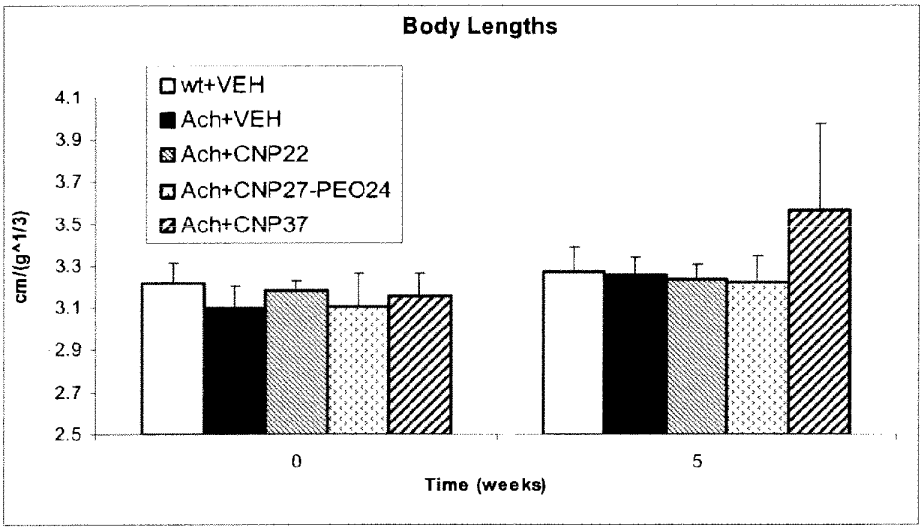


FIGURE 52

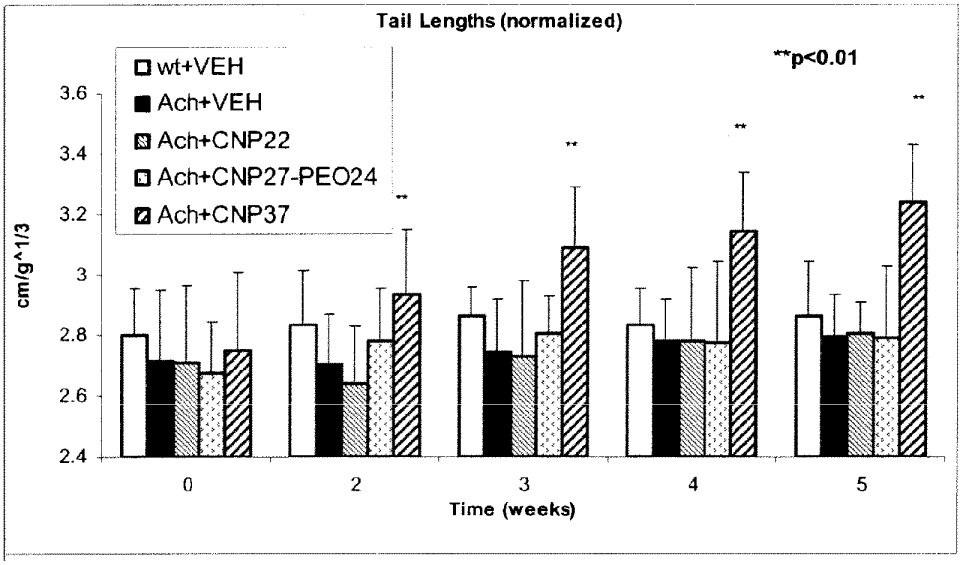
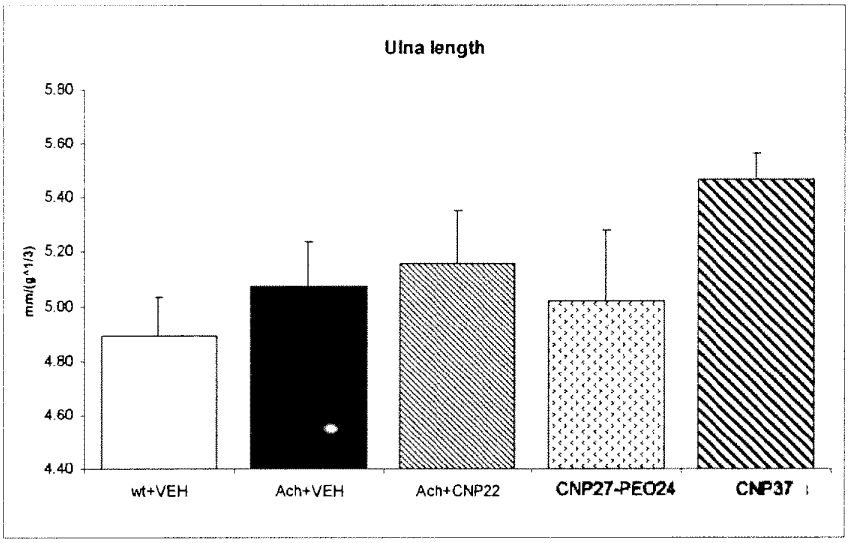


FIGURE 53

A.


Ulna length
5.80
5.60
5.40
5.20
5.00
4.80
4.60
4.40
mm/(g^1/3)
wt+VEH
Ach+VEH
Ach+CNP22
CNP27-PEO24
CNP37

B.

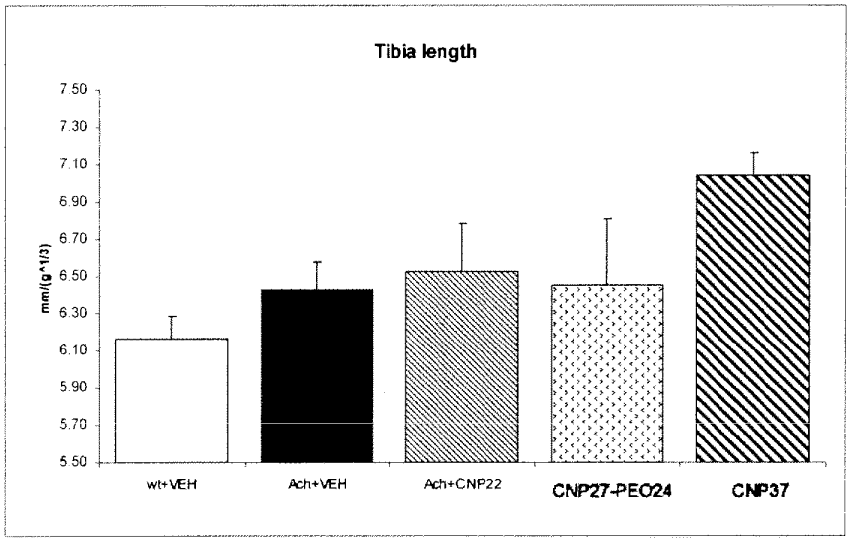
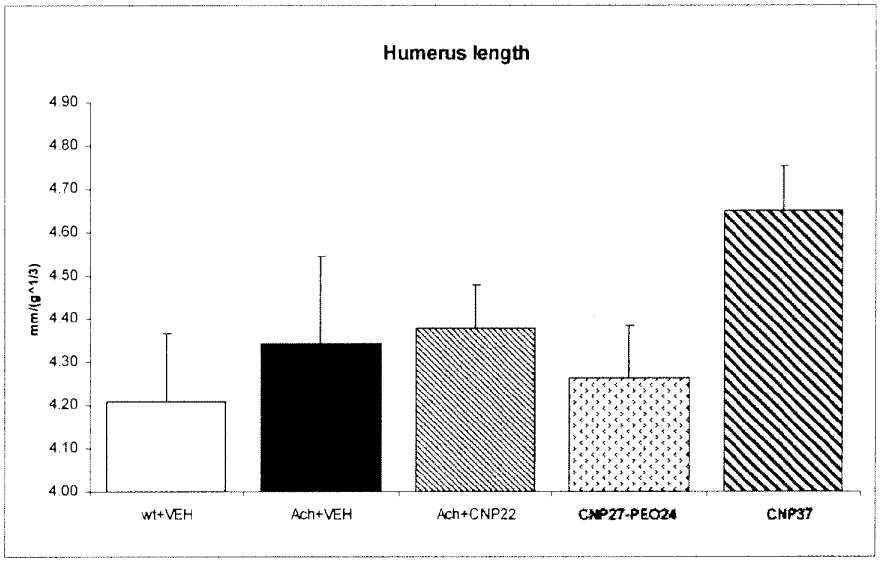

Tibia length
7.50
7.30
7.10
6.90
6.70
6.50
6.30
6.10
5.90
5.70
5.50
mm/(g^1/3)
wt+VEH
Ach+VEH
Ach+CNP22
CNP27-PEO24
CNP37

FIGURE 54

A.


Humerus length
mm/(g^1/3)
4.90
4.80
4.70
4.60
4.50
4.40
4.30
4.20
4.10
4.00
wt+VEH
Ach+VEH
Ach+CNP22
CNP27-PEO24
CNP37

B.

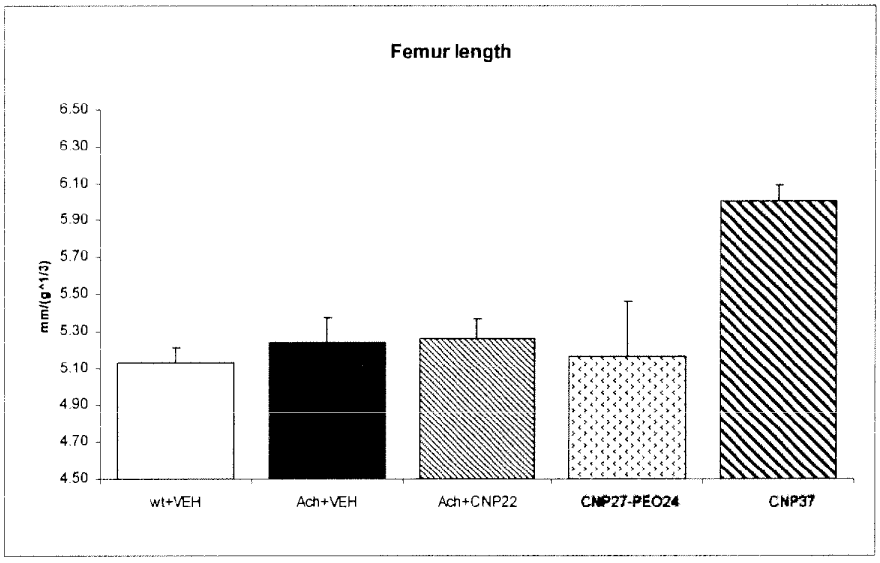

Femur length
mm/(g^1/3)
6.50
6.30
6.10
5.90
5.70
5.50
5.30
5.10
4.90
4.70
4.50
wt+VEH
Ach+VEH
Ach+CNP22
CNP27-PEO24
CNP37

FIGURE 55

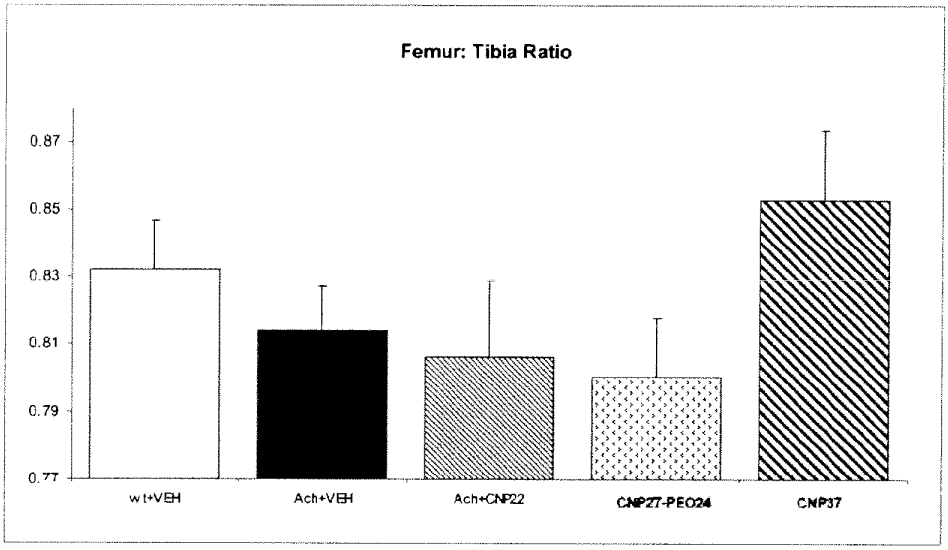


FIGURE 56

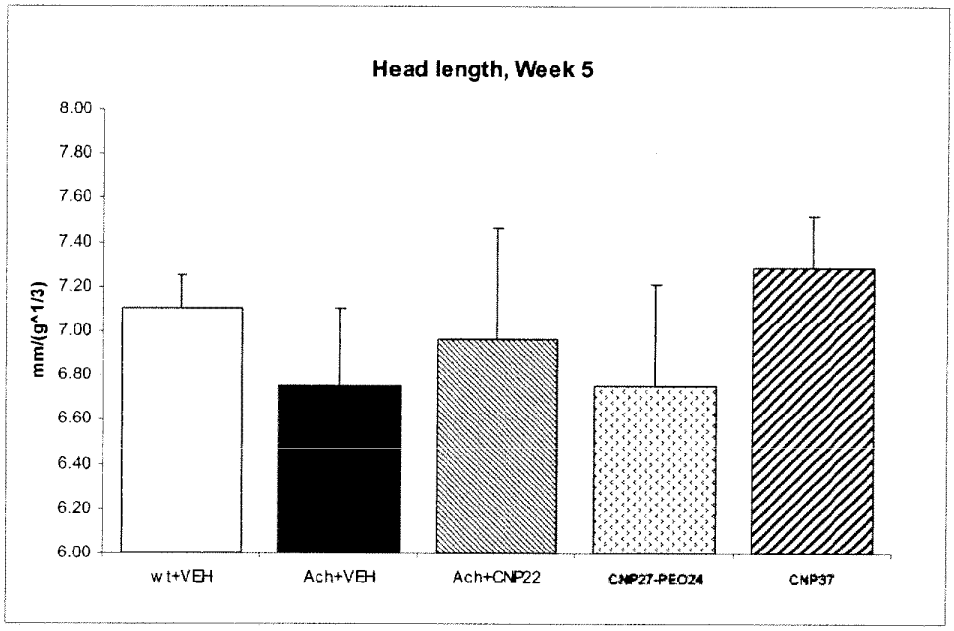


FIGURE 57

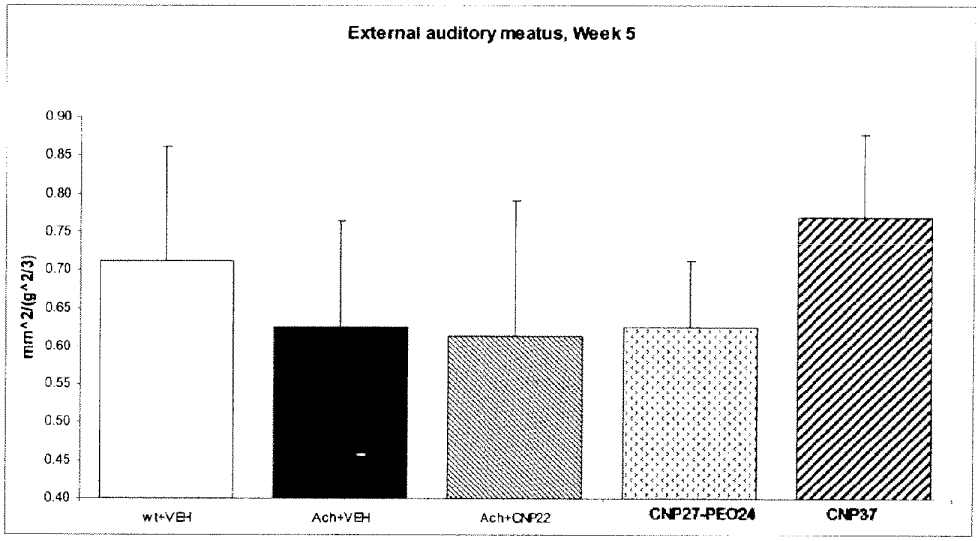


FIGURE 58

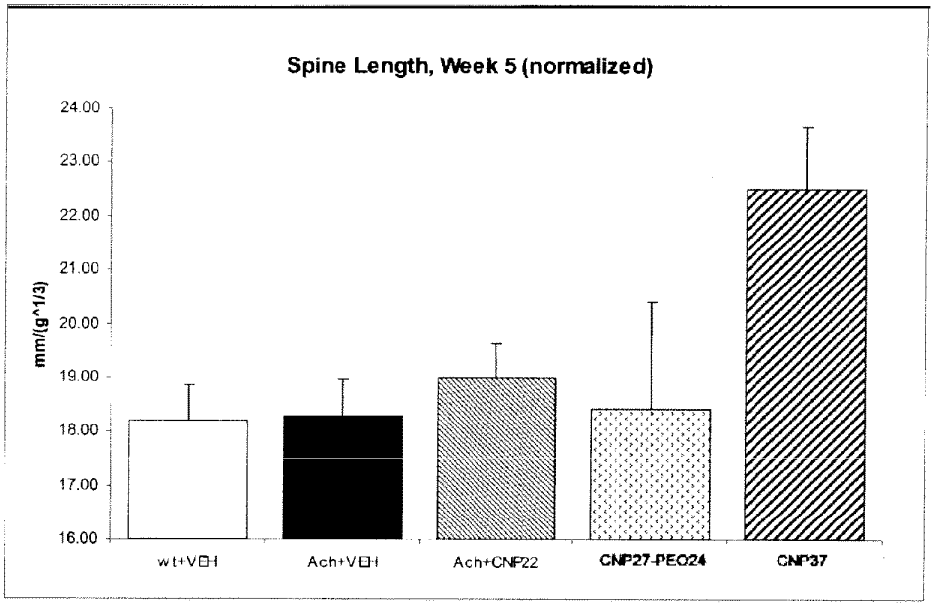


FIGURE 59

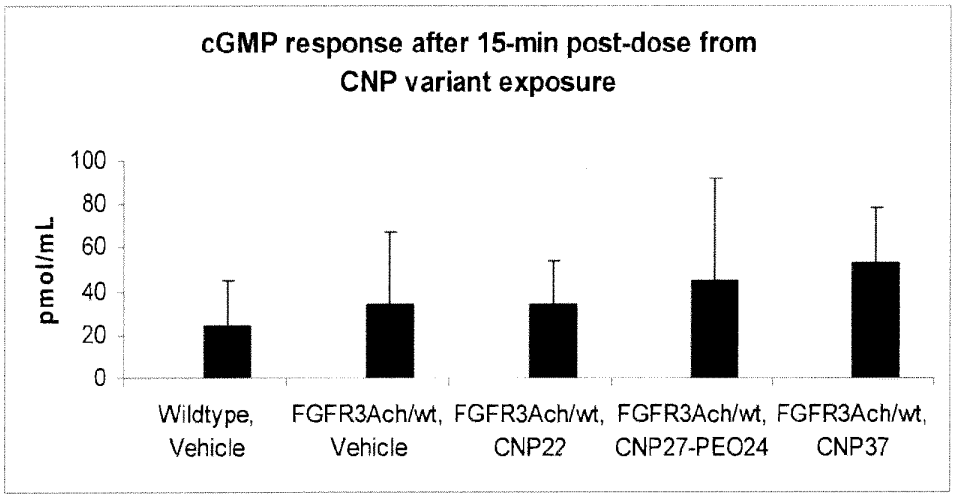


FIGURE 60

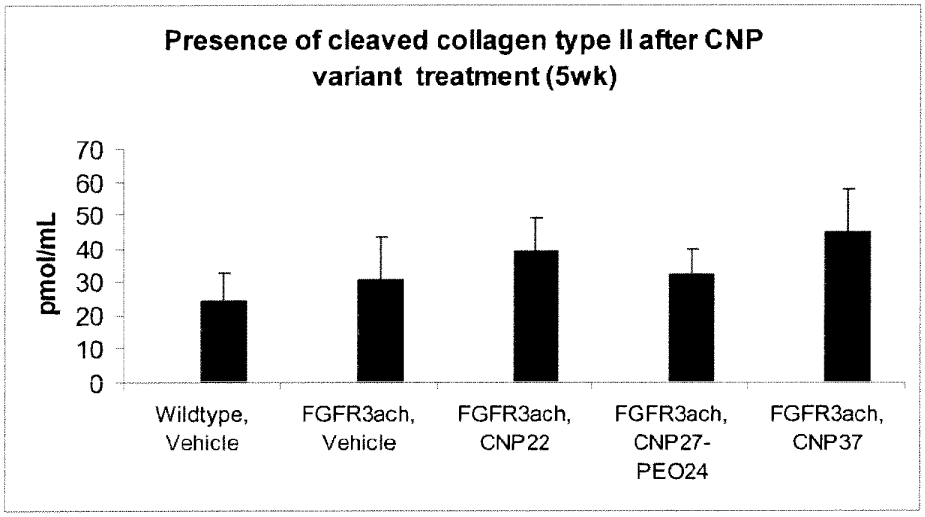


FIGURE 61

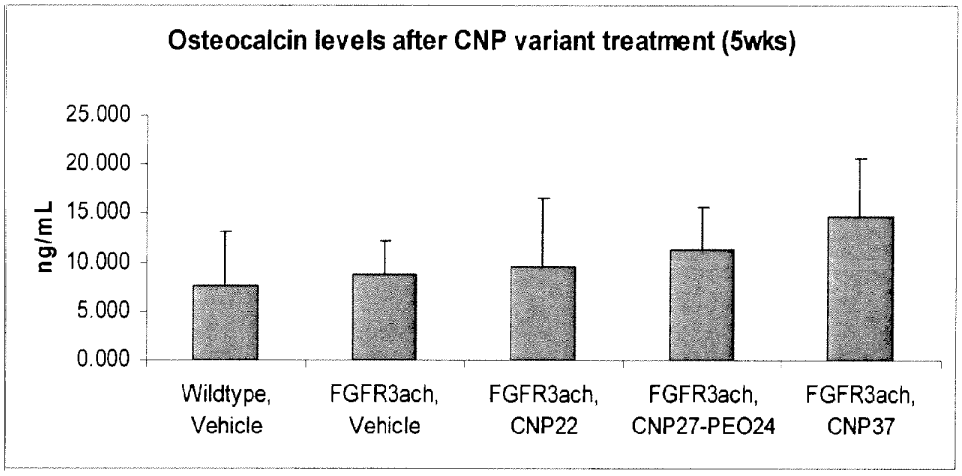


FIGURE 62

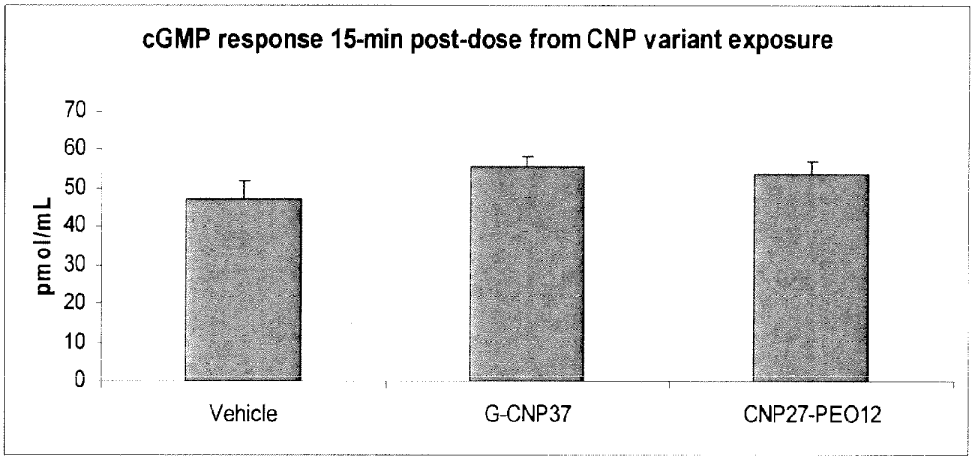


FIGURE 63

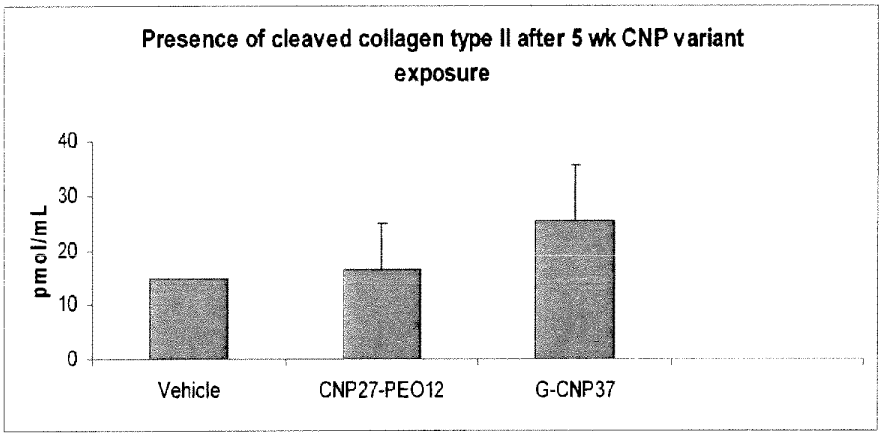


FIGURE 64

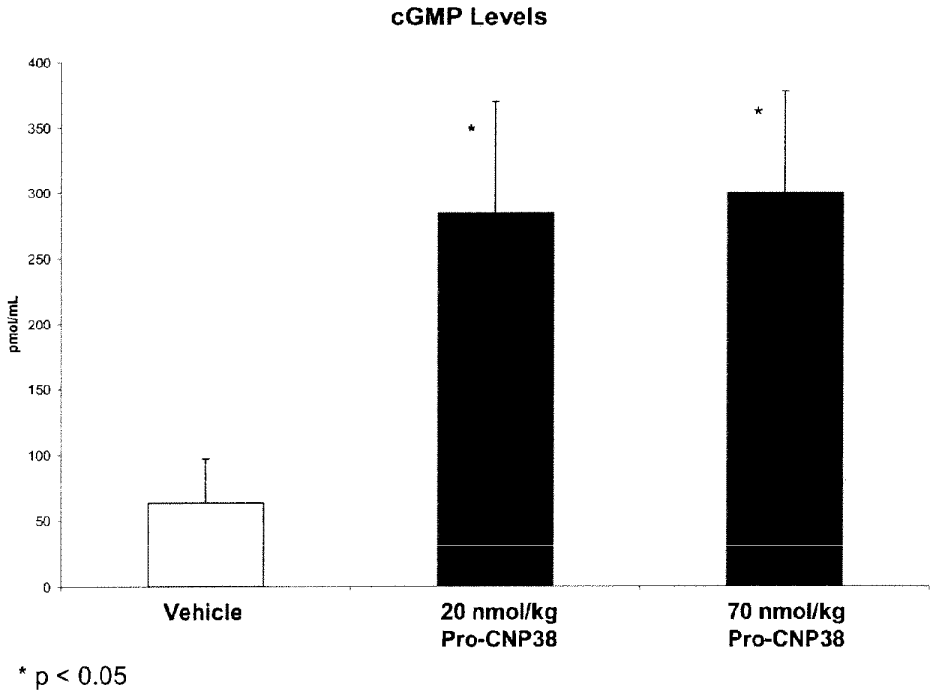


FIGURE 65

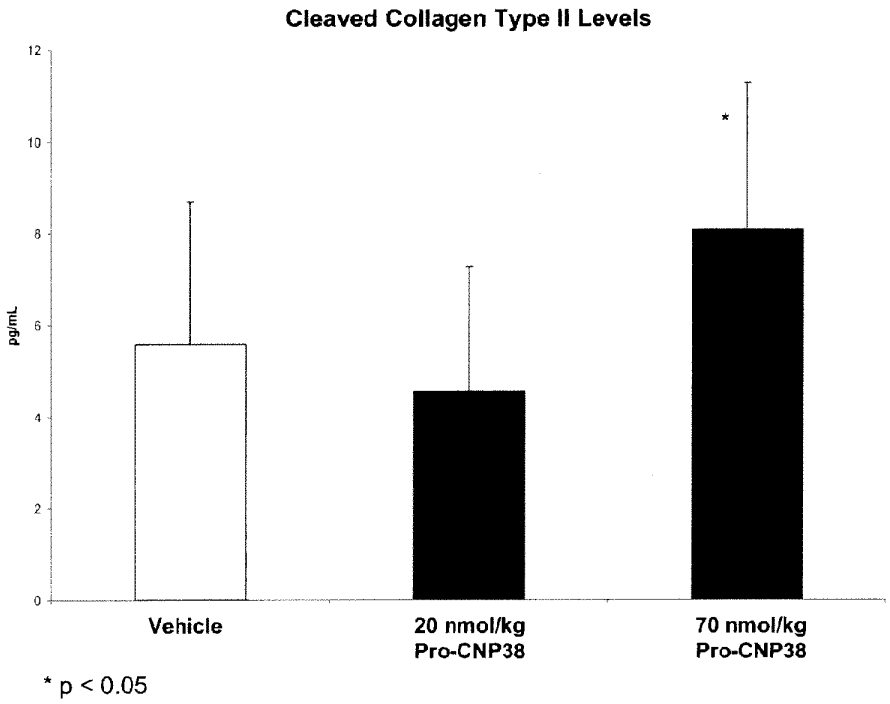

Cleaved Collagen Type II Levels
pg/mL
12
10
8
6
4
2
0
*
Vehicle
20 nmol/kg Pro-CNP38
70 nmol/kg Pro-CNP38
* p < 0.05

FIGURE 66

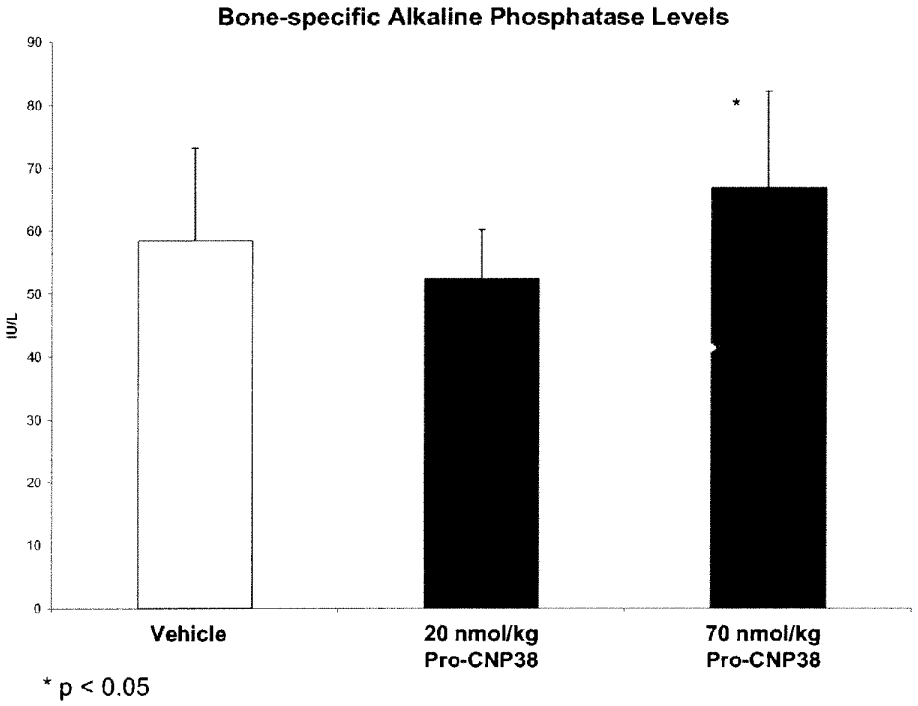

Bone-specific Alkaline Phosphatase Levels
IU/L
90
80
70
60
50
40
30
20
10
0
*
Vehicle
20 nmol/kg Pro-CNP38
70 nmol/kg Pro-CNP38
* p < 0.05

FIGURE 67

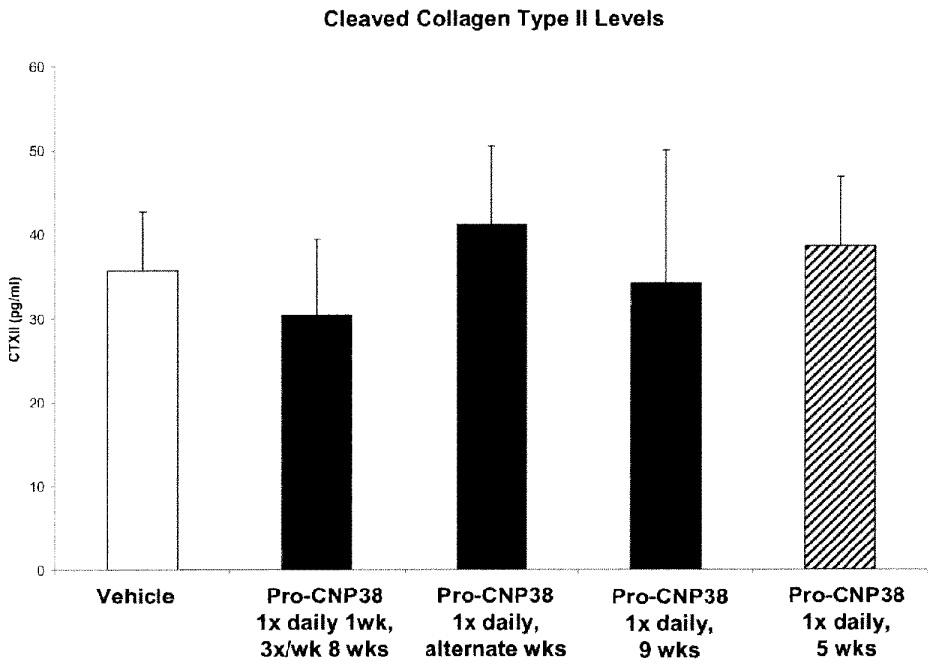

Cleaved Collagen Type II Levels
CTXII (pg/ml)
60
50
40
30
20
10
0
Vehicle
Pro-CNP38 1x daily 1wk, 3x/wk 8 wks
Pro-CNP38 1x daily, alternate wks
Pro-CNP38 1x daily, 9 wks
Pro-CNP38 1x daily, 5 wks

FIGURE 68

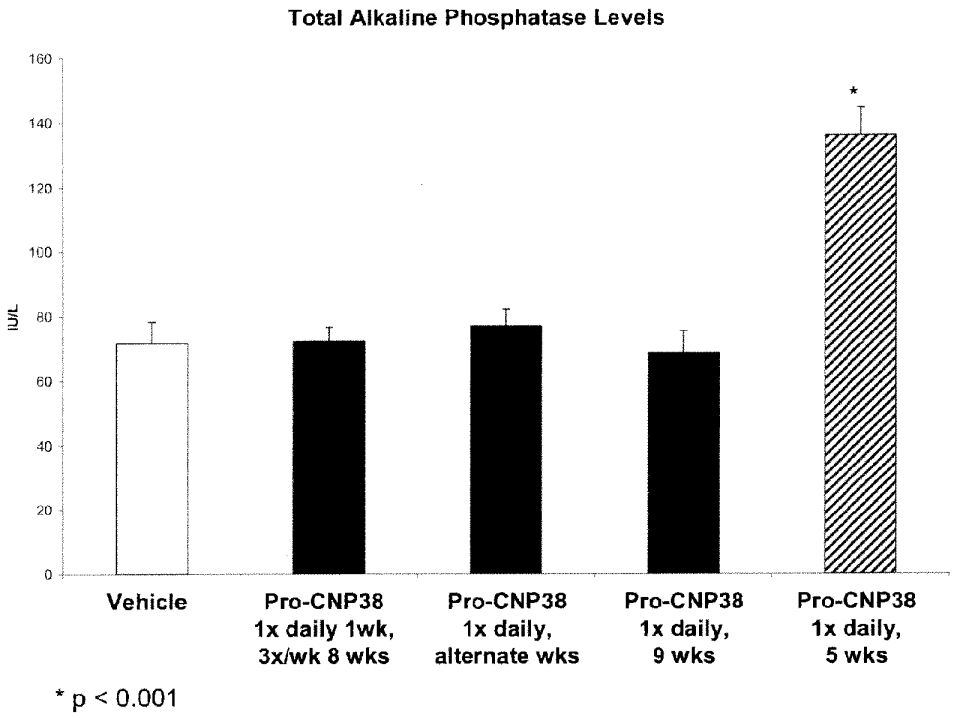

Total Alkaline Phosphatase Levels
IU/L
160
140
120
100
80
60
40
20
0
*
Vehicle
Pro-CNP38 1x daily 1wk, 3x/wk 8 wks
Pro-CNP38 1x daily, alternate wks
Pro-CNP38 1x daily, 9 wks
Pro-CNP38 1x daily, 5 wks
* p < 0.001

FIGURE 69

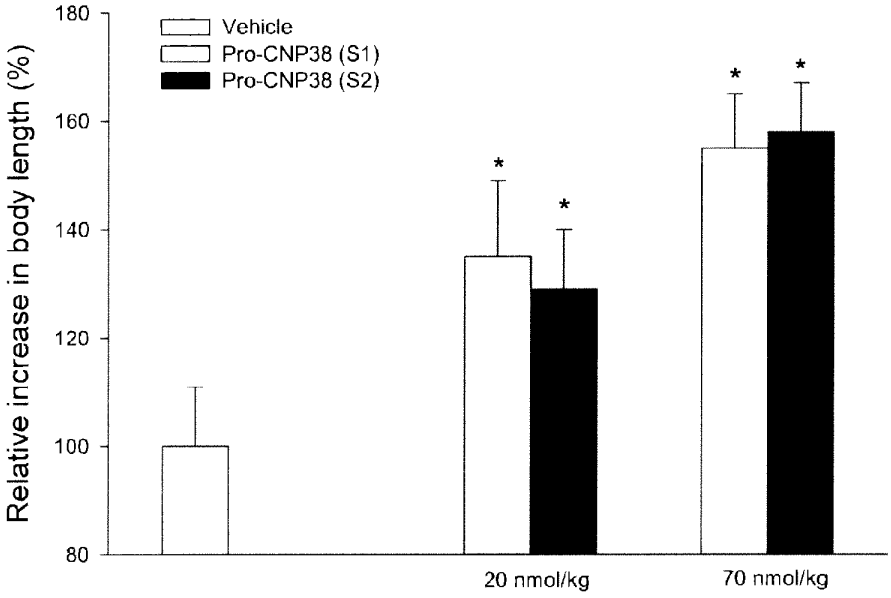
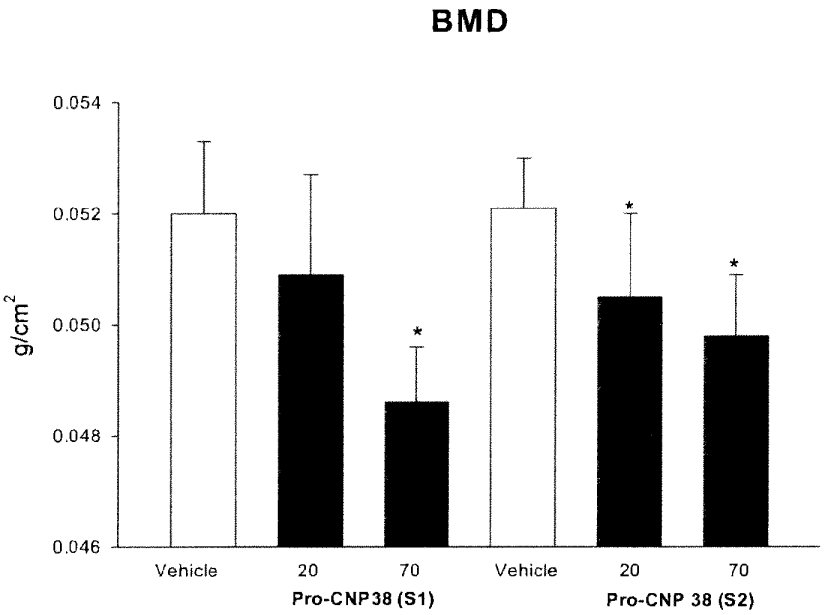

Vehicle
Pro-CNP38 (S1)
Pro-CNP38 (S2)
Relative increase in body length (%)
180
160
140
120
100
80
*
*
*
*
20 nmol/kg
70 nmol/kg

FIGURE 70

A.


BMD
0.054
0.052
0.050
0.048
0.046
g/cm2
*
*
*
Vehicle
20
70
Vehicle
20
70
Pro-CNP38 (S1)
Pro-CNP 38 (S2)

B.

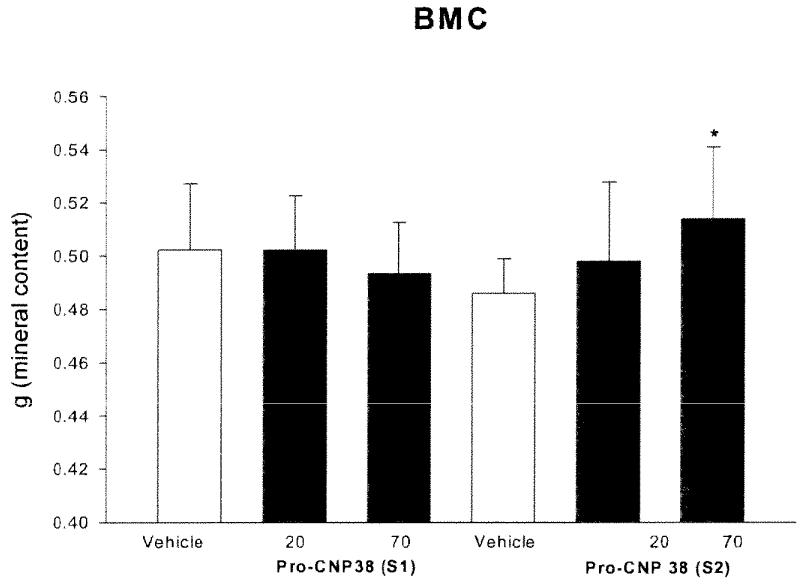

BMC
0.56
0.54
0.52
0.50
0.48
0.46
0.44
0.42
0.40
g (mineral content)
*
Vehicle
20
70
Vehicle
20
70
Pro-CNP38 (S1)
Pro-CNP 38 (S2)

FIGURE 71

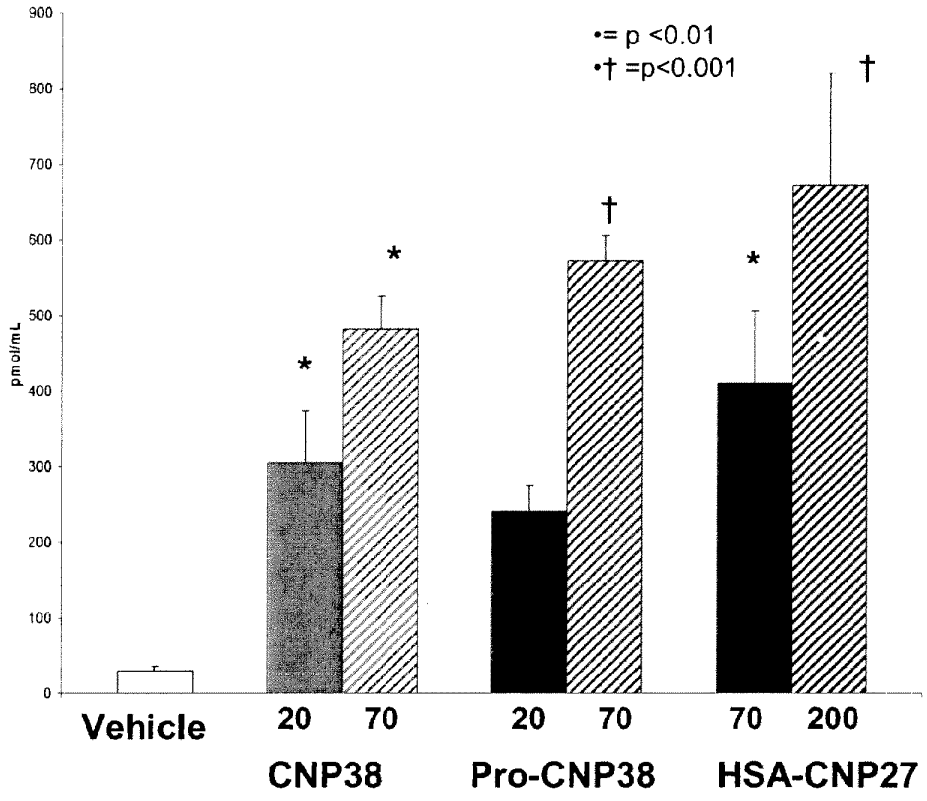

•= p <0.01
•† =p<0.001
pmol/mL
900
800
700
600
500
400
300
200
100
0
*
*
†
*
†
Vehicle
20
70
20
70
70
200
CNP38
Pro-CNP38
HSA-CNP27

FIGURE 72

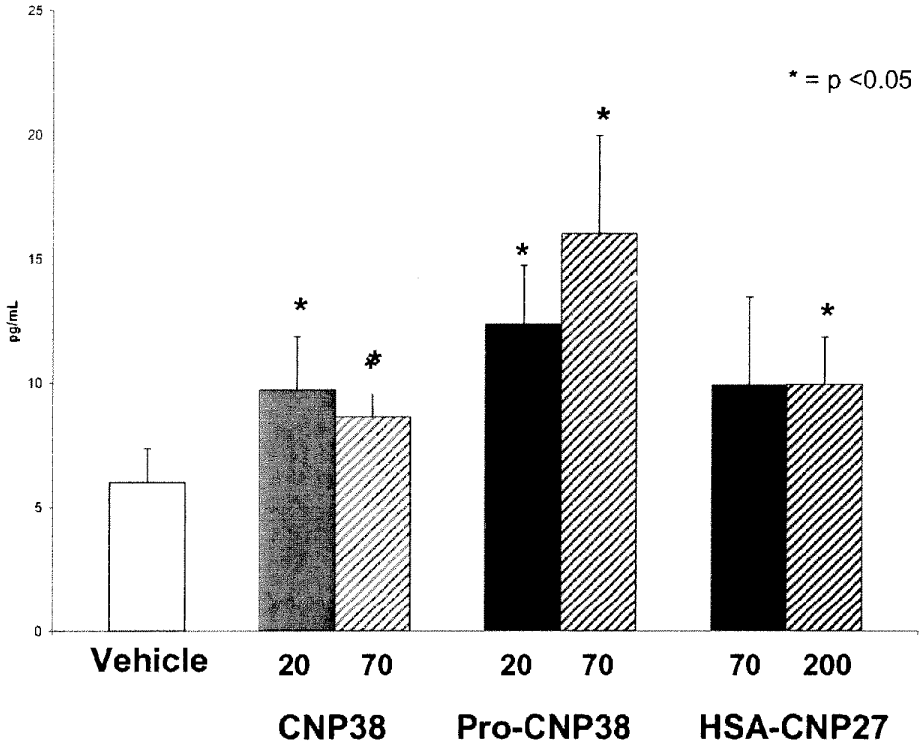

25
20
15
10
5
0
pg/mL
* = p <0.05
*
*
*
*
*
Vehicle
20
70
20
70
70
200
CNP38
Pro-CNP38
HSA-CNP27

FIGURE 73

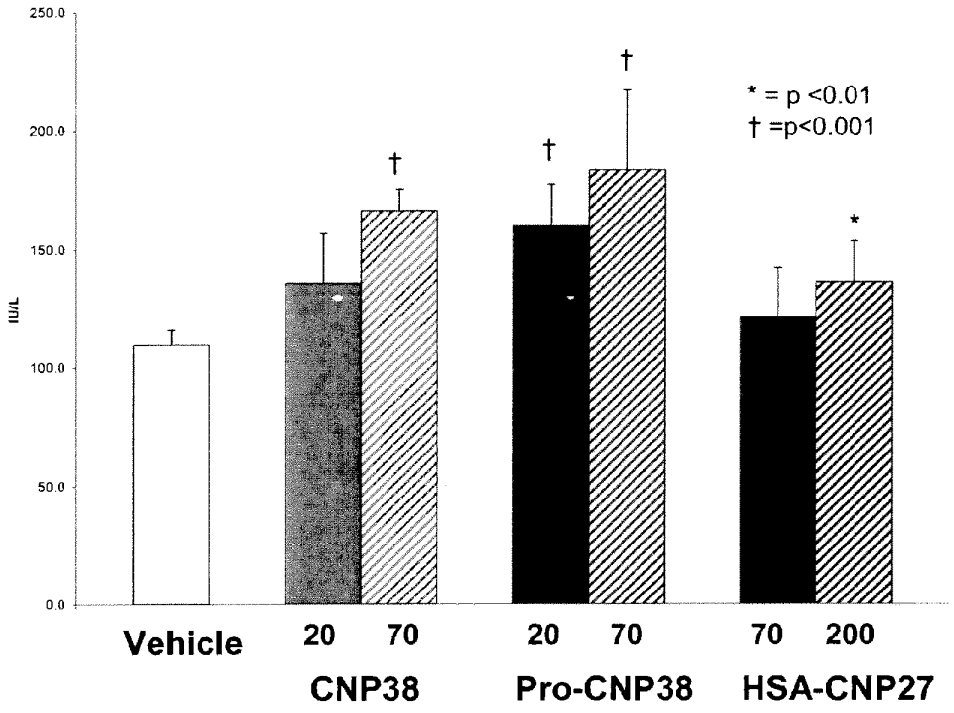
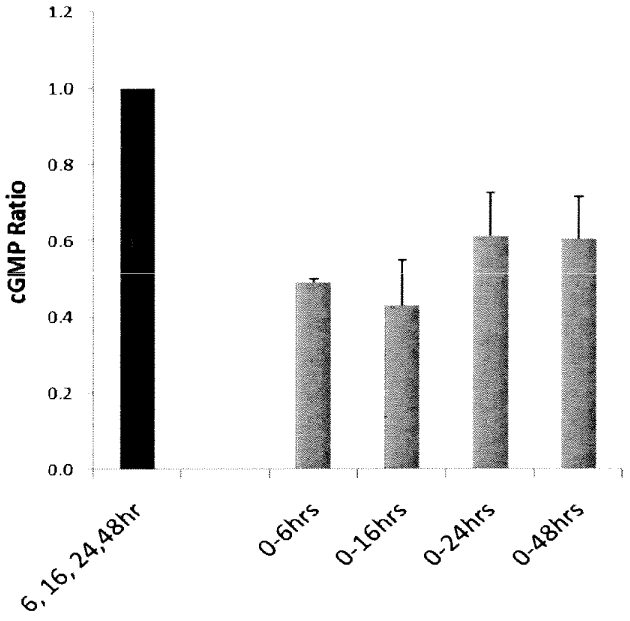

250.0
200.0
150.0
100.0
50.0
0.0
IU/L
* = p <0.01
† =p<0.001
†
†
†
*
Vehicle
20
70
20
70
70
200
CNP38
Pro-CNP38
HSA-CNP27

FIGURE 74

A.


1.2
1.0
0.8
0.6
0.4
0.2
0.0
cGMP Ratio
6, 16, 24, 48hr
0-6hrs
0-16hrs
0-24hrs
0-48hrs

B.

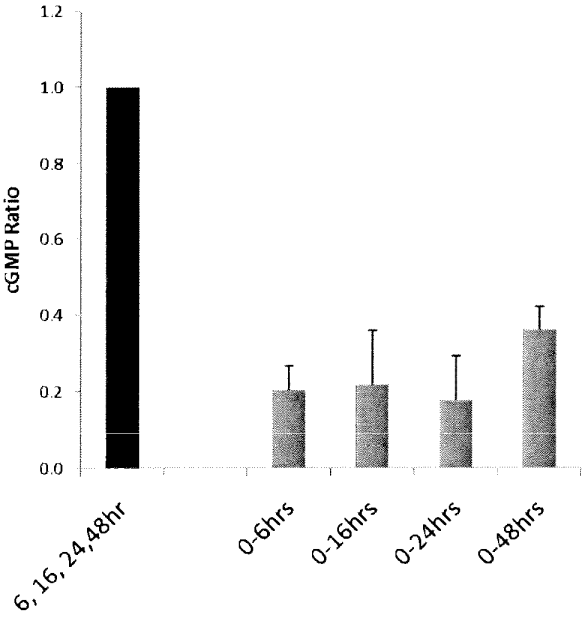

1.2
1.0
0.8
0.6
0.4
0.2
0.0
cGMP Ratio
6, 16, 24, 48hr
0-6hrs
0-16hrs
0-24hrs
0-48hrs

FIGURE 75

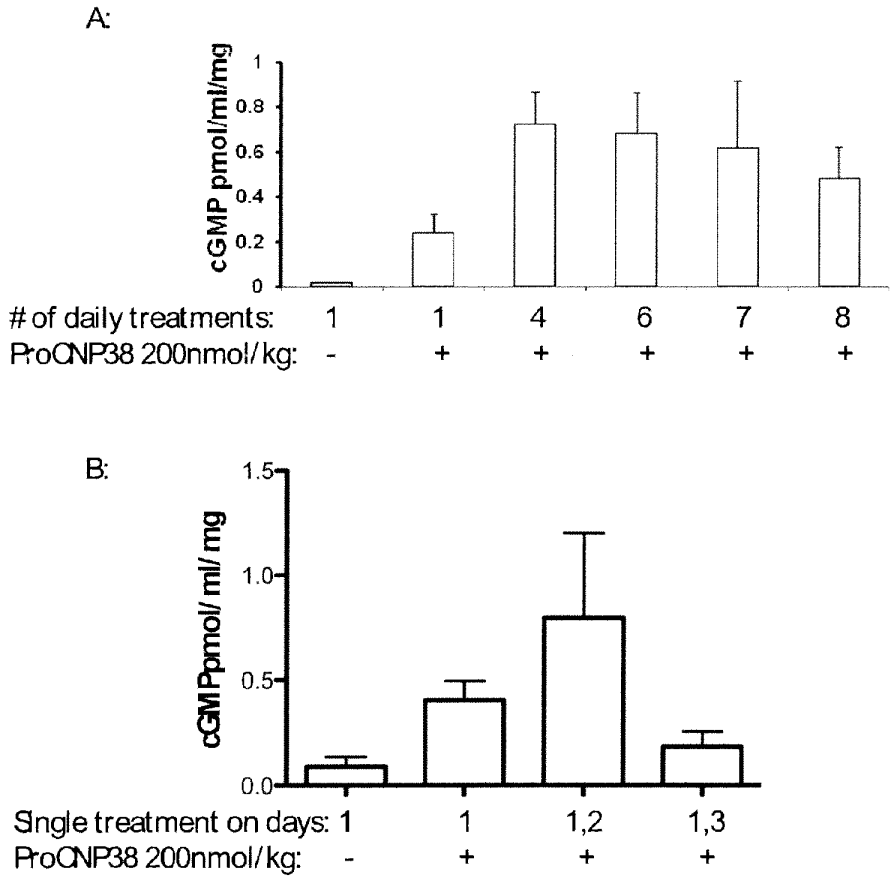

A:
cGMP pmol/ml/mg
1
0.8
0.6
0.4
0.2
0
# of daily treatments: 1 1 4 6 7 8
ProCNP38 200nmol/kg: - + + + + +
B:
cGMPpmol/ml/mg
1.5
1.0
0.5
0.0
Single treatment on days: 1 1 1,2 1,3
ProCNP38 200nmol/kg: - + + +

FIGURE 76

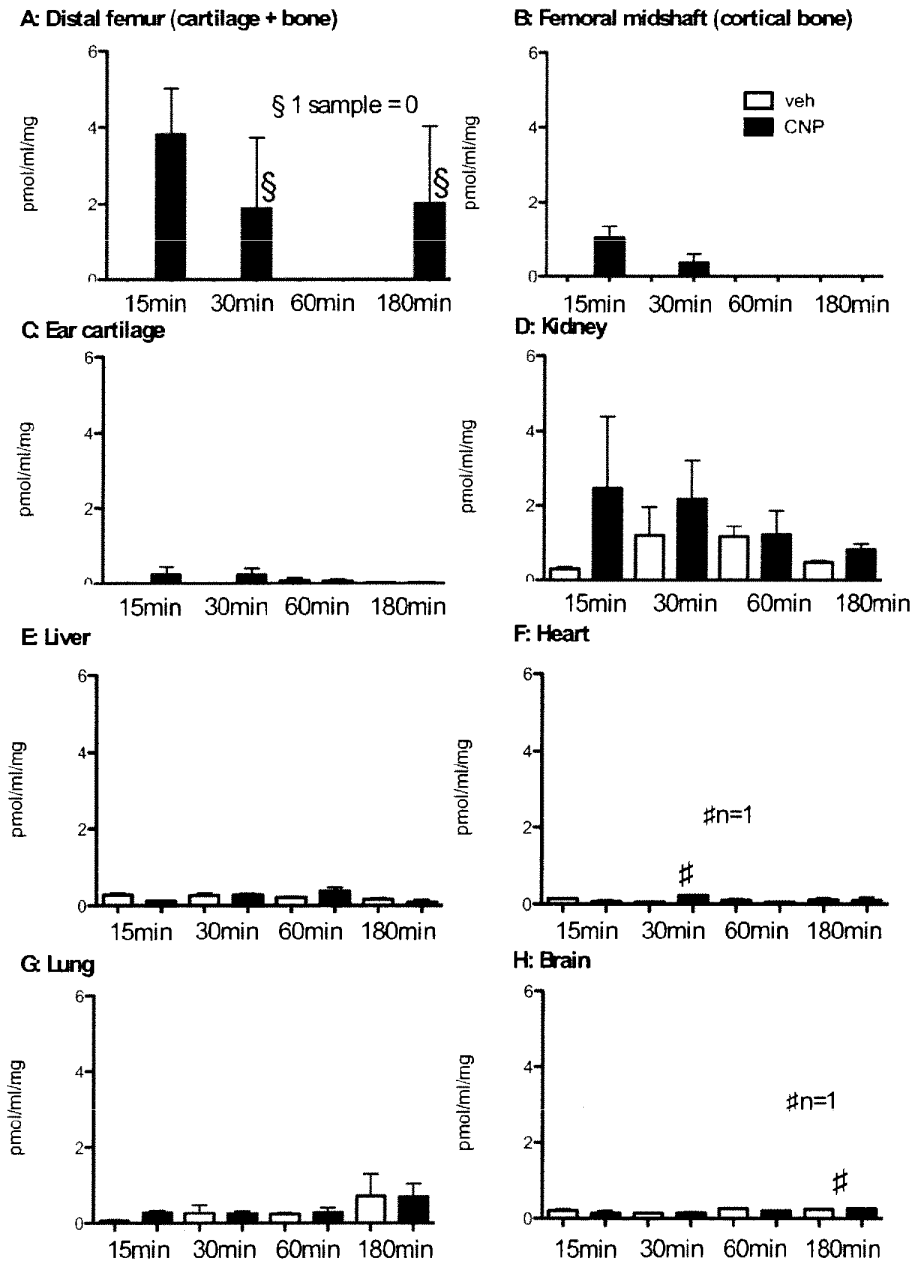

A: Distal femur (cartilage + bone)
§ 1 sample = 0
§
§
pmol/ml/mg
15min 30min 60min 180min
B: Femoral midshaft (cortical bone)
veh
CNP
C: Ear cartilage
D: Kidney
E: Liver
F: Heart
#n=1
#
G: Lung
H: Brain
#n=1
#

FIGURE 77

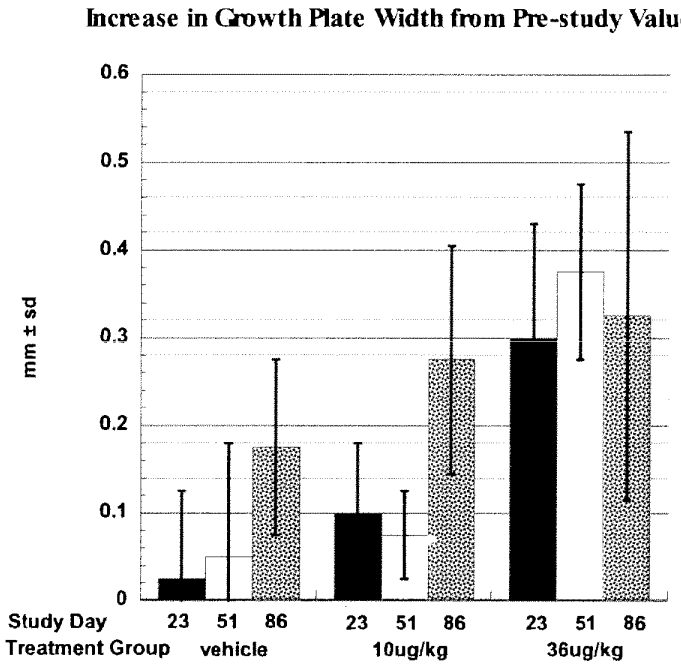


FIGURE 79

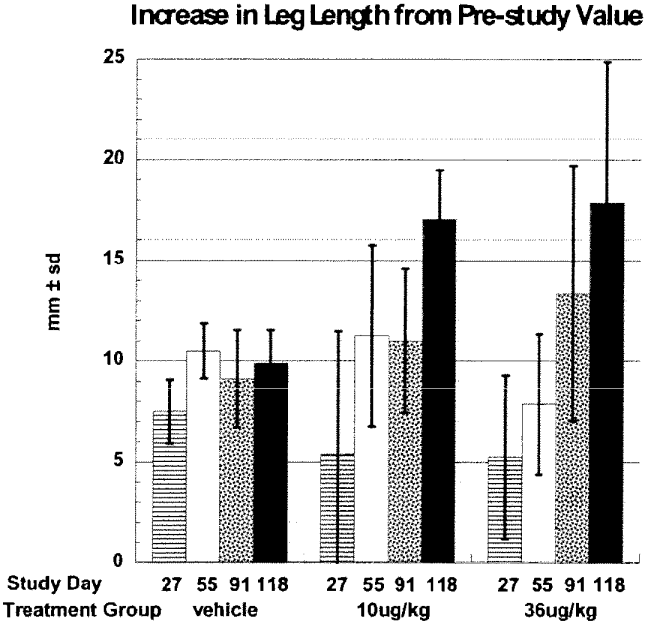
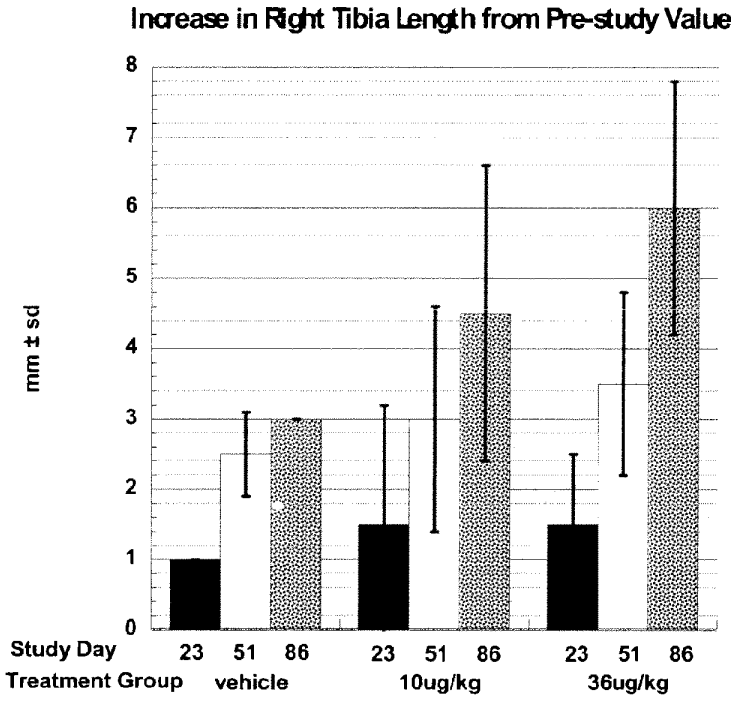


FIGURE 78

A.


Increase in Right Tibia Length from Pre-study Value
mm ± sd
0
1
2
3
4
5
6
7
8
Study Day
23 51 86 23 51 86 23 51 86
Treatment Group
vehicle
10ug/kg
36ug/kg

B.

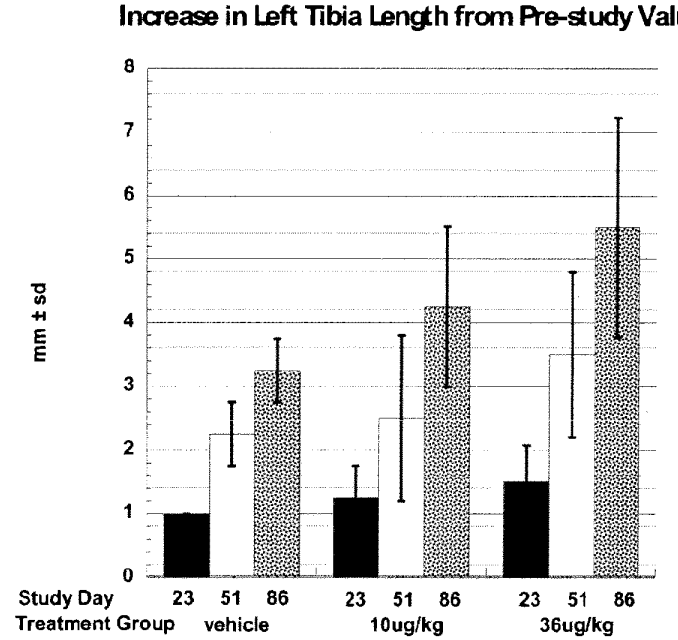

Increase in Left Tibia Length from Pre-study Value
mm ± sd
0
1
2
3
4
5
6
7
8
Study Day
23 51 86 23 51 86 23 51 86
Treatment Group
vehicle
10ug/kg
36ug/kg

FIGURE 80

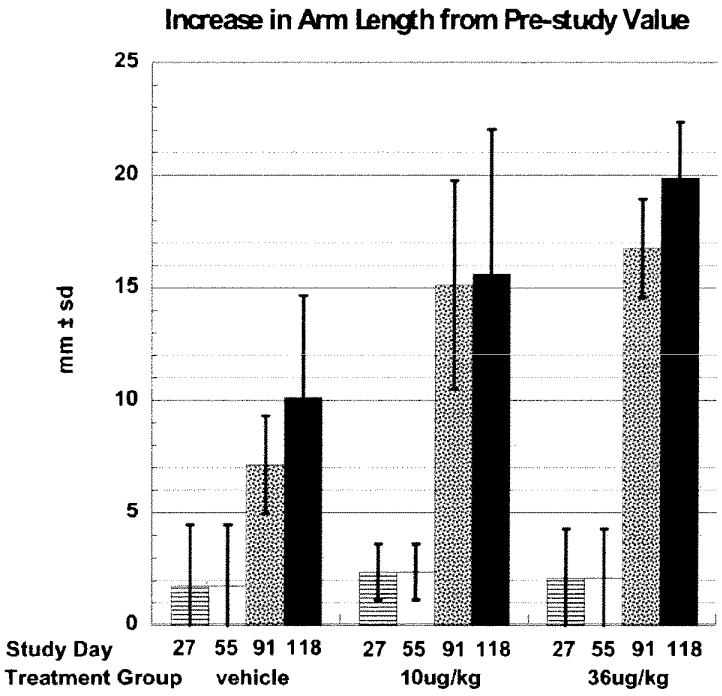


FIGURE 81

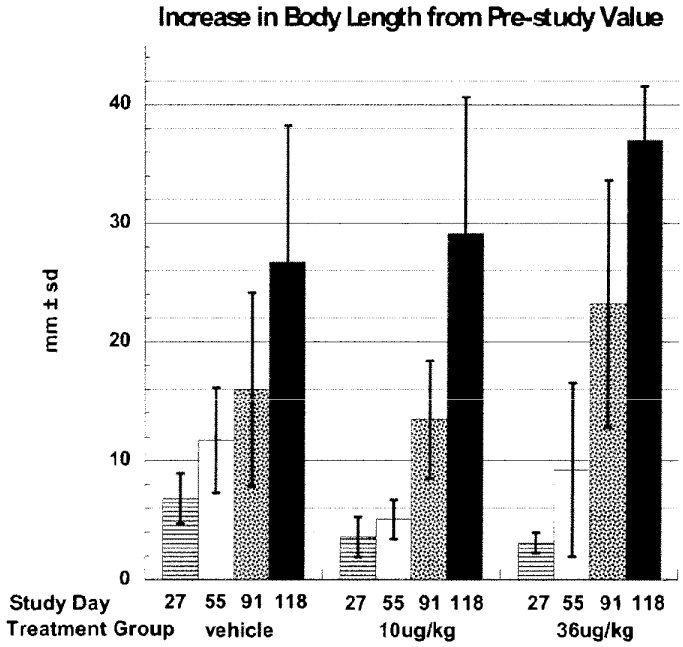


FIGURE 82

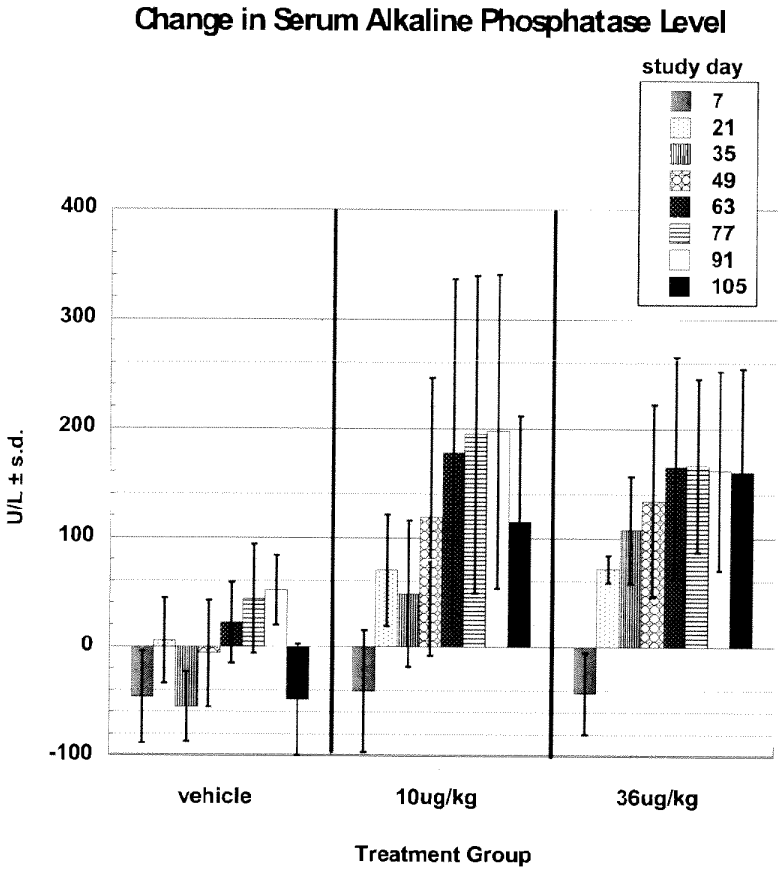
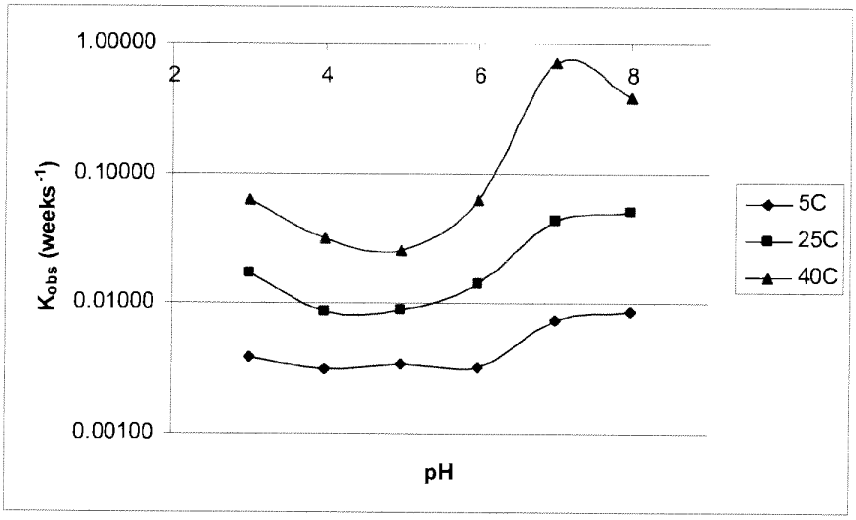


FIGURE 83



VARIANTS OF C-TYPE NATRIURETIC PEPTIDE

Matter enclosed in heavy brackets [ ] appears in the original patent but forms no part of this reissue specification; matter printed in italics indicates the additions made by reissue; a claim printed with strikethrough indicates that the claim was canceled, disclaimed, or held invalid by a prior post-patent action or proceeding.

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application claims the priority and benefit of U.S. Provisional Application No. 61/254,563, filed on Oct. 23, 2009, and U.S. Provisional Application No. 61/180,112, filed on May 20, 2009, the disclosure of each of which is incorporated herein by reference in its entirety.

FIELD OF THE DISCLOSURE

The field of the disclosure relates, in general, to variants of C-type natriuretic peptide (CNP), compositions comprising CNP variants, methods of making CNP variants, and methods of using CNP variants to treat disorders responsive to CNP, including but not limited to bone-related disorders such as skeletal dysplasias (e.g., achondroplasia) and vascular smooth muscle disorders.

BACKGROUND OF THE DISCLOSURE

The natriuretic peptide family consists of three structurally related peptides: atrial natriuretic peptide (ANP) (Genbank Accession No. NP_006163, for the ANP precursor protein, NPPA), brain natriuretic peptide (BNP) (GenBank Accession No. NP_002512, for the BNP precursor protein, NPPB), and C-type natriuretic peptide (CNP) (Biochem. Biophys. Res. Commun., 168: 863-870 (1990) (GenBank Accession No. NP_077720, for the CNP precursor protein, NPPC) (J. Hypertens., 10: 907-912 (1992)). These small, single chain peptides (ANP, BNP, CNP) have a 17-amino acid loop structure (Levin et al., N. Engl. J. Med., 339: 863-870 (1998)) and have important roles in multiple biological processes. ANP and BNP bind to and activate the natriuretic peptide receptor A (NPR-A), also termed guanalyl cyclase A (GC-A), resulting in higher intracellular cyclic guanosine monophosphate (cGMP) levels. Likewise, CNP interacts with NPR-B (GC-B) to stimulate the generation of cGMP (J. Hypertens., 10: 1111-1114 (1992)). A third type of receptor, NPR-C, binds each of the natriuretic peptides with high affinity and functions primarily to capture the peptides from the extracellular compartment and deposit the peptides into lysosomes, where they are degraded (Science, 238: 675-678 (1987)). ANP and BNP are produced primarily within the muscle cells of the heart, and are believed to have important roles in cardiovascular homeostasis (Science, 252: 120-123 (1991)). CNP is expressed more widely, including in the central nervous system, reproductive tract, bone and endothelium of blood vessels (Hypertension, 49: 419-426 (2007)).

In humans, CNP is initially produced from the natriuretic peptide precursor C(NPPC) gene as a single chain 126-amino acid pre-pro polypeptide (Biochem. Biophys. Res. Commun., 168: 863-870 (1990)). Removal of the signal peptide yields pro-CNP, and further cleavage by the endoprotease furin generates an active 53-amino acid peptide

(CNP-53), which is secreted and cleaved again by an unknown enzyme to produce the mature 22-amino acid peptide (CNP-22) (Wu, J. Biol. Chem. 278: 25847-852 (2003)). CNP-53 and CNP-22 differ in their distribution, with CNP-53 predominating in tissues, while CNP-22 is mainly found in plasma and cerebrospinal fluid (J. Alfonzo, Recept. Signal. Transduct. Res., 26: 269-297 (2006)). The predominant CNP form in cartilage is unknown. Both CNP-53 and CNP-22 bind similarly to NPR-B. Furthermore, they both induce cGMP production in a dose-dependent and similar fashion (V T Yeung, Peptides, 17: 101-106 (1996)).

Natural CNP gene and polypeptide have been previously described. U.S. Pat. No. 5,352,770 discloses isolated and purified CNP-22 from porcine brain identical in sequence to human CNP and its use in treating cardiovascular indications. U.S. Pat. No. 6,034,231 discloses the human gene and polypeptide of proCNP (126 amino acids) and the human CNP-53 gene and polypeptide.

Clearance of CNP from the extracellular space occurs through the action of membrane-bound neutral endopeptidase (NEP), which rapidly degrades CNP (Biochem. J., 291 (Pt 1): 83-88 (1993)), and through NPR-C, which binds to and deposits CNP into lysosomes, where CNP is degraded. CNP has been shown to have an in vivo half-life of 2.6 min in the normal human (J. Clin. Endocrinol. Metab., 78: 1428-35 (1994)). The low plasma concentration of CNP (J. Bone Moner. Res., 19 (Suppl. 1) S20 (2004)) and its co-expression with NPR-B in a number of tissues suggests that CNP functions primarily through an autocrine/paracrine mechanism.

As stated above, CNP binds to and activates natriuretic peptide receptor B (NPR-B), also termed guanylyl cyclase B (GC-B), resulting in higher intracellular cyclic guanosine monophosphate (cGMP) levels. Downstream signaling mediated by cGMP generation influences a diverse array of biological processes that include endochondral ossification. Accordingly, elevated or depressed levels of any of the components in this pathway may lead to aberrant bone growth. For example, knockout of either CNP or NPR-B in mouse models results in animals having a dwarfed phenotype with shorter long bones and vertebrae. Mutations in human NPR-B that block proper CNP signaling have been identified and result in dwarfism (Olney, et al., J. Clin. Endocrinol. Metab. 91(4): 1229-1232 (2006); Bartels, et al., Am. J. Hum. Genet. 75: 27-34 (2004)). In contrast, mice engineered to produce elevated levels of CNP display elongated long bones and vertebrae.

Achondroplasia is a result of an autosomal dominant mutation in the gene for fibroblast growth factor receptor 3 (FGFR-3), which causes an abnormality of cartilage formation. FGFR-3 normally has a negative regulatory effect on chondrocyte growth, and hence bone growth. In achondroplasia, the mutated form of FGFR-3 is constitutively active, which leads to severely shortened bones. Both chondrocyte proliferation and differentiation appear to be disturbed, leading to remarkably short growth plate cartilage (P. Krejci et al., J. Cell Sci. 118: 5089-5100 (2005)). Endochondral ossification is the process that governs longitudinal long-bone growth. There are four zones of the growth plate—resting, proliferative, hypertrophic and zone of calcification. In the growth plate, NPR-B is expressed by proliferative cells while NPR-C is expressed by hypertrophic cells (Yamashite et al., J. Biochem. 127: 177-179 (2000)). In normal endochondral bone growth, chondrocytes organize in columns and proliferate in the proliferative zone of the growth plate. These columns are disorganized in achondroplasia patients. Additionally, the hypertrophic zone is where the

cells become large and eventually apoptose (lyse), leading to osteocyte invasion and mineralization. The hypertrophic chondrocytes and the overall size of the zone are much smaller in achondroplasia patients than in normal patients. CNP is an agonist for NPR-B, a positive regulator of chondrocyte and bone growth. Downstream signaling of CNP/NPR-B inhibits the FGFR-3 pathway at the level of mitogen-activated protein kinase (MAP K). Inhibition at MAP K promotes proliferation and differentiation of the chondrocytes in the proliferative and hypertrophic zones of the growth plate, resulting in bone growth.

In humans activating mutations of FGFR-3 are the primary cause of genetic dwarfism. Mice having activated FGFR-3 serve as a model of achondroplasia, the most common form of the skeletal dysplasias, and overexpression of CNP rescues these animals from dwarfism. Accordingly, CNP and functional variants of CNP are potential therapeutics for treatment of the various skeletal dysplasias.

Therapeutic use of CNP is currently limited by its short plasma half-life, which has been shown to be 2.6 minutes in vivo in humans (J. Clin. Endocrinol. Metab., 78: 1428-35 (1994)). To increase CNP concentration above intrinsic levels (about 5 pM) typically found in human plasma, continuous infusion has been necessary in all human and animal studies using systemically administered CNP. A CNP variant having a longer in vivo serum half-life and exhibiting similar or improved activity to that of wild-type CNP is important for a sustainable therapeutic strategy. Two mechanisms by which the half-life of CNP is reduced in human plasma are degradation by neutral endopeptidase (NEP) and clearance by natriuretic peptide receptor C(NPR-C) (Growth Horm. & IGF Res., 16: S6-S14 (2006)). Modifications of peptides reportedly can improve resistance to endopeptidase and exopeptidase cleavage (Amino Acids, 30: 351-367 (2006); Curr. Opin. Biotech., 17: 638-642 (2006)).

The biological activities of various analogs and derivatives of CNP have been evaluated. By substituting S-methyl Cys in place of both Cys_6 and Cys_{22}, cyclization of the peptide via a Cys6-Cys22 disulfide linkage was reportedly shown to be important for the activity of CNP in stimulating cGMP formation (Biochem. Biophys. Res. Comm., 183: 964-969 (1992), also using alanine scanning to identify amino acids important for CNP functionality). A significant additional enhancement of activity reportedly results from the combined presence of the amino acids Leu_9, Lys_{10}, and Leu_{11}. U.S. Pat. No. 5,434,133 describes CNP analogs comprising CNP-22 with substitutions at amino acid position 6, 7, 9, 11, or 22, wherein the amino acid is selected from Cys or Pmp (penta-cyclomercaptopropionic acid) at position 6, Phe, 4-chloro-Phe, 4-fluoro-Phe, 4-nitro-Phe, or Cha (3-cyclohexyl-Ala) at position 7, Gly, Val, Aib, or tLeu at position 9, Leu or Ile at position 11, and Cys or Pmp at position 22.

U.S. Patent Publication No. 2004/0138134 (now U.S. Pat. No. 7,276,481) describes CNP variants comprising amino acids Cys_6 to Cys_{22} of CNP-22 ("CNP-17") which include at least one substitution for another natural amino acid at position 9, 10, 11, 16, 17, 19, or 20, CNP variants with insertions and deletions, such as addition of a His residue at the reported primary site of NEP cleavage, between Cys_6 and Phe_7, and methods of using such variants for increasing the size of a bone growth plate in abnormal bone and elongation of an abnormal bone. However, no significant gains in activity as measured by cGMP production were obtained for these variants, and activity was diminished for nearly all of the variants, as observed in an in vitro cell-based method (Example 7). Further no supportive data, such as for example in vitro stability or in vivo determination of improved pharmacokinetics (PK) were provided to substantiate the asserted NEP resistance and NPR-C resistance of the CNP analogs. U.S. Pat. No. 6,743,425 discloses substances for treating achondroplasia which activate NPR-B/GC-B and are peptides or low molecular weight compounds, including the C-type natriuretic peptides CNP-22 and CNP-53. PCT Publication No. WO 94/20534 discloses a chimera of CNP-22 and the 5-amino acid C-terminus of ANP designated as the vasonatrin peptide (VNP), a limited number of amino acid substitutions and cyclic chimeric peptides that result from formation of a disulfide or double bond.

Approaches for improving the half-life of other natriuretic peptide family members include decreasing the affinity of ANP for NPR-C (U.S. Pat. No. 5,846,932), utilizing pentapeptide antagonists of NPR-C (WO 00/61631), and co-administering NEP inhibitors such as thiorphan and candoxatril (Clin. Exp. Pharma. Physiol., 25: 986-991 (1997), Hyperten., 30: 184-190 (1997)). WO 2004/047871 describes conjugates of BNP and BNP variants to polyalkylene glycol moieties, sugar moieties, polysorbate moieties, polycationic moieties, and other hydrophilic polymer moieties that reportedly exhibit improved half-life in circulation and reportedly are useful for the treatment of acute congestive heart failure.

There have heen no puhlished reports, however, on a successful strategy for making CNP resistant to NEP while retaining its functionality.

SUMMARY OF THE DISCLOSURE

The present disclosure relates to variants of C-type natriuretic peptide (CNP) which are useful in the treatment of bone-related disorders (e.g., achondroplasia) and vascular smooth muscle disorders. The disclosure encompasses CNP variants having increased serum half-life, e.g. as a result of reduced ability to bind to neutral endopeptidase (NEP), greater resistance to proteolysis by NEP and/or reduced affinity to the clearance natriuretic peptide receptor C(NPR-C), while retaining the functionality of CNP.

The wild-type sequence of human CNP-22 (referred to herein as "hCNP22", "wtCNP22" or "CNP22") is set forth below:

(SEQ ID NO: 1)
(N-terminus) Gly_1-Leu_2-Ser_3-Lys_4-Gly_5-Cys_6-Phe_7-Gly_8-Leu_9-Lys_{10}-Leu_{11}-Asp_{12}-Arg_{13}-Ile_{14}-Gly_{15}-Ser_{16}-Met_{17}-Ser_{18}-Gly_{19}-Leu_{20}-Gly_{21}-Cys_{22}.

Positions 6 to 22 of CNP22 form a cyclic domain by means of a disulfide bond between Cys6 and Cys22. The 17-amino acid cyclic structure has been shown to be important for binding of CNP to NPR-B (Schiller, Biochem. Biophys. Res. Commun., 138: 880-886 (1986)). The amino acid sequence of positions 6 to 22 of CNP22 is referred to herein as "CNP17" (SEQ ID NO: 2).

CNP is susceptible to NEP cleavage at a number of sites: Cys6-Phe7, Gly8-Leu9, Lys10-Leu11, Arg13-Ile14, Ser16-Met17 and Gly19-Leu20. In one embodiment, the disclosure encompasses a CNP variant that is (1) modified to increase its overall size or molecular weight, e.g., to a range from about 2.6 kDa or 2.8 kDa to about 4 kDa, 4.2 kDa, 4.4 kDa, 4.6 kDa, 4.8 kDa, 5 kDa, 5.2 kDa, 5.4 kDa, 5.6 kDa, 5.8 kDa, 6 kDa, 6.2 kDa, 6.4 kDa, or to about 7 kDa, 7.2 kDa or about 8.2 kDa, and/or (2) modified at certain amino acid positions to reduce its susceptibility to NEP cleavage at 1, 2, 3, 4, 5 or all 6 of the sites listed above. The size or molecular

weight of the CNP variant can be increased by various means, e.g., by conjugating additional amino acids and/or other kinds of chemical (e.g., natural or synthetic polymeric) groups to the peptide sequence at, e.g., the N-terminus, the C-terminus and/or side chain(s), and/or by using natural amino acids, unnatural amino acids, and/or peptidomimetics with bulkier side chains. The CNP variant is optionally further conjugated to other functional or structural moieties. Optionally in combination with any of the embodiments described herein, mutation(s) (e.g., substitution(s), addition(s), and/or deletion(s)) may be introduced to certain position(s) of CNP22 to reduce the CNP variants' affinity to NPR-C. Further modifications may be made without affecting NEP resistance or CNP activity, e.g., conservative substitutions, or other modifications known in the art.

In one embodiment, the CNP variant is represented by the general formula: (x)-Gly_1-Leu_2-Ser_3-Lys_4-Gly_5-Cys_6-Phe_7-Gly_8-Leu_9-Lys_{10}-Leu_{11}-Asp_{12}-Arg_{13}-Ile_{14}-Gly_{15}-Ser_{16}-Met_{17}-Ser_{18}-Gly_{19}-Leu_{20}-Gly_{21}-Cys_{22}-(z) (SEQ ID NO: 5), wherein:

the CNP variant comprises one or more modified amino acids, which may result in modified peptide bonds (e.g., through use of peptide bond isosteres), at a position corresponding to one or more of the following CNP residues: Gly1, Lys4, Gly5, Cys6, Phe7, Gly8, Leu9, Lys10, Leu11, Ile14, Gly15, Ser16, Met17, Gly19, Leu20 and Gly21; and

(x) and (z) independently may be absent or may be an amino acid sequence derived from a natriuretic polypeptide (e.g., NPPC, ANP, BNP) or a non-natriuretic polypeptide (e.g., human serum albumin (HSA), IgG, etc.).

In an embodiment, the CNP variant includes: (1) a modification at an amino acid position corresponding to one of positions 6, 7 or 8 (Cys6, Phe7 or Gly8) of CNP22, (2) optionally deletion, addition and/or substitution of any or all of the amino acids at positions 1-5 (Gly1, Leu2, Ser3, Lys4, and Gly5) and (3) optionally up to 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 further modifications (deletions, additions and/or substitutions) at positions corresponding to positions 6-22, of which 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 may be conservative substitutions or other substitutions described herein or known in the art.

It is understood that a reference to a particular amino acid position by number (e.g. position 7 of CNP22) refers to the corresponding amino acid position in any CNP variant, even if the number of the position in that CNP variant has changed due to preceding insertions or deletions. For example, a reference to "position 7" or "Phe7" would mean the corresponding position 2 for a CNP variant in which the first five amino acids had been deleted. Similarly, a reference to "position 7" would mean the corresponding position 8 for a CNP variant in which one amino acid had been added to the N-terminus.

In any of the embodiments described herein, the CNP variant may be cyclized through a covalent bond between positions corresponding to 6 and 22 of CNP22. It is contemplated that the covalent bond is formed using any methods known in the art. In another embodiment, the CNP variant may be cyclized through a covalent bond formed between an amino acid at or toward the N-terminus and an amino acid at or toward the C-terminus (referred to as "terminal" amino acids for this purpose) of the peptide. In one embodiment, the covalent bond is formed between the side chains of the two terminal amino acids or the amino acids at positions corresponding to 6 and 22 of CNP22. In another embodiment, the covalent bond is formed between the side chain of one terminal amino acid and the terminal group of the other terminal amino acid, or between the terminal groups of each terminal amino acid. For example, head-to-tail, side chain-to-side chain, side chain-to-head, or side chain-to-tail bonds are possible for the covalent bond formed between the terminal amino acids or between the amino acids at positions corresponding to 6 and 22 of CNP22.

In one embodiment, the disclosure provides a CNP variant having reduced affinity to NEP, and/or greater resistance to cleavage by NEP and/or increased in vivo serum half-life, while retaining functionality of CNP (e.g., stimulation of cGMP production). NEP preferably recognizes substrates smaller than about 3 kDa, due to the limited size of its active site cavity (Oefner, J. Mol. Biol., 296: 341-349 (2000)). In an embodiment, the CNP variants are modified to increase their overall molecular weight to a range from about 2.6 or 2.8 kDa to about 4, 4.6, 5, 5.2, 5.8, 6, 6.4 or 7 kDa, e.g., by adding about 0.6 to about 5 kDa of amino acids, hydrophilic or water-soluble polymers, hydrophobic acids (including fatty acids), and/or carbohydrates. In specific exemplary embodiments, the CNP variants have a molecular weight between about 2.6 kDa and about 7 kDa, or between about 2.8 kDa and 6 kDa, or between about 2.8 kDa and about 5.8 kDa. In certain embodiments, at least about 0.6, 0.8, 1, 1.2, 1.4, 1.6 or 1.8 kDa, or up to 2, 2.2, 2.4, 2.6, 2.8, 3, 3.2, 3.4, 3.6, 3.8, 4, 4.2, 4.4, 4.6, 4.8 or 5 kDa are added, to increase the total molecular weight of the CNP variant, e.g., to a range from about 2.6 or 2.8 kDa up to about 4 kDa, 4.2 kDa, 4.4 kDa, 4.6 kDa, 4.8 kDa, 5 kDa, 5.2 kDa, 5.4 kDa, 5.6 kDa, 5.8 kDa, 6 kDa, 6.2 kDa, 7.2 kDa, 8.2 kDa or higher. In some embodiments, such CNP variants comprise an amino acid sequence at least about 70%, 75%. 80%, 85%, 90%, or 95% identical or homologous to amino acids 6-22 of CNP22. In other embodiments, such CNP variants comprise a substitution, insertion or deletion of 1, 2, 3, 4, 5, 6 or 7 amino acids with another natural or unnatural amino acid or peptidomimetic. While both conservative and non-conservative substitutions or insertions are envisioned at any position, introduction of modifications may commence, e.g., by conservative substitutions in regions that have been identified in the art as involved in CNP activity or NPR-B binding, while non-conservative substitutions may be made in those regions that have been previously shown to be tolerant of modification.

In another embodiment, the CNP variants comprise a CNP having an intact cyclized portion between Cys6 and Cys22, and N-terminal and/or C-terminal tails that contain about 1-40, 1-20, 5-40, 5-35, 10-35, 15-35, 5-31, 10-31, or 15-31 amino acids and are fragments derived from a CNP polypeptide and/or a non-CNP polypeptide. In an embodiment, such CNP variants have a molecular weight in a range from about 2.8 kDa to about 4, 4.6, 5, 5.2, 5.8, 6, 6.4 or 7 kDa. Non-limiting examples of such CNP variants include wild-type CNP22 or CNP22 with one or more amino acid substitutions (e.g., a K4R substitution), having an N-terminal and/or C-terminal extension derived from natriuretic peptide precursor sequences (e.g., ANP, BNP or CNP) from human or other species, a natriuretic peptide precursor C (NPPC) variant with amino acid substitutions, additions and/or deletions (e.g., the CNP variants may be truncations of CNP-53 which result in peptides with a molecular weight between about 2.8 kDa and 5.8 kDa), or other non-CNP polypeptides such as, e.g., serum albumin or IgG protein (e.g., the CNP variants may be CNP chimeras containing fragments of serum albumin or IgG from human or other species).

In one embodiment, CNP variants having a total mass characterized by the ranges described generally herein, e.g.,

from about 2.6 kDa or 2.8 kDa to about 6 or 7 kDa, designed for increased resistance to NEP degradation, are represented by the general formula:

(x)-Gly_1-Leu_2-Ser_3-(b)$_4$-Gly_5-Cys_6-Phe_7-Gly_8-Leu_9-(h)$_{10}$-Leu_{11}-Asp_{12}-Arg_{13}-Ile_{14}-Gly_{15}-Ser_{16}-Met_{17}-Ser_{18}-Gly_{19}-Leu_{20}-Gly_{21}-Cys_{22}-(z) (SEQ ID NO: 6), wherein:

(x) is a synthetic or natural polymeric group, or a combination thereof, wherein a non-limiting example of a synthetic polymeric group is polyethylene glycol (PEG, also called polyethylene oxide (PEO)), and a non-limiting example of a natural polymeric group is an amino acid sequence containing from 1 to 35 amino acids and derived from NPPC or variants thereof with substitutions and/or deletions, ANP, BNP, or other non-CNP (poly)peptides such as, e.g., serum albumin, IgG, histidine-rich glycoproteins, fibronectin, fibrinogen, zinc finger-containing polypeptides, osteocrin or fibroblast growth factor 2 (FGF2);

(z) may be absent or may be a synthetic or natural polymeric group, or a combination thereof, wherein a non-limiting example of a synthetic polymeric group is PEG, and a non-limiting example of a natural polymeric group is an amino acid sequence derived from a natriuretic polypeptide (e.g., NPPC, CNP, ANP or BNP) or non-natriuretic polypeptide (e.g., serum albumin or IgG); and

(b) and (h) independently may each be the wild type Lys at that position or may be replaced with a conservative amino acid substitution or any natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amine on a side chain, including but not limited to Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Glu or Ser. In one embodiment, (b) is Arg. In another embodiment, for improved NEP resistance, (b) is not Gly. In yet another embodiment, (h) is not Arg.

Non-limiting examples of amino acid sequences derived from NPPC or variants thereof include:

Arg,

Glu-Arg,

(SEQ ID NO: 7)
Gly-Ala-Asn-Lys-Lys,

(SEQ ID NO: 8)
Gly-Ala-Asn-Arg-Arg,

(SEQ ID NO: 9)
Gly-Ala-Asn-Pro-Arg,

(SEQ ID NO: 10)
Gly-Ala-Asn-Gln-Gln,

(SEQ ID NO: 11)
Gly-Ala-Asn-Ser-Ser,

(SEQ ID NO: 12)
Gly-Ala-Asn-Arg-Gln,

(SEQ ID NO: 13)
Gly-Ala-Asn-Arg-Met,

(SEQ ID NO: 14)
Gly-Ala-Asn-Arg-Thr,

(SEQ ID NO: 15)
Gly-Ala-Asn-Arg-Ser,

(SEQ ID NO: 16)
Ala-Ala-Trp-Ala-Arg-Leu-Leu-Gln-Glu-His-Pro-Asn-Ala,

-continued

(SEQ ID NO: 17)
Ala-Ala-Trp-Ala-Arg-Leu-Leu-Gln-Glu-His-Pro-Asn-Ala-Arg,

(SEQ ID NO: 18)
Asp-Leu-Arg-Val-Asp-Thr-Lys-Ser-Arg-Ala-Ala-Trp-Ala-Arg,

(SEQ ID NO: 19)
Gln-Glu-His-Pro-Asn-Ala-Arg-Lys-Tyr-Lys-Gly-Ala-Asn-Lys-Lys,

(SEQ ID NO: 20)
Gln-Glu-His-Pro-Asn-Ala-Arg-Lys-Tyr-Lys-Gly-Ala-Asn-Arg-Arg,

(SEQ ID NO: 21)
Asp-Leu-Arg-Val-Asp-Thr-Lys-Ser-Arg-Ala-Ala-Trp-Ala-Arg-Leu-Leu-Gln-Glu-His-Pro-Asn-Ala-Arg-Lys-Tyr-Lys-Gly-Ala-Asn-Lys-Lys,
and

(SEQ ID NO: 22)
Asp-Leu-Arg-Val-Asp-Thr-Lys-Ser-Arg-Ala-Ala-Trp-Ala-Arg-Leu-Leu-Gln-Glu-His-Pro-Asn-Ala-Arg-Lys-Tyr-Lys-Gly-Ala-Asn-Arg-Arg.

Non-limiting examples of amino acid sequences derived from non-CNP polypeptides such as, e.g., ANP, BNP, serum albumin and IgG include:

(SEQ ID NO: 23)
Ser-Leu-Arg-Arg-Ser-Ser;

(SEQ ID NO: 24)
Asn-Ser-Phe-Arg-Tyr;

(SEQ ID NO: 25)
Ser-Pro-Lys-Met-Val-Gln-Gly-Ser-Gly;

(SEQ ID NO: 26)
Met-Val-Gln-Gly-Ser-Gly;

(SEQ ID NO: 27)
Lys-Val-Leu-Arg-Arg-Tyr;

(SEQ ID NO: 28)
Lys-Val-Leu-Arg-Arg-His;

(SEQ ID NO: 29)
Gly-Gln-His-Lys-Asp-Asp-Asn-Pro-Asn-Leu-Pro-Arg;

(SEQ ID NO: 30)
Gly-Val-Pro-Gln-Val-Ser-Thr-Ser-Thr;

(SEQ ID NO: 31)
Gly-Glu-Arg-Ala-Phe-Lys-Ala-Trp-Ala-Val-Ala-Arg-Leu-Ser-Gln;
and

(SEQ ID NO: 32)
Gly-Gln-Pro-Arg-Glu-Pro-Gln-Val-Tyr-Thr-Leu-Pro-Pro-Ser.

In an embodiment, the N-terminus and/or C-terminus of CNP22 or a variant thereof independently may be conjugated to an amino acid extension containing 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39 or 40 amino acids. In one embodiment, the amino acid extension is derived from NPPC, CNP53, ANP or BNP. In a specific embodiment, the amino acid extension is Gln-Glu-His-Pro-Asn-Ala-Arg-Lys-Tyr-Lys-Gly-Ala-Asn-Lys-Lys (SEQ ID NO: 33). In a related embodiment, this 15-amino acid extension is added to the N-terminus to provide a CNP variant of the formula Gln-Glu-His-Pro-Asn-Ala-Arg-Lys-Tyr-Lys-Gly-Ala-Asn-Lys-Lys-Gly_1-Leu_2-

Ser_3-(b)$_4$-Gly_5-Cys_6-Phe_7-Gly_8-Leu_9-(h)$_{10}$-Leu_{11}-Asp_{12}-Arg_{13}-Ile_{14}-Gly_{15}-Ser_{16}-Met_{17}-Ser_{18}-Gly_{19}-Leu_{20}-Gly_{21}-Cys_{22}-(z) (SEQ ID NO: 34).

In one embodiment, the CNP variants comprise wtCNP22 or a variant thereof (e.g., one having addition(s), deletion(s), and/or substitution(s) such as, e.g., a K4R substitution) (SEQ ID NO: 35) conjugated at the N-terminus and/or C-terminus to a hydrophilic polymer (e.g., PEG) to increase their overall molecular size to a range from about 2.6 kDa or 2.8 kDa to about 4, 5, 6 or 7 kDa. Such CNP variants are optionally further conjugated at the N-terminus and/or C-terminus to a polymeric group comprising, e.g., amino acids, carbohydrates, hydrophobic acids and/or phospholipids, a non-limiting example of which is an N-terminal amino acid extension containing 1 to 35, or 5 to 31, amino acids. In an embodiment, a hydrophilic polymeric (e.g., PEG) moiety of at least about 0.4, 0.6, 0.8, 1, 1.2, 1.4, 1.6 or 1.8 kDa, or up to about 2, 2.2, 2.4, 2.6, 2.8, 3, 3.2, 3.4, 3.6, 3.8, 4, 4.2, 4.4, 4.6, 4.8 or 5 kDa, is added to the N-terminus and/or C-terminus of wtCNP22 or a variant thereof.

As shown herein, conjugation of a hydrophilic or water-soluble PEG (or PEO) polymer of about 0.6 kDa or more to CNP22 or variants thereof generally increases resistance to NEP cleavage markedly. However, addition of PEG, even as small as 0.6 kDa, to wtCNP22 may reduce CNP functionality (e.g., stimulation of cGMP signaling), and addition of greater than about 2 or 3 kDa of PEG to CNP22 or variants thereof may reduce CNP functional activity in a size-dependent manner. But CNP functionality (at least comparable to that of wtCNP22) is retained when a PEG (or PEO) polymer of about 0.6 kDa to about 1.2 kDa, or potentially to about 2 kDa, is conjugated to a CNP variant having an N-terminal amino acid extension in which at least one relatively large amino acid that may potentially be positively charged under physiological conditions (e.g., arginine) immediately precedes the position corresponding to Gly1 of CNP22, such as, e.g., GANRR-CNP22(K4R) (SEQ ID NO: 36), GANPR-CNP22(K4R) (SEQ ID NO: 37), ER-CNP22 (SEQ ID NO: 38), ER-CNP22(K4R) (SEQ ID NO: 39), R-CNP22 (SEQ ID NO: 40) and R-CNP22(K4R) (SEQ ID NO: 41).

Accordingly, in one embodiment, PEGylated CNP variants comprise at the N-terminus of CNP22 or a variant thereof (e.g., one having a K4R substitution) an amino acid extension containing at least 1, 2, 3, 4 or 5 amino acids, wherein the PEG polymer is conjugated to the N-terminus of the amino acid-extended CNP variant to result in a total mass characterized by the ranges described generally herein, e.g., from about 2.6 kDa or 2.8 kDa to about 6 or 7 kDa for increased resistance to NEP cleavage. In an embodiment, for enhanced CNP functionality, such pegylated, amino acid-extended CNP variants contain at least one relatively large natural or unnatural amino acid that may potentially be positively charged under physiological conditions, immediately preceding the position corresponding to Gly1 of CNP22. In a specific embodiment, the pegylated, amino acid-extended CNP variants contain at least one arginine residue immediately preceding the position corresponding to Gly1 of CNP22.

In addition to CNP variants conjugated at the N-terminus and/or C-terminus to a hydrophilic or water-soluble polymer such as, e.g., PEG (or PEO), the disclosure encompasses CNP variants conjugated to such a polymer at an internal site. For purposes of brevity here, PEG (or PEO) will be used as a representative example of a hydrophilic or water-soluble polymer. Various sites of PEGylation of a CNP variant are possible, including but not limited to: (1) PEGylation only at the N-terminus; (2) PEGylation only at the C-terminus; (3) PEGylation only at an internal site (e.g., Lys4); (4) PEGylation at both the N-terminus and the C-terminus; (5) PEGylation at the N-terminus and an internal site; and (6) PEGylation at the C-terminus and an internal site. For increased resistance to NEP degradation and retention of CNP functionality, in certain embodiments the total mass of PEGylatedCNP variants is characterized by the ranges described generally herein, e.g., in the range from about 2.6 kDa or 2.8 kDa to about 4, 5, 6 or 7 kDa. In a particular embodiment, CNP17, CNP22, CNP37 (defined below) or variants thereof (including those having amino acid additions, substitutions and/or deletions) are PEGylated only at the N-terminus. In another embodiment, the CNP variants are PEGylated only at an internal site (e.g., Lys4). In yet another embodiment, the CNP variants are PEGylated at the N-terminus and an internal site (e.g., Lys4). In still another embodiment, for better functionality the CNP variants are not PEGylated at a site (e.g., Lys10) within the cyclic domain (corresponding to Cys6 to Cys22 of CNP22). To prevent PEGylation at an internal site, Lys4 and/or Lys10 can be substituted with a natural or unnatural amino acid or peptidomimetic that does not contain a reactive primary amino group on a side chain, such as, e.g., Gly, Ser, Arg, Asn, Gln, Asp, Glu or citrulline (Cit). In a particular embodiment, Lys4 and/or Lys10 are replaced with Arg. In another embodiment, Lys10 is not replaced with Arg.

The disclosure contemplates use of hydrophilic or water soluble polymers (e.g., PEG molecules) that may vary in type (e.g., homopolymer or copolymer; random, alternating or block copolymer; linear or branched; monodispersed or polydispersed), linkage (e.g., hydrolysable or stable linkage such as, e.g., amide, imine, aminal, alkylene, or ester bond), conjugation site (e.g., at the N-terminus and/or C-terminus, preferably not at any of the residues in the cyclized region of CNP (corresponding to residues 6-22 of CNP22)), and length (e.g., from about 0.2, 0.4 or 0.6 kDa to about 2, 3, 4 or 5 kDa). The hydrophilic or water-soluble polymer can be conjugated to the CNP peptide by means of N-hydroxy succinimide (NHS)- or aldehyde-based chemistry or other chemistry, as is known in the art. Such CNP variants can be generated using, e.g., wtCNP-22 (2.2 kDa), CNP-17 retaining only the cyclized region (residues 6-22) of wtCNP22, CNP variants having an amino acid extension at the N-terminus and/or C-terminus of CNP22, or variants with amino acid substitutions, additions and/or deletions, for example, GANRR-CNP22(K4R) (SEQ ID NO: 36), GANPR-CNP22 (K4R) (SEQ ID NO: 37), R-CNP22 (SEQ ID NO: 40), R-CNP22(K4R) (SEQ ID NO: 41), ER-CNP22 (SEQ ID NO: 38) and ER-CNP22(K4R) (SEQ ID NO: 39). In an embodiment, the PEG-CNP variants having a total mass characterized by the ranges described generally herein, e.g., from about 2.6 kDa or 2.8 kDa to about 6 or 7 kDa, contain a monodispersed, linear PEG (or PEO) group conjugated at the N-terminus and/or C-terminus via NHS- or aldehyde-based chemistry, or a two-arm or three-arm branched PEG group conjugated at the N-terminus and/or C-terminus via NHS-based chemistry. The disclosure further contemplates negatively charged PEG-CNP variants designed for reduced renal clearance, including but not limited to carboxylated, sulfated and phosphorylated compounds.

In a related embodiment, the disclosure contemplates PEG-CNP conjugates comprising NHS- or aldehyde-based PEG of the formula $(CH_2CH_2O)_n$, wherein n is an integer from 12 to 50, and the PEG polymer is up to about 2.5 kDa in molecular weight. In a specific embodiment, n is 12 or 24. In an embodiment, the terminal hydroxyl group of the PEG

polymer is capped with a non-reactive group. In a particular embodiment, the capping group is an alkyl group, e.g., a lower alkyl group such as methyl.

In an additional embodiment, the PEG polymers or derivatives thereof have a polymer number-average molecular weight in the range from about 0.4 kDa to about 2.5 kDa or from about 0.6 kDa to about 1.5 kDa.

In a further embodiment, the wtCNP or CNP variant peptide is conjugated to a moiety including, e.g., bisphosphonates, carbohydrates, hydrophobic acids (including fatty acids) or amino acid sequences. Such amino acid sequences include for example polyAsp or polyGlu useful in bone/cartilage targeting, or can be derived from bone proteins with elucidated bone-targeting domains or derivatives thereof, such as for example fusion proteins or peptide sequences of osteopontin, osteocalcin, sialoprotein, etc. In embodiments described berein where CNP22 or a variant thereof is attached to a bone- or cartilage-targeting moiety, such a moiety is designed to promote getting the modified CNP peptide to chondrocytes of bone growth plates, where the peptide can bind and activate NPR-B on the chondrocytes.

In another embodiment, the disclosure provides CNP variants with a peptide bond that is less susceptible to cleavage by peptidases including NEP. The disclosure encompasses a CNP variant comprising at least one modified residue at a site of endopeptidase cleavage. In one embodiment, the Cys6-Phe7 peptide bond (—C(═O)—NH—) at an NEP cleavage site in CNP can be replaced with anyone of the following peptide-bond isosteres:

—CH_2—NH—,

—C(═O)—N(R)—, where the amide group is alkylated with any of the following R groups: methyl, ethyl, n-propyl, isopropyl, cyclopropyl, n-butyl, isobutyl, sec-butyl or tert-butyl,

—C(═O)—NH—CH_2—,

—CH_2—S—,

—CH_2—S(O)$_n$—, where n is 1 or 2,

—CH═CH—,

—C(═O)—CH_2—,

—CH(CN)—NH—,

—CH(OH)—CH_2—,

—O—C(═O)—NH—, and

—NHC(═O)NH—.

In another embodiment, Phe7 is substituted with its enantiomer D-Phe. In yet another embodiment, D-enantiomers are introduced to one or more, up to all 22, positions within wtCNP-22. In a further embodiment, a beta amino acid such as 3-amino-2-phenylpropionic acid is substituted for Phe7, which increases the length of the backbone while reducing the length of the side chain. In yet another embodiment, the disclosure contemplates Cys analogs at the Cys6 position including but not limited to homocysteine, penicillamine, 2-mercaptopropionic acid, and 3-mercaptopropionic acid.

Even in the presence of a NEP-resistant bond between Cys6 and Phe7, other peptide bonds can be hydrolyzed by NEP, including Gly8-Leu9, Lys10-Leu11, Arg13-Ile14, Ser16-Met17 and Gly19-Leu20. Accordingly, the disclosure encompasses CNP analogs containing peptide bond isosteres at multiple locations in the backbone of the CNP analogs. In one embodiment, CNP analogs or variants comprise modifications at more than one peptidase cleavage site. In a further embodiment, such variant comprises a CNP with substitutions at amino acid residues important in binding to the NEP active site, thereby increasing resistance to NEP degradation. One or more NEP-binding residues, including but not limited to Gly8, Gly15, Ser18, Gly19 and/or Gly21, are replaced with larger-size natural or unnatural amino acid residues to reduce affinity to the NEP active site. In yet another embodiment, one or more hydrophobic residues essential in NEP recognition, including but not limited to Phe7, Leu9, Leu11, Ile14, Met17 and Leu20, are substituted with natural or unnatural amino acids and/or peptidomimetics that decrease NEP binding. In yet another embodiment, one to five of the first five amino acids of CNP can be deleted or substituted with any other natural amino acids or unnatural amino acids or peptidomimetics, or one or more natural or unnatural amino acids or peptidomimetics can be added to any one or to all of the first five positions of CNP.

In a further embodiment, the CNP variants have a total mass characterized by the ranges described generally herein, e.g., from about 2.6 kDa or 2.8 kDa to about 6 or 7 kDa for increased resistance to NEP, and are represented hy the formula:

(x)-Gly_1-Leu_2-Ser_3-(a)$_4$-Gly_5-(b)$_6$-(c)$_7$-(d)$_8$-(e)$_9$-(f)$_{10}$-(g)$_{11}$-Asp_{12}-Arg_{13}-(h)$_{14}$-Gly_{15}-Ser_{16}-(i)$_{17}$-Ser_{18}-Gly_{19}-(j)$_{20}$-Gly_{21}-Cys_{22}-(z) (SEQ ID NO: 46), wherein:

(x) may be absent or may be selected from the group consisting of synthetic bone-targeting compounds such as, e.g., bisphosphonates; amino acid sequences useful in bone or cartilage targeting such as, e.g., polyAsp and polyGlu; amino acid sequences derived from bone proteins with elucidated bone-targeting domains, such as, e.g., fusion proteins or peptide sequences of osteopontin, osteocalcin, sialoprotein, etc.; polymeric or non-polymeric molecules that reduce renal clearance such as, e.g., charged PEG molecules; and extensions comprising, e.g., polymers (e.g., PEGs), carbohydrates, hydrophobic acids (including fatty acids), and/or amino acids, and wherein such amino acid extensions can contain, e.g., from 1 to 31, or 1 to 35, or 5 to 35, or 10 to 35, or 15 to 35 amino acid residues, and can be derived from NPPC, ANP, BNP, other non-CNP (poly) peptides such as, e.g., serum albumin or IgG, or variants of the aforementioned polypeptides having substitutions, additions and/or deletions, or combinations thereof;

(z) may be absent or may be selected from the group consisting of amino acid sequences useful in bone or cartilage targeting such as for example polyAsp and polyGlu, amino acid sequences from bone-targeting domains of bone proteins such as, e.g., osteopontin, osteocalcin and sialoprotein, and amino acid sequences derived from non-CNP (poly)peptides such as, e.g., ANP or BNP;

(a) may be the wild type Lys at that position or may be replaced with a conservative amino acid substitution or a natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amine on a side chain, including but not limited to Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu, wherein in one embodiment (a) is Arg;

(b) is selected from the group consisting of Cys and peptide-bond isosteres between Cys6 and Phe7 such as, e.g., Cys-CH_2—NH;

(c) is selected from the group consisting of L-Phe; D-Phe; 3-amino-2-phenylpropionic acid; N-alkylated derivatives of Phe, wherein the N-alkyl group is methyl, ethyl, n-propyl, isopropyl, cyclopropyl, n-butyl, isobutyl, sec-butyl or tert-butyl; and Phe analogs, wherein one or more ortho-, meta-, and/or para-positions of the benzene ring of the Phe analog are substituted with one or more substituents selected from the group consisting of halogen, hydroxyl, cyano, straight or branched C_{1-6}alkyl, straight or branched C_{1-6}alkoxy, straight or branched halo-C_{1-6} alkyl, C_{3-10} cycloalkyl, heterocyclyl, C_{6-14} aryl and heteroaryl (examples include, but are not limited to, tyrosine, 3-chlorophenylalanine, 2,3-chloro-phe-

nylalanine, 3-chloro-5-fluoro-phenylalanine, 2-chloro-6-fluoro-3-methyl-phenylalanine), or wherein the benzene ring of the Phe analog can be replaced with another aryl group (non-limiting examples include 1- and 2-naphthylalanine) or with a heteroaryl group (non-limiting examples include pyridylalanine, thienylalanine and furylalanine);

(d) is selected from the group consisting of Gly, tert-butyl-Gly (tBu-Gly), Thr, Ser, Val and Asn;

(e) is selected from the group consisting of Leu, Ser, Thr and peptide-bond isosteres such as, e.g., N-Me-Leu;

(f) may be the wild type Lys at that position or may be replaced with a conservative amino acid substitution or a natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amine on a side chain, including but not limited to Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu, wherein in one embodiment (f) is not Arg;

(g) is selected from the group consisting of Leu and peptide-bond isosteres such as, e.g., N-Me-Leu;

(h) is selected from the group consisting of Ile, tBu-Gly, and peptide-bond isosteres such as, e.g., N-Me-Ile;

(i) is selected from the group consisting of Met, Val, Asn, beta-Cl-Ala, 2-aminobutyric acid (Abu) and 2-amino-isobutyric acid (Aib); and

(j) is selected from the group consisting of Leu, norleucine (Nle), homoleucine (Hleu), Val, tert-butyl-Ala (tBu-Ala), Ser, Thr, Arg, and peptide-bond isosteres such as, e.g., N-Me-Leu.

In one embodiment, the CNP variants comprise a modification at one or more of positions 6, 7, 8, 9, 10, 11, 13, 14, 16, 17, 19 and/or 20, and may optionally have modifications at any of the other positions disclosed herein.

In embodiments described herein where CNP22 or variants thereof can be attached to a hydrophobic acid, the CNP peptides can be attached to one or hydrophobic acids. Non-limiting examples of hydrophobic acids include straight-chain or branched, saturated or unsaturated C_5-C_{12} carboxylic acids (e.g., pentanoic acid, heptanoic acid, etc.) and natural fatty acids. The hydrophobic acids can be attached to the N-terminus, the C-terminus, and/or the side chain of one or more amino acid residues. In one embodiment, the hydrophobic acids are conjugated to the N-terminus. In an embodiment, conjugation of CNP22 or a variant thereof to a hydrophobic acid is designed, inter alia, to promote non-specific interaction between the modified CNP peptide and serum albumin, thereby increasing the size of the CNP peptide and protecting it from cleavage by proteases such as, e.g., NEP. The interaction between the hydrophobic acid-conjugated CNP peptide and albumin is designed to be not too strong, so that the modified CNP peptide can diffuse through cartilage, get to chondrocytes of bone growth plates, and bind and activate NPR-B.

In a further embodiment, the disclosure provides CNP variants that in vitro or in vivo stimulate the production of at least about 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140% or 150% of the cGMP level produced under the same concentration of wtCNP22 (e.g., 1 uM), comprise at least one modified amino acid at position $(b)_6$, $(c)_7$ and/or $(d)_8$, and are represented by the general formula: (x)-Gly_1-Leu_2-Ser_3-$(a)_4$-Gly_5-$(b)_6$-$(c)_7$-$(d)_8$-$(e)_9$-$(f)_{10}$-$(g)_{11}$-Asp_{12}-Arg_{13}-$(h)_{14}$-Gly_{15}-Ser_{16}-$(i)_{17}$-Ser_{18}-Gly_{19}-$(j)_{20}$-Gly_{21}-Cys_{22}-(z) (SEQ ID NO: 47), wherein:

(x) may be absent or may be a peptide sequence containing one to five amino acids which is derived from a natriuretic polypeptide (e.g. NPPC, CNP, ANP or BNP) or a non-natriuretic polypeptide as described herein (e.g., HSA, IgG, a bone-targeting protein, etc.);

(z) may be absent or may be selected from the group consisting of synthetic bone-targeting compounds such as, e.g., bisphosphonates; amino acid sequences useful in bone/cartilage targeting such as, e.g., polyAsp and polyGlu; bone proteins with bone-targeting domains and derivatives thereof, such as fusion proteins or peptides sequences of osteopontin, osteocalcin, and sialoprotein; molecules that reduce renal clearance, such as, e.g., charged PEGs; and molecules that increase resistance of CNP to NEP-mediated degradation, as described herein;

(a) may be the wild type Lys at that position or may be replaced with a conservative amino acid substitution or a natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amine on a side chain, including but not limited to Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu, wherein in one embodiment (a) is Arg;

(b) may be Cys or descarboxy cysteine, or the $(b)_6$-$(c)_7$ peptide bond (—C(═O)—NH—) may be replaced with any one of the following peptide bond isosteres:

—CH_2—NH—,
—C(═O)—N(R)—, where the amide group is alkylated with any of the following R groups: methyl, ethyl, n-propyl, isopropyl, cyclopropyl, n-butyl, isobutyl, sec-butyl or tert-butyl,
—C(═O)—NH—CH_2—,
—CH_2—S—,
—CH_2—$S(O)_n$—, where n is 1 or 2,
—CH_2—CH_2—,
—CH═CH—,
—C(═O)—CH_2—,
—CH(CN)—NH—,
—CH(OH)—CH_2—,
—O—C(═O)—NH—, or
—NHC(═O)NH—;

(c) is selected from the group consisting of L-Phe; D-Phe; 3-amino-2-phenylpropionic acid; peptide bond isosteres of Phe such as N-alkylated derivatives of Phe wherein the N-alkyl group is selected from the group consisting of methyl, ethyl, n-propyl, isopropyl, cyclopropyl, n-butyl, isobutyl, sec-butyl and tert-butyl; and Phe analogs, wherein one or more ortho-, meta-, and/or para-positions of the benzene ring of the Phe analog are substituted with one or more substituents selected from the group consisting of halogen, hydroxyl, cyano, straight or branched C_{1-6} alkyl, straight or branched C_{1-6} alkoxy, straight or branched halo-C_{1-6} alkyl, C_{3-10} cycloalkyl, C_{6-14} aryl, heterocyclyl and heteroaryl (examples include, but are not limited to, tyrosine, 3-chlorophenylalanine, 2,3-chloro-phenylalanine, 3-chloro-5-fluoro-phenylalanine, 2-chloro-6-fluoro-3-methyl-phenylalanine), or wherein the benzene ring of the Phe analog can be replaced with another aryl group (non-limiting examples include 1- and 2-naphthylalanine) or with a heteroaryl group (non-limiting examples include pyridylalanine, thienylalanine and furylalanine);

(d) is selected from the group consisting of Gly, tert-butyl-Gly (tBu-Gly), Val, Ser, Thr and Asn;

(e) is selected from the group consisting of Leu, Ser, Thr, and peptide-bond isosteres such as, e.g., N-Me-Leu;

(f) is any natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amino group on a side chain, including but not limited to Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu, wherein in one embodiment (f) is not Arg;

(g) is selected from the group consisting of Leu and peptide-bond isosteres such as, e.g., N-Me-Leu;

(h) is selected from the group consisting of Ile, tBu-Gly and peptide-bond isosteres such as, e.g., N-Me-Ile;

(i) is selected from the group consisting of Met, Val, Asn, beta-Cl-Ala, 2-aminobutyric acid (Abu) and 2-amino-isobutyric acid (Aib); and

(j) is selected from the group consisting of Leu, norleucine (Nle), homoleucine (Hleu), Val, tert-butyl-Ala (tBu-Ala), Ser, Thr, Arg, and peptide-bond isosteres such as, e.g., N-Me-Leu.

In a further embodiment, the disclosure encompasses CNP variants that in vitro or in vivo stimulate the production of at least about 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140% or 150% of the cGMP level produced under the same concentration of wtCNP22 (e.g., 1 uM), have a total mass characterized by the ranges described generally herein, e.g., from about 2.6 kDa or 2.8 kDa to about 6 or 7 kDa for increased resistance to NEP degradation, and are represented by the general formula:

(x)-(y)-Cys_6-Phe_7-Gly_8-Leu_9-$(h)_{10}$-Leu_{11}-Asp_{12}-Arg_{13}-Ile_{14}-Gly_{15}-Ser_{16}-Met_{17}-Ser_{18}-Gly_{19}-Leu_{20}-Gly_{21}-Cys_{22}-(z) (SEQ ID NO: 48), wherein:

(x) is a synthetic or natural polymeric group, or a combination thereof, wherein a non-limiting example of a synthetic polymeric group is polyethylene glycol (PEG), and a non-limiting example of a natural polymeric group is an amino acid sequence containing from 1 to 35 amino acids and derived from NPPC or variants thereof with substitutions and/or deletions, ANP, BNP, or other non-CNP (poly) peptides such as, e.g., serum albumin, IgG, histidine-rich glyco-proteins, fibronectin, fibrinogen, zinc finger-containing polypeptides, osteocrin or FGF2;

(y) may be absent or may be one or more amino acids from Gly_1-Leu_2-Ser_3-Lys_4-Gly_5 (corresponding to positions 1 to 5 of CNP22) (SEQ ID NO: 1) and/or substitutions at one or more of those positions using natural or unnatural amino acids (e.g., K4R substitution);

(h) may be the wild-type Lys at that position or may be replaced with a conservative amino acid substitution or a natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amine on a side chain, including but not limited to Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu, wherein in one embodiment (h) is not Arg; and

(z) may be absent or may be a synthetic or natural polymeric group, or a combination thereof, wherein a non-limiting example of a synthetic polymeric group is PEG, and a non-limiting example of a natural polymeric group is an amino acid sequence derived from a natriuretic polypeptide (e.g., NPPC, CNP, ANP or BNP) or non-natriuretic polypeptide (e.g., serum albumin or IgG).

In an embodiment, (x), (y) and (z) together contain from about 10 to about 40, or from about 15 to about 35 amino acids. In another embodiment, (x) is an amino acid sequence comprising from 1 to 40 amino acids, or from 1 to 20 amino acids.

Further contemplated are CNP variants that in vitro or in vivo stimulate the production of at least about 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140% or 150% of the cGMP level produced under the same concentration of wtCNP22 (e.g., 1 uM), and comprise the sequence:

(y)-Cys_6-Phe_7-Gly_8-Leu_9-Lys_{10}-Leu_{11}-Asp_{12}-Arg_{13}-Ile_{14}-Gly_{15}-Ser_{16}-Met_{17}-Ser_{18}-Gly_{19}-Leu_{20}-Gly_{21}-Cys_{22} (SEQ ID NO: 138), wherein:

(y) comprises one or more amino acids selected from Gly_1-Leu_2-Ser_3-Lys_4-Gly_5 (SEQ ID NO: 1) and/or substitutions at one or more of those positions using natural or unnatural amino acids (e.g., K4R substitution), and further comprises a hydrophilic or water soluble polymer of molecular weight from about 0.6 kDa to about 5 kDa. In an embodiment, the hydrophilic or water-soluble polymer is conjugated to the N-terminus of such amino acid-extended CNP variant. In a particular embodiment, the hydrophilic or water-soluble polymer is PEG (or PEO).

In yet another embodiment, the disclosure provides CNP variants that in vitro or in vivo stimulate the production of at least about 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140% or 150% of the cGMP level produced under the same concentration of wtCNP22 (e.g. 1 uM), wherein the CNP variants comprise an N-terminal and/or C-terminal peptide extension containing from 1 to 15 amino acids, and are conjugated to a hydrophilic or water soluble polymer. In an embodiment, the peptide extension contains from 5 to 10 amino acids. In a specific embodiment, the peptide extension contains 5 amino acids. In another specific embodiment, the hydrophilic or water-soluble polymer is PEG (or PEO).

In a still further embodiment, the CNP variants of the disclosure in vitro or in vivo stimulate the production of at least about 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140% or 150% of the cGMP level produced under the same concentration of wtCNP22 (e.g. 1 uM), and comprise at least a 15 amino acid fragment derived from natriuretic peptide precursor C(NPPC), wherein the fragment is at least 70% homologous to a sequence from wild type NPPC containing the same number of amino acid residues.

In still another embodiment, the CNP variants have a total mass characterized by the ranges described generally herein, e.g., from about 2.6 kDa or 2.8 kDa to about 6 or 7 kDa for increased NEP resistance, and are represented by the formula:

(x)-$(b)_6$-$(c)_7$-$(d)_8$-$(e)_9$-$(f)_{10}$-$(g)_{11}$-Asp_{12}-Arg_{13}-$(h)_{14}$-Gly_{15}-Ser_{16}-$(i)_{17}$-Ser_{18}-Gly_{19}-$(j)_{20}$-Gly_{21}-Cys_{22}-(z) (SEQ ID NO: 49), wherein:

(x) may be absent (i.e., the N-terminus ends with an —NH_2 group) or may be selected from the group consisting of a sequence of 1, 2, 3, 4 or 5 amino acids from the peptide Gly_1-Leu_2-Ser_3-Lys_4-Gly_5 (SEQ ID NO: 1); amino acid sequences useful in bone/cartilage targeting such as for example polyAsp or polyGlu; bone-targeting domains from bone proteins such as for example osteopontin, osteocalcin or sialoprotein; molecules that reduce renal clearance such as hydrophilic or water-soluble polymers, including but not limited to charged PEG molecules; and moieties comprising PEG, carbohydrates, hydrophobic acids, amino acids, or combinations thereof, wherein such moieties can be amino acid extensions including but not limited to amino acid sequences derived from NPPC or non-CNP (poly)peptides such as, e.g., BNP, ANP, serum albumin or IgG;

(z) may be absent or may be selected from the group consisting of amino acid sequences useful in bone/cartilage targeting such as for example polyAsp or polyGlu; amino acid sequences derived from bone-targeting proteins, such as for example osteopontin, osteocalcin or sialoprotein; and amino acid sequences derived from NPPC or non-CNP (poly) peptides, as described herein;

(b) is selected from the group consisting of Cys and peptide bond isosteres between Cys6 and Phe7 such as, e.g., Cys-CH_2—NH;

(c) is selected from the group consisting of L-Phe; D-Phe; 3-amino-2-phenylpropionic acid; N-alkylated derivatives of Phe, wherein the N-alkyl group is methyl, ethyl, n-propyl, isopropyl, cyclopropyl, n-butyl, isobutyl, sec-butyl or tert-butyl; and Phe analogs, wherein one or more ortho-, meta-, and/or para-positions of the benzene ring of the Phe analog are substituted with one or more substituents selected from the group consisting of halogen, hydroxyl, cyano, straight or

branched C_{1-6} alkyl, straight or branched C_{1-6} alkoxy, straight or branched halo-C_{1-6} alkyl, C_{3-10} cycloalkyl, C_{6-14} aryl, heterocyclyl and heteroaryl (examples include, but are not limited to, tyrosine, 3-chlorophenylalanine, 2,3-chlorophenylalanine, 3-chloro-5-fluoro-phenylalanine, 2-chloro-6-fluoro-3-methyl-phenylalanine), or wherein the benzene ring of the Phe analog can be replaced with another aryl group (non-limiting examples include 1- and 2-naphthylalanine) or with a heteroaryl group (non-limiting examples include pyridylalanine, thienylalanine and furylalanine);

(d) is selected from the group consisting of Gly, tert-butyl-Gly (tBu-Gly), Val, Ser, Thr and Asn;

(e) is selected from the group consisting of Leu, Ser, Thr, and peptide-bond isosteres such as, e.g., N-Me-Leu;

(f) may be the wild type Lys at that position or may be replaced with a conservative amino acid substitution or a natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amine on a side chain, including but not limited to Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu, wherein in one embodiment (f) is not Arg;

(g) is selected from the group consisting of Leu and peptide-bond isosteres such as, e.g., N-Me-Leu;

(h) is selected from the group consisting of Ile, tBu-Gly and peptide-bond isosteres such as, e.g., N-Me-Ile;

(i) is selected from the group consisting of Met, Val, Asn, beta-Cl-Ala, 2-aminobutyric acid (Abu) and 2-amino-isobutyric acid (Aib); and

(j) is selected from the group consisting of Leu, norleucine (Nle), homoleucine (Hleu), Val, tert-butyl-Ala (tBu-Ala), Ser, Thr, Arg, and peptide-bond isosteres such as, e.g., N-Me-Leu.

In a further embodiment, the CNP variants have a total mass characterized by the ranges described generally herein, e.g., from about 2.6 kDa or 2.8 kDa to about 6 or 7 kDa for increased NEP resistance, and are represented by the formula:

(x)-Gly_1-Leu_2-Ser_3-(a)$_4$-Gly_5-(b)$_6$-(c)$_7$-(d)$_8$-(e)$_9$-(f)$_{10}$-(g)$_{11}$-Asp_{12}-Arg_{13}-(h)$_{14}$-(i)$_{15}$-Ser_{16}-(j)$_{17}$-Ser_{18}-Gly_{19}-(k)$_{20}$-Gly_{21}-Cys_{22}-(z) (SEQ ID NO: 50), wherein:

(x) and (z) independently may be absent or may be selected from the group consisting of synthetic bone-targeting compounds such as, e.g., bisphosphonates; amino acid sequences useful in bone/cartilage targeting such as for example polyAsp or polyGlu; amino acid sequences derived from bone-targeting domains of bone proteins and derivatives thereof, such as for example fusion proteins or peptide sequences of osteopontin, osteocalcin, sialoprotein, etc.; moieties that reduce renal clearance, including but not limited to hydrophilic or water-soluble polymers such as, e.g., charged PEG molecules; and moieties comprising, e.g., hydrophilic polymers (e.g., PEG), carbohydrates, hydrophobic acids, and/or amino acids;

(a) may be the wild type Lys at that position or may be replaced with a conservative amino acid substitution or a natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amine on a side chain, including but not limited to Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu, wherein in one embodiment (a) is Arg;

(b) is selected from the group consisting of Cys and peptide bond isosteres between Cys6 and Phe7 such as, e.g., Cys-CH_2—NH;

(c) is selected from the group consisting of L-Phe; D-Phe; 3-amino-2-phenylpropionic acid; N-alkylated derivatives of Phe, wherein the N-alkyl group is methyl, ethyl, n-propyl, isopropyl, cyclopropyl, n-butyl, isobutyl, sec-butyl or tert-butyl; and Phe analogs, wherein one or more ortho-, meta-, and/or para-positions of the benzene ring of the Phe analog are substituted with one or more substituents selected from the group consisting of halogen, hydroxyl, cyano, straight or branched C_{1-6} alkyl, straight or branched C_{1-6} alkoxy, straight or branched halo-C_{1-6} alkyl, C_{3-10} cycloalkyl, C_{6-14} aryl, heterocyclyl and heteroaryl (examples include, but are not limited to, tyrosine, 3-chlorophenylalanine, 2,3-chlorophenylalanine, 3-chloro-5-fluoro-phenylalanine, 2-chloro-6-fluoro-3-methyl-phenylalanine), or wherein the benzene ring of the Phe analog can be replaced with another aryl group (non-limiting examples include 1- and 2-naphthylalanine) or with a heteroaryl group (non-limiting examples include pyridylalanine, thienylalanine and furylalanine);

(d) is selected from the group consisting of Gly, tert-butyl-Gly, Thr, Ser, Val and Asn;

(e) is selected from the group consisting of Leu, Ser, Thr, and peptide bond isosteres such as, e.g., N-Me-Leu;

(f) may be the wild type Lys at that position or may be replaced with a conservative amino acid substitution or a natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amine on a side chain, including but not limited to Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu, wherein in one embodiment (f) is not Arg;

(g) is selected from the group consisting of Leu, Asn, and peptide bond isosteres such as, e.g., N-Me-Leu;

(h) is selected from the group consisting of Ile, tert-butyl-Gly (tBu-Gly), Asn, and peptide bond isosteres such as, e.g., N-Me-Ile;

(i) is selected from the group consisting of Gly, Arg, Ser and Asn;

(j) is selected from the group consisting of Met, Val, Asn, beta-Cl-Ala, 2-aminobutyric acid (Abu) and 2-amino-isobutyric acid (Aib); and

(k) is selected from the group consisting of Leu, norleucine (Nle), homoleucine (Hleu), Val, tert-butyl-Ala (tBu-Ala), Arg, Thr, Ser, and peptide bond isosteres such as, e.g., N-Me-Leu.

In a further embodiment, the CNP variants can have amino acid substitution(s) at one or more of any of positions 1 to 22 of CNP22. In one embodiment, Gly1 is substituted with Arg or Glu. In another embodiment, Lys4 is replaced with Arg. In still another embodiment, Gly5 is substituted with Arg, Gln or Ser. In yet another embodiment, Gly15 is substituted with Ser, Asn, Arg or Cit. In a further embodiment, Gly19 is substituted with Ser, Arg or Asn. In yet another embodiment, Gly21 is substituted with Ser, Thr, or Arg.

In one embodiment, the CNP variant is selected from the group consisting of GLSKGC(CH_2NH)FGLKLDRIGSMSGLGC (formed using descarboxy-Cys) (SEQ ID NO: 56), GLSKGC-(N-Me-Phe)-GLKLDRIGSMSGLGC (SEQ ID NO: 57), GLSKGC-(D-Phe)-GLKLDRIGSMSGLGC(SEQ ID NO:136), GLSKGCF-(tBuG)-LKLDRIGSMSGLGC (SEQ ID NO: 58), GLSKGC-(3-Cl-Phe)-GLKLDRIGSMSGLGC (SEQ ID NO:137), and GLSKGC-[NHCH_2CH(Ph)CO]-GLKLDRIGSMSGLGC (formed using 3-amino-2-phenylpropionic acid) (SEQ ID NO: 59). In a further embodiment, a disulfide bond exists between Cys6, descarboxy-Cys or another sulfhydryl-containing cysteine analog at the Cys6 position, and Cys22 of any CNP variant described herein.

In another embodiment, the CNP variants contain an amino acid extension at the N-terminus and/or C-terminus of CNP22 or CNP17, including but not limited to:

(SEQ ID NO: 4)
DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMS
GLGC (CNP-53);

(SEQ ID NO: 60)
QEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC
(CNP-37, Analog BL);

(SEQ ID NO: 61)
AAWARLLQEHPNAGLSKGCFGLKLDRIGSMSGLGC (Analog CA);

(SEQ ID NO: 62)
AAWARLLQEHPNARGLSKGCFGLKLDRIGSMSGLGC (Analog CB);

(SEQ ID NO: 63)
DLRVDTKSRAAWARGLSKGCFGLKLDRIGSMSGLGC (Analog CC);

(SEQ ID NO: 40)
RGLSKGCFGLKLDRIGSMSGLGC;

(SEQ ID NO: 38)
ERGLSKGCFGLKLDRIGSMSGLGC;

(SEQ ID NO: 64)
GANQQGLSKGCFGLKLDRIGSMSGLGC;

(SEQ ID NO: 65)
GANRRGLSKGCFGLKLDRIGSMSGLGC;

(SEQ ID NO: 66)
GANPRGLSKGCFGLKLDRIGSMSGLGC;

(SEQ ID NO: 67)
GANSSGLSKGCFGLKLDRIGSMSGLGC;

(SEQ ID NO: 144)
GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC
(sometimes designated "CNP27-HSA" or "HSA-CNP27"
in the Examples and figures);

(SEQ ID NO: 68)
SPKMVQGSG-CNP17-KVLARH (Analog CD) (CNP17 having
N-terminal and C-terminal tails derived from BNP).

In a further embodiment, the CNP variants have a K4R substitution at position 4 of CNP22. Non-limiting examples of CNP(K4R) variants include:

((SEQ ID NO: 36)
GANRRGLSRGCFGLKLDRIGSMSGLGC (Analog AY);

(SEQ ID NO: 37)
GANPRGLSRGCFGLKLDRIGSMSGLGC (Analog CI);

(SEQ ID NO: 41)
RGLSRGCFGLKLDRIGSMSGLGC (Analog AZ);

(SEQ ID NO: 39)
ERGLSRGCFGLKLDRIGSMSGLGC (Analog BA);

(SEQ ID NO: 69)
GANQQGLSRGCFGLKLDRIGSMSGLGC (Analog CH);
and

(SEQ ID NO: 70)
GANSSGLSRGCFGLKLDRIGSMSGLGC (Analog CG).

In one embodiment, CNP variants having a PEG (or PEO) moiety and an amino acid extension at the N-terminus contain arginine at the position immediately preceding the position corresponding to Gly1 of CNP22. Such PEGylated CNP variants are designed for increased resistance to NEP degradation, reduced binding to serum albumin, and enhanced CNP functional activity (e.g., activation of cGMP signaling). Non-limiting examples of PEGylated CNP variants include PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36), PEO12-GANRR-CNP22(K4R) (SEQ ID NO: 36), PEO24-GANRR-CNP22(SEQ ID NO: 65), PEO12-GANRR-CNP22(SEQ ID NO: 65), PEO24-GANPR-CNP22(K4R) (SEQ ID NO: 37), PEO12-GANPR-CNP22 (K4R) (SEQ ID NO: 37), PEO24-GANPR-CNP22(SEQ ID NO: 37), PEO12-GANPR-CNP22 (SEQ ID NO: 66), PEO24-GANQQ-CNP22(SEQ ID NO: 64), PEO12-GANQQ-CNP22(SEQ ID NO: 64), PEO24-ER-CNP22 (K4R) (SEQ ID NO: 39), PEO12-ER-CNP22(K4R) (SEQ ID NO: 39), PEO24-ER-CNP22(SEQ ID NO: 38), PEO12-ER-CNP22(SEQ ID NO: 38), PEO24-R-CNP22(K4R) (SEQ ID NO: 41), PEO12-R-CNP22(K4R) (SEQ ID NO: 41), PEO24-R-CNP22(SEQ ID NO: 40), and PEO12-R-CNP22(SEQ ID NO: 40), wherein PEO24 is a monodispersed 1.2 kDa PEG polymer and PEO12 is a monodispersed 0.6 kDa PEG polymer. In an embodiment, the PEG (or PEO) polymer is attached to the N-terminus of the CNP variants.

Additional CNP variants include, but are not limited to, derivatives of CNP37 having mutation(s) at the furin cleavage site (underlined), designed to improve in vivo resistance to the furin protease, and/or having glycine (underlined) preceding glutamine, designed to prevent pyroglutamine formation, including but not limited to:

(SEQ ID NO: 71)
GQEHPNARKYKGANPKGLSKGCFGLKLDRIGSMSGLGC (An. CS);

(SEQ ID NO: 72)
GQEHPNARKYKGANQKGLSKGCFGLKLDRIGSMSGLGC (An. CT);

(SEQ ID NO: 73)
GQEHPNARKYKGANQQGLSKGCFGLKLDRIGSMSGLGC (An. CU);

(SEQ ID NO: 74)
GQEHPNARKYKGANKPGLSKGCFGLKLDRIGSMSGLGC (An. CW);

(SEQ ID NO: 75)
GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC
(Gly-CNP37, An. DB);

(SEQ ID NO: 145)
PGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC
(Pro-Gly-CNP37).

In another embodiment, the CNP variants are chimera comprising CNP22 and an N-terminal peptide fragment, including but not limited to:

(SEQ ID NO: 76)
GHHSHEQHPHGANQQGLSKGCFGLKLDRIGSMSGLGC (Analog CQ)
(histidine-rich glycoprotein (HRGP) fragment-
CNP22 chimera);

(SEQ ID NO: 77)
GAHHPHEHDTHGANQQGLSKGCFGLKLDRIGSMSGLGC (Analog CR)
(HRGP fragment-CNP22 chimera);

(SEQ ID NO: 78)
GHHSHEQHPHGANPRGLSKGCFGLKLDRIGSMSGLGC (Analog CX)
(HRGP fragment-CNP22 chimera);

(SEQ ID NO: 79)
GQPREPQVYTLPPSGLSKGCFGLKLDRIGSMSGLGC (Analog CF)
(IgG_1(F) fragment-CNP22 chimera);

(SEQ ID NO: 80)
GQHKDDNPNLPRGANPRGLSKGCFGLKLDRIGSMSGLGC (Analog
CY) (human serum albumin (HSA) fragment-CNP22
chimera);

(SEQ ID NO: 81)
GERAFKAWAVARLSQGLSKGCFGLKLDRIGSMSGLGC (Analog CE)
(HSA fragment-CNP22 chimera);

-continued

(SEQ ID NO: 82)
FGIPMDRIGRNPRGLSKGCFGLKLDRIGSMSGLGC (Analog CZ)
(osteocrin "NPR C inhibitor" fragment-CNP22
chimera);
and

(SEQ ID NO: 83)
GKRTGQYKLGSKTGPGPKGLSKGCFGLKLDRIGSMSGLGC
(Analog DA) (FGF2 "heparin-binding domain"
fragment-CNP22 chimera).

In a further embodiment, the CNP variants are chimera comprising an N-terminal peptide fragment and CNP22 in which arginine is substituted for Lys4 of CNP22 ("CNP22 (K4R)"), including but not limited to:

(SEQ ID NO: 84)
GQPREPQVYTGANQQGLSRGCFGLKLDRIGSMSGLGC (Analog CK)
(IgG_1(F) fragment-CNP22(K4R) chimera);

(SEQ ID NO: 85
GVPQVSTSTGANQQGLSRGCFGLKLDRIGSMSGLGC (Analog CL)
(HSA fragment-CNP22(K4R) chimera)

(SEQ ID NO: 86)
GQPSSSSQSTGANQQGLSRGCFGLKLDRIGSMSGLGC
(Analog CM) (fibronectin fragment-CNP22(K4R)
chimera);

(SEQ ID NO: 87)
GQTHSSGTQSGANQQGLSRGCFGLKLDRIGSMSGLGC
(Analog CN) (fibrinogen fragment-CNP22(K4R)
chimera);

(SEQ ID NO: 88)
GSTGQWHSESGANQQGLSRGCFGLKLDRIGSMSGLGC
(Analog CO) (fibrinogen fragment-CNP22(K4R)
chimera);
and

(SEQ ID NO: 89)
GSSSSSSSSSGANQQGLSRGCFGLKLDRIGSMSGLGC
(Analog CP) (zinc finger fragment-CNP22(K4R)
chimera).

In yet another embodiment, the CNP variants are chimera, or fusion proteins, comprising a CNP peptide or variant, and a cleavable peptide or protein, or peptide tag. Exemplary cleavable proteins or peptides include, but are not limited to, histidine (e.g., hexa-His) tags; TAF12: human transcription factor TAF12; KSI: ketosteroid isomerase; MBP: maltose-binding protein; β-Gal: β-galactosidase; GST: glutathione-S-transferase; Trx: thioredoxin; CBD: chitin binding domain; BMPM: BMP-2 mutation, SUMO, CAT, TrpE, staphylococcal protein A, streptococcal proteins, starch-binding protein, cellulose-binding domain of endoglucanase A, cellulose-binding domain of exoglucanase Cex, biotin-binding domain, recA, Flag, c-Myc, poly(His), poly(Arg), poly(Asp), poly (Gln), poly(Phe), poly(Cys), green fluorescent protein, red fluorescent protein, yellow fluorescent protein, cyan fluorescent protein, biotin, avidin, streptavidin, antibody epitopes, and fragments thereof.

In yet another embodiment, the CNP variant may be a monomer or a dimer. In a related embodiment the monomers of dimeric CNP variants can be attached N-terminus to N-terminus via a linker or no linker, N-terminus to C-terminus via a linker or no linker, or C-terminus to C-terminus via a linker or no linker.

Chimera comprising an IgG fragment and CNP22 or a variant thereof are designed for, inter alia, increased resistance to NEP degradation and reduced binding to serum albumin. CNP chimera comprising a surface fragment of HSA are designed for, inter alia, reduced immunogenicity and reduced binding to serum albumin. HRGP-CNP22 and HRGP-CNP22(K4R) chimera containing a cationic, histidine-rich, non-lysine, non-arginine sequence at the N-terminus are designed for, inter alia, increased stability to proteases. Chimera containing an osteocrin fragment are designed to release, upon protease (e.g., furin) cleavage, the osteocrin fragment at bone growth plates, where the fragment would inhibit the clearance receptor NPR-C. With respect to chimera comprising an FGF2 heparin-binding fragment, heparin binding to the fragment is designed to protect the chimera from degradation, thereby providing a longer serum half-life. Chimera containing a fibronectin, fibrinogen, or zinc-finger fragment are designed for reduced binding to serum albumin, among other advantageous features.

Not intending to be bound by theory, a CNP variant of molecular weight from about 2.6 kDa or 2.8 kDa to about 6 or 7 kDa which has increased resistance to NEP degradation and has similar or improved functionality (e.g., binding to NPR-B and stimulation of cGMP signaling) as compared to wtCNP22, may be more effective if it does not bind tightly to plasma proteins such as serum albumin. A CNP variant that does not bind tightly to plasma proteins (e.g., serum albumin) may be more effective in diffusing through cartilage, getting to chondrocytes of bone growth plates, and binding to and activating NPR-B for cGMP signaling. In one embodiment, CNP variants designed for reduced binding to plasma proteins (e.g., serum albumin) are chimeras comprising CNP22 or a variant thereof and a peptide fragment from IgG. In another embodiment, CNP variants designed for reduced binding to plasma proteins are chimeras comprising CNP22 or CNP22(K4R) and a fragment from a polypeptide (e.g., IgG, HSA, fibronectin, fibrinogen, a zinc finger-containing polypeptide, etc.). In yet another embodiment, CNP variants designed for reduced binding to plasma proteins comprise CNP22 or a variant thereof conjugated to a hydrophilic or water-soluble polymer. In one embodiment, the hydrophilic or water-soluble polymer is PEG (or PEO). In another embodiment, the hydrophilic or water-soluble polymer (e.g., PEG) is functionalized with one or more functional groups that impart a negative charge to the polymer under physiological conditions, such as, e.g, carboxyl, sulfate or phosphate groups, or a combination thereof.

In any of the embodiments disclosed herein, the CNP variants may have substantially the same or better biological activity than wild-type CNP22. For example, the CNP variants may retain at least 50%, 60%, 70%, 80%, 90%, 95% or more of the activity of wild-type CNP22, or may have greater activity than CNP22, e.g., with respect to interaction with NPR-B (GC-B) to stimulate the generation of cGMP. Alternatively, or in addition, the CNP variants may retain at least 50%, 60%, 70%, 80%, 90%, 95% or more of the activity of wild-type CNP22, or may have greater activity than CNP22, with respect to regulating endochondral bone growth and chondrocyte activity, including but not limited to chondrocyte proliferation, chondrocyte differentiation, inhibition of the mitogen activated protein (MAP) kinase/MEK (Raf-1) kinase signaling pathway, and promoting endochondral ossification. In any of the embodiments described herein, the CNP variants may comprise an amino acid sequence that is at least 40%, 50%, 60%, 70%, 80%, 90%, 95% or more identical or homologous to amino acids 6-22 or 1-22 of wild-type CNP22.

In a further embodiment, the disclosure provides variants of CNP22 having less affinity to the NPR-C clearance receptor while retaining the ability to bind and activate

NPR-B. The present disclosure encompasses variants that were, or can be, generated from a homology-based structural model of the NPR-B/CNP complex as described in the Detailed Description. In another embodiment, the CNP variants have substitution(s) at one or more Gly sites at positions 1, 5, 8, 15, 19 and 21, to reduce conformational flexibility, which may increase their specificity for binding to NPR-B over NPR-C. Variants of CNP having potentially reduced affinity to the NPR-C include but are not limited to those having one or more of the following substitutions: G1R, G1E, G5R, G5Q, G5S, F7Y, G8T, G8S, G8V, G8N, L9S, L9T, K10Cit, K10Q, K10S, I14N, G15R, G15S, G15N, G15Cit, S16Q, M17V, M17N, G19S, G19R, G19N, L20V, L20R, L20T, L20S, G21S, G21T and G21R.

In yet another embodiment, the CNP variants have a modification and/or substitution at one or more of positions 5, 7, 8, 9, 10, 14, 15, 16, 17, 19, 20 and 21, and may optionally have modifications and/or substitutions at any of the other positions disclosed herein. In a further embodiment, the CNP variants can optionally have conjugation(s) or extension(s), e.g., at the N- and/or C-terminus to facilitate bone/cartilage targeting, reduce renal clearance, and/or increase resistance to NEP degradation. Such conjugation(s) or extension(s) can comprise molecules or sequences formed or derived from, e.g., polyAsp, polyGlu, bone- or cartilage-targeting peptides, osteopontin, osteocalcin, sialoprotein, PEGs, carbohydrates, hydrophobic acids, NPPC or non-CNP (poly)peptides, or combinations thereof.

In still another embodiment, the CNP variants are prepared by standard solid-phase peptide synthesis methods with natural or unnatural amino acid(s) or peptidomimetic(s) being substituted and/or added where appropriate. In another embodiment, the CNP variants are produced by recombinant synthesis processes, e.g., via fusion proteins containing a tag or carrier protein, wherein use of the tag or carrier protein facilitates, e.g., detection, isolation and/or purification of the fusion protein, and selective chemical or proteolytic cleavage of the tag or carrier protein from the fusion protein provides the target CNP variant. In a further embodiment, PEGylation of the CNP variants occurs following, or part of, chemical or biological synthesis with the conjugation reaction being performed by NHS- or aldehyde-based chemistry or other chemistry known in the art. In another embodiment, the CNP variants comprise a disulfide bond. In a related embodiment, the disulfide bond forms a cyclic peptide. In a particular embodiment, the disulfide bond is formed between cysteine residues at positions corresponding to positions 6 and 22 of CNP22.

It is further contemplated that the CNP variants can be conjugated to a hydrophobic polymeric or non-polymeric moiety, such as, e.g., heptanoic acid, pentanoic acid, or fatty acids. The hydrophobic moiety can be conjugated to the side chain of an amino acid residue, including but not limited to a lysine, a serine, a cysteine or a threonine, or can be attached to the N-terminus and/or C-terminus of the CNP variant.

In an embodiment, the CNP variants as described herein have a pI in the range from about 8 to about 10.5 or from about 8.5 to about 10.

In a further embodiment, the disclosure provides a pharmaceutical composition comprising a CNP variant, optionally another biologically active agent, and optionally a pharmaceutically acceptable excipient, carrier or diluent. In some embodiments, the compositions are sterile pharmaceutical compositions suitable for parenteral injection. In some embodiments, the compositions comprise substantially pure CNP variant, e.g. at least about 90% or 95% pure. In some embodiments, the compositions contain less than about 5%, 4%, 3%, 2%, 1% or 0.5% contaminants, such as other human proteins, porcine proteins, or CNP53 or fragments thereof (other than the desired CNP variant). In certain embodiments, the sterile composition is administered to a subject for treating or preventing any of the CNP-responsive conditions or disorders disclosed herein.

CNP variants of the disclosure advantageously retain CNP activity and exhibit increased serum half-life. Retention of CNP activity can be shown, for example, as retention of desired in vivo biological effect, or retention of at least about 50%, 60%, 70%, 80%, 90%, 95% or at least about 100% of the cGMP stimulating activity of CNP22, under the same concentration (e.g., 1 uM of CNP peptide or greater than the ED80). In some embodiments, CNP variants exhibit at least about 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, 15-fold, 20-fold, 25-fold, 30-fold, 35-fold or 40-fold increase in serum half-life compared to CNP22.

In a related embodiment, the CNP variants described herein have increased NEP resistance and exhibit increased half-life compared to wild-type CNP22. In one embodiment, the half-life of the CNP variants is increased by about 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or about 100% compared to wild-type CNP22.

In certain embodiments, the CNP variants described herein increase cGMP production in vitro, increase cGMP production in vivo, increase in vivo the level of one or more biomarkers associated with cartilage or bone formation or growth, increase resistance to NEP cleavage in vitro, increase plasma or serum half-life in vivo, increase bioavailability in vivo, or increase the length of particular bones in vivo, or effect combinations of such increases, by about 1.5-fold, about 2-fold, about 2.5-fold, about 3-fold, about 3.5-fold, about 4-fold, about 4.5-fold, or about 5-fold or more compared to wild-type CNP22.

In yet another embodiment, the disclosure provides methods of treating conditions or disorders responsive to CNP, comprising administering a therapeutically effective amount of a CNP variant or a composition comprising the same to a subject in need thereof. In one embodiment, disorders responsive to CNP are disorders of bone growth, including but not limited to skeletal dysplasias and inherited skeletal malformations such as disorders associated with fibroblast growth factor receptor 3 (FGFR-3) mutations. In a specific embodiment, the disorder associated with FGFR-3 mutation(s) is achondroplasia. In another embodiment, the disorders responsive to CNP are disorders associated with vascular smooth muscle cells and tissues. In a further embodiment, the CNP variants are useful for increasing the size of the growth plate of a bone (e.g., a limb bone). In another embodiment, the CNP variants are useful for elongating a bone or increasing long bone growth. In still another embodiment, the CNP variants are useful for enhancing matrix production, proliferation and differentiation of chondrocytes.

In certain embodiments, the CNP variants described herein are administered at a dose in the range from about 5 or 10 nmol/kg to about 300 nmol/kg, or from about 20 nmol/kg to about 200 nmol/kg. In some embodiments, the CNP variants are administered at a dose of about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 125, 150, 175, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 350, 400, 450, 500, 750, 1000, 1250, 1500, 1750 or 2000 nmol/kg or other dose deemed appropriate by the treating physician. In other embodiments, the CNP variants are administered at a dose of about 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170,

180, 190, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750 or 800 ug/kg, or about 1 mg/kg, 1.25 mg/kg, 1.5 mg/kg, 2 mg/kg or other dose deemed appropriate by the treating physician. The doses of CNP variants described herein can be administered according to the dosing frequency/frequency of administration described herein, including without limitation daily, 2 or 3 times per week, weekly, every 2 weeks, every 3 weeks, monthly, etc.

In another embodiment, the CNP variants are administered in a single treatment or in multiple doses. The multiple doses may be administered daily, or in multiple doses over the course of treatment. In certain embodiments, it is contemplated that the CNP variant is administered, in a single dose or in multiple doses, daily, every other day, every 3 days, 2 times per week, 3 times per week, weekly, bi-weekly, every 3 weeks, monthly, every 6 weeks, every 2 months, every 3 months or as deemed appropriate hy a treating physician.

In certain embodiments, administration of the CNP variant is adjusted to allow for periods of growth (e.g., chondrogenesis), followed by a recovery period (e.g., osteogenesis). For example, the CNP variant may be administered subcutaneously, intravenously, or by another mode daily or multiple times per week for a period of time, followed by a period of no treatment, then the cycle is repeated. In some embodiments, the initial period of treatment (e.g., administration of the CNP variant daily or multiple times per week) is for 3 days, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks or 12 weeks. In a related embodiment, the period of no treatment lasts for 3 days, 1 week, 2 weeks, 3 weeks or 4 weeks. In certain embodiments, the dosing regimen of the CNP variant is daily for 3 days followed by 3 days off; or daily or multiple times per week for 1 week followed by 3 days or 1 week off; or daily or multiple times per week for 2 weeks followed by 1 or 2 weeks off; or daily or multiple times per week for 3 weeks followed by 1, 2 or 3 weeks off; or daily or multiple times per week for 4, 5, 6, 7, 8, 9, 10, 11 or 12 weeks followed by 1, 2, 3 or 4 weeks off.

In additional embodiments, the disclosure provides a method of treating a CNP-responsive condition or disorder, comprising administering a CNP peptide or variant to a subject, and monitoring the level of at least one bone- or cartilage-associated biomarker in the subject (e.g., in a biological sample from the subject), wherein an increase or decrease in the level of the bone- or cartilage-associated biomarker indicates a therapeutic effect of the CNP peptide or variant on the subject. In some embodiments, when the level of a biomarker increases in association with bone or cartilage formation or growth, an increase in the level of that biomarker indicates a therapeutic effect of the CNP peptide or variant on the subject. In other embodiments, when the level of a biomarker decreases in association with bone or cartilage formation or growth, a decrease in the level of that biomarker indicates a therapeutic effect of the CNP peptide or variant on the subject.

In further embodiments, the therapeutic method further comprises adjusting the amount (or dose) or frequency of administration of the CNP peptide or variant, wherein:

(i) the amount (or dose) or frequency of administration of the CNP peptide or variant is increased if the level of the at least one bone- or cartilage-associated biomarker is below a target level, where the level of the biomarker increases in association with bone or cartilage formation or growth; or

(ii) the amount (or dose) or frequency of administration of the CNP peptide or variant is decreased if the level of the at least one bone- or cartilage-associated biomarker is above a target level, where the level of the biomarker increases in association with bone or cartilage formation or growth; or

(iii) the amount (or dose) or frequency of administration of the CNP peptide or variant is increased if the level of the at least one bone- or cartilage-associated biomarker is above a target level, where the level of the biomarker decreases in association with bone or cartilage formation or growth; or

(iv) the amount (or dose) or frequency of administration of the CNP peptide or variant is decreased if the level of the at least one bone- or cartilage-associated biomarker is below a target level, where the level of the biomarker decreases in association with bone or cartilage formation or growth.

It is contemplated that the target level of a biomarker refers to the level or range of levels of the biomarker that is associated with therapeutic effect in the subject and/or beneficial effect in alleviating or ameliorating symptoms of the disorder or condition. In certain embodiments, a level of a biomarker above or below a target level may be deleterious to the subject.

In other embodiments, the disclosure contemplates a method for assessing the effect of administration of a CNP peptide or variant on bone or cartilage formation or growth. In one embodiment, the method provides for assaying or measuring the level of at least one bone- or cartilage-associated biomarker in a subject that has been administered a CNP peptide or variant in order to assess the effect of the CNP peptide or variant on bone and cartilage formation and growth in vivo. In a related embodiment, an increase in the level of the at least one bone- or cartilage-associated biomarker may indicate that administration of a CNP peptide or variant has a positive effect on bone or cartilage formation or growth and is a useful treatment for skeletal dysplasias and other bone- or cartilage-related diseases or disorders associated with decreased CNP activity. Exemplary bone- or cartilage-associated biomarkers include, but are not limited to, CNP (e.g, endogenous level of CNP-22 or CNP-53), cGMP, osteocalcin, proliferating cell nuclear antigen (PCNA), propeptides of type I procollagen (PINP) and fragments thereof, collagen type I and fragments thereof, propeptides of collagen type II and fragments thereof, collagen type II and fragments thereof, aggrecan chondroitin sulfate, and alkaline phosphatase.

In further embodiments, the disclosure contemplates a method for assessing the effect of a CNP peptide or variant on the level of at least one bone- or cartilage-associated biomarker in a subject, comprising assaying or measuring the level of the bone- or cartilage-associated biomarker in a biological sample from a subject that has been administered a CNP peptide or variant. In some embodiments, the method further comprises administering the CNP peptide or variant to the subject before assaying or measuring the level of the bone- or cartilage-associated biomarker.

In certain embodiments, the at least one bone- or cartilage-associated biomarker is selected from the group consisting of CNP (e.g, endogenous level of CNP-22 or CNP-53), cGMP, propeptides of collagen type II and fragments thereof, collagen type II and fragments thereof, osteocalcin, proliferating cell nuclear antigen (PCNA), propeptides of type I procollagen (PINP) and fragments thereof, collagen type I and fragments thereof, aggrecan chondroitin sulfate, and alkaline phosphatase.

In some embodiments of methods (e.g., therapeutic, diagnostic and assay methods) relating to bone- or cartilage-

associated biomarkers, the CNP peptide or variant is CNP-22, CNP-53, or any of the CNP peptides and variants described herein. In certain embodiments of such methods, the CNP peptide or variant is not CNP-22 or CNP-53.

In other embodiments, the disclosure provides a method for recombinant production of a CNP variant, comprising culturing in a medium a host cell comprising a polynucleotide encoding a CNP variant peptide linked to a polynucleotide encoding a cleavable peptide or protein, under conditions that result in expression of a fusion polypeptide encoded by the polynucleotides. In a related embodiment, the host cell is transformed with an expression vector comprising a polynucleotide encoding a CNP variant peptide linked to a polynucleotide encoding a cleavable peptide or protein.

In one embodiment, the vector is a plasmid. In still another embodiment, the plasmid is selected from the group consisting of pET-21a, pJexpress, pET-31b, pET-15b, pET-32a, pET-41a, pMAL, pQE-30, pET-SUMO, pET-22b, and pTYB11.

In certain embodiments, the cleavable peptide or protein comprises a polypeptide that is selected from the group consisting of a histidine tag, human transcription factor TAF12, ketosteroid isomerase, maltose-binding protein, β-galactosidase, glutathione-S-transferase, thioredoxin, chitin binding domain, and BMP-2 mutation, or fragments thereof.

In a related embodiment, the cleavable peptide or protein is cleaved by a cleaving agent. In some embodiments, the cleaving agent is selected from the group consisting of formic acid, cyanogen bromide (CNBr), hydroxylamine, protein self cleavage, Factor Xa, enterokinase, ProTEV, and SUMO protease. Additional exemplary cleaving agents include, but are not limited to, palladium, clostripain, thrombin, chymotrypsin, trypsin, trypsin-like proteases, carboxypeptidase, enteropeptidase, Kex 2 protease, Omp T protease, subtilisin, V8 protease, HIV protease, rhinovirus protease, furilisin protease, IgA proteases, human Pace protease, collagenase, Nia protease, poliovirus 2Apro protease, poliovirus 3C protease, genenase, furin, elastase, Proteinase K, pepsin, rennin (chymosin), microbial aspartic proteases, papain, calpain, chymopapain, ficin (ficain), bromelain (bromelase), cathespisin B, caspases, thermolysin, Endoprotease Arg-C, Endoprotease Glu-C, Endoprotease Lys-C, kallikrein, and plasmin.

In certain embodiments, the fusion polypeptide is expressed as a soluble protein or as an inclusion body. In a related embodiment, the disclosure contemplates isolating the expressed fusion polypeptide from the host cell or culture medium. In a further embodiment, the isolated fusion polypeptide is contacted with a cleaving agent as described herein.

In one embodiment, the disclosure provides a bacterial host cell comprising an expression vector, said vector comprising a polynucleotide encoding a CNP variant peptide linked to a polynucleotide encoding a cleavable peptide or protein. In some embodiments, the cleavable peptide or protein is selected from the group consisting of a histidine tag, human transcription factor TAF12, ketosteroid isomerase, maltose-binding protein, β-galactosidase, glutathione-S-transferase, thioredoxin, chitin binding domain, and BMP-2 mutation, or fragments thereof.

In another embodiment, the host cell is a bacteria, such as E. coli. In a related embodiment, the E. coli cell is selected from the group consisting of BL21(DE3), BL21(DE3)pLysS, BL21(DE3)pGro7, ArcticExpress(DE3), C41 [also called C41(DE3)], C43 [also called C43(DE3)], Origami B(DE3), Origami B(DE3)pLysS, KRX, and Tuner(DE3). In still a further embodiment, the host cell comprises a vector as described above. In some embodiments, the host cell is transformed with the vector prior to cell culture.

In certain embodiments, it is contemplated that the host cell is cultured in a medium under conditions suitable for expression of a fusion polypeptide encoded by the polynucleotides. In one embodiment, the fusion polypeptide is expressed as a soluble protein or as inclusion body. In a related embodiment, the expressed fusion polypeptide is isolated from the host cell or culture medium. In still another embodiment, the isolated fusion polypeptide is contacted with a cleaving agent as described herein.

BRIEF DESCRIPTION OF THE FIGURES

FIG. **1** shows expression of CNP fusion proteins in E. coli (FIG. **1**A: Coomassie blue stain, FIG. **1**B: Western blot). M: protein marker; T: total cell lysates; S: soluble supernatants; P: 2 ug CNP22; KSI: KSI-CNP(M/N) fusion protein expression (insoluble); N: Un-induced KSI-CNP fusion protein total lysates; KSI': KSI-P-CNP fusion protein expression (insoluble); Trx: Trx-P-CNP fusion protein expression (soluble); MBP: MBP-P-CNP fusion protein expression (soluble); TAF: TAF-P-CNP fusion protein expression (insoluble) (BL21); TAF': TAF-P-CNP fusion protein expression (insoluble) BL21(DE3), where CNP is Gly-CNP37.

FIG. **2** shows formic acid cleavage of TAF-CNP inclusion bodies. M: protein marker; 1: Gly-CNP37 positive control; 2: uncleaved TAF-CNP inclusion bodies; 3: 2% formic acid cleaved TAF-CNP inclusion bodies, where CNP is Gly-CNP37.

FIGS. **3**A-E depict expression of CNP fusion proteins in E. coli. M: protein marker; Tu: total un-induced cell lysates; Su: un-induced soluble supernatants; T: total induced cell lysates; S: soluble supernatants; Cl: CNP22; C: Gly-wtCNP37 ("CNP38"); P: insoluble pellets. A: KSI: KSI-CNP38(M/N) fusion protein expression (Insoluble); KSI': KSI-Pro-CNP38 (Pro-Gly-wtCNP37 is designated "Pro-CNP38") fusion protein expression (Insoluble); Trx: Trx-Pro-CNP38 fusion expression (Soluble); MBP: MBP-Pro-CNP38 fusion protein expression (Soluble); TAF: TAF-Pro-CNP38 fusion protein expression (Insoluble) from BL21 cell; TAF': TAF-Pro-CNP38 fusion protein expression (Insoluble) from BL21(DE3) cell. B: TAF-Pro-CNP37 and BMP-Pro-CNP37 fusion protein expression. C: BMP-Pro-CNP38 fusion protein and BMP protein expression. D: TAF-Pro-HSA-CNP ("Pro-GH-KSEVAHRFK-wtCNP27 (SEQ ID NO: 188) is designated "Pro-HSA-CNP") fusion protein expression. E: TAF-Pro-CNP38 fusion protein and TAF protein expression.

FIGS. **4**A-C depict formic acid cleavage of TAF-Pro-CNP38 inclusion bodies. A: 50% formic acid cleavage of TAF-Pro-CNP38 inclusion bodies. M: protein marker; U: un-cleaved TAF-Pro-CNP38 inclusion bodies; 25° C., 37° C., 42° C., 55° C.: TAF-Pro-CNP38 inclusion bodies were cleaved in 50% formic acid at 25° C., 37° C., 42° C. or 55° C. for 24 hours. 37° C.-S and 55° C.-S: soluble supernatants from the cleavage reactions at 37° C. and 55° C. neutralized with 10N NaOH and centrifuged at 14,000 rpm for 15 minutes. B: 10% and 2% formic acid cleavage of TAF-Pro-CNP38 inclusion bodies. M: protein marker; U: un-cleaved TAF-Pro-CNP38 inclusion bodies; C: formic acid cleaved TAF-Pro-CNP38; S: soluble supernatant after centrifuged at 14,000 rpm for 5 minutes without neutralization; P: insoluble pellet after centrifuged at 14,000 rpm for 5 minutes

without neutralization. C: LC/MS analysis of 2% and 10% formic acid cleaved products from TAF-Pro-CNP38 inclusion bodies.

FIGS. **5**A-C depict formic acid cleavage of TAF-Pro-CNP38 inclusion bodies at different temperature and time of formic acid cleavage. M: protein marker; C: Gly-wtCNP37 ("CNP38") positive control; U: un-cleaved TAF-Pro-CNP38 inclusion bodies. A: 2% formic acid cleaved TAF-Pro-CNP38 at 42° C., 55° C. or 70° C. for 6, 24 or 48 hours. B: 2% formic acid cleaved TAF-Pro-CNP38 at 55° C., 60° C., 65° C. or 70° C. for 17 or 24 hours. C: LC/MS analysis of 2% formic acid cleaved products from TAF-Pro-CNP38 inclusion bodies.

FIG. **6** is an SDS-PAGE (Coomassie blue stain) for the expression of a TAF-CNP34 fusion protein. M: protein marker; C: control [Gly-CNP37 ("CNP38")]; T: total cell lysates; S: supernatant; TI: total cell lysates induced; SI: supernatant induced.

FIG. **7** is an SDS-PAGE (Coomassie blue stain) for the expression of fusion proteins TAF-NL-(C/A & 6D/6E)-Pro-CNP38, TAF(C/A & 10D/10E)-Pro-CNP38, and TAF-Pro-CNP53, where "Pro-CNP38" denotes Pro-Gly-CNP37. M: protein marker; C: control [Gly-CNP37 ("CNP38")]; T: total cell lysates; TI: total cell lysates induced; S: supernatant; SI: supernatant induced.

FIG. **8** is an SDS-PAGE (Coomassie blue stain) of the products of formic acid cleavage of the fusion proteins TAF-CNP34 and TAF-Pro-CNP53. M: protein marker; P: positive control [Gly-CNP37 ("CNP38")]; U: uncleaved; C: cleaved; CS: cleaved supernatant; CP: cleaved pellet.

FIG. **9** is an LC/MS chromatogram showing the peak for CNP-34 after formic acid cleavage of TAF-CNP34.

FIG. **10** is an LC/MS chromatogram showing the peak for Pro-CNP53 after formic acid cleavage of TAF-Pro-CNP53.

FIG. **11** is an SDS-PAGE (Coomassie blue stain) for the expression of fusion proteins TAF(C/A & 4D/4E)-Pro-CNP38 and TAF(4D/4E)-Pro-CNP38, where "Pro-CNP38" denotes Pro-Gly-CNP37. M: protein marker; C: control [Gly-CNP37 ("CNP38")]; T: total cell lysates; TI: total cell lysates induced; S: supernatant; SI: supernatant induced.

FIG. **12** is an SDS-PAGE (Coomassie blue stain) of the products of formic acid cleavage of the fusion proteins TAF (4D/4E)-Pro-CNP38 and TAF(C/A & 4D/4E)-Pro-CNP38, where "Pro-CNP38" denotes Pro-Gly-CNP37. M: protein marker; P: positive control [Gly-CNP37 ("CNP38")]; U: uncleaved; C: cleaved; CS: cleaved supernatant; CP: cleaved pellet.

FIG. **13** is an SDS-PAGE (Coomassie blue stain) of the products of formic acid cleavage of the fusion proteins TAF-NL-(C/A & 6D/6E)-Pro-CNP38 and TAF(C/A & 10D/10E)-Pro-CNP38, where "Pro-CNP38" denotes Pro-Gly-CNP37. M: protein marker; P: positive control [Gly-CNP37 ("CNP38")]; U: uncleaved; C: cleaved; CS: cleaved supernatant; CP: cleaved pellet.

FIG. **14** is a Western blot, using an anti-CNP antibody, of a TAF-Pro-CNP38 fusion protein produced in a 10 L fermentation of BL21(DE3) cells, where the cells were induced at OD_{600}=64 and hour 17 and "Pro-CNP38" denotes Pro-Gly-CNP37.

FIG. **15** is an SDS-PAGE (Coomassie blue stain) of eluate fractions from SP-Sepharose cation-exchange column chromatography of a cruder Pro-Gly-CNP37 ("Pro-CNP38") product. A: TAF-Pro-CNP38 inclusion body (IB) in water; B: IB in formate; C: IB in formate, neutralized; D: neutralized pellet; E: neutralized supernatant; F: TMAE Hi-CAP load; G: TMAE Hi-CAP flow through/SP-Sepharose load; H: SP-Sepharose flow through; SP-Sepharose eluate fractions 1-47: 10 uL/lane.

FIG. **16** shows the degree of resistance of N-terminal PEGylated CNP22 conjugates to neutral endopeptidase (NEP) in vitro.

FIG. **17** depicts the degree of NEP resistance of CNP variants having an amino acid extension at the N-terminus ["CNP27" is GANRR-CNP22(K4R) (SEQ ID NO: 36)].

FIG. **18** illustrates the degree of NEP resistance of N-terminal PEGylated CNP17 and GANRR-CNP22(K4R) ("CNP27") (SEQ ID NO: 36).

FIG. **19** illustrates the degree of NEP resistance of wtCNP22 and CNP variants Gly-CNP37, GHKSEVAH-RFK-wtCNP27 ("CNP27-HSA" in the figures) (SEQ ID NO: 144) and PEO12-GANRR-CNP22(K4R) ("CNP27-PEO12" in the figures) (SEQ ID NO: 36).

FIG. **20** shows the ability of CNP variants having an N-terminal amino acid extension to stimulate cGMP production in NIH3T3 cells in vitro. The results are relative to the level of cGMP produced in the presence of 1 uM CNP22. "CNP27" is GANRR-CNP22(K4R) (SEQ ID NO: 36)

FIG. **21** displays the ability of N-terminal PEGylated CNP17 and GANRR-CNP22(K4R) ("CNP27") (SEQ ID NO: 36) to stimulate cGMP production in NIH3T3 cells.

FIG. **22** illustrates the effects of N-terminal PEGylation of CNP22 on cGMP production.

FIG. **23** illustrates the cGMP production induced by wtCNP22 and CNP variants Gly-CNP37, GHKSEVAH-RFK-wtCNP27 ("CNP27-HSA") (SEQ ID NO: 144), wtCNP29 and PEO12-GANRR-CNP22(K4R) ("CNP27-PEO12") (SEQ ID NO: 36) in NIH3T3 cells.

FIGS. **24**A and B show that CNP-22 and Pro-Gly-CNP37 ("Pro-CNP38") stimulated cGMP production through NPR-B with similar dose-response curves, and to a much greater extent than through NPR-A, and exhibited a similar profile for NPR-B vs. NPR-C selectivity in in vitro signaling competition assays.

FIG. **25** demonstrates that exposure of rat chondrosarcoma cells to CNP22 1 hour once daily or 2 hours once daily has substantially similar effectiveness in reversing FGF2-induced arrest of chondrocyte growth as continuous exposure to CNP22.

FIGS. **26**A and B show results from a dose response study of CNP22 effects on FGF2-arrested rat chondrosarcoma (RCS) cells.

FIGS. **27**A-D show that addition of CNP22 to RCS cells arrested by FGF2 increases matrix synthesis and partly inhibits FGF2, as assessed by ^{35}S-sulfate and ^{3}H-Pro incorporation into or decrease from matrix. Panels A and C, synthesis; B and D, degradation; A and B, ^{35}S measurement; C and D, ^{3}H measurement. Statistically significant differences are high-lighted (ANOVA; *$p<0.05$, **$p<0.01$).

FIGS. **28**A-C show the levels of aggrecan and fibronectin production (mRNA, panels A and C, and protein, panel B) in RCS cells cultured with FGF2 and CNP22.

FIG. **29** shows the efficacy of CNP37 and PEO24-GANRR-CNP22(K4R) ("CNP27-PEO24" in the figures) (SEQ ID NO: 36) in stimulating longitudinal growth of wild-type femur in an ex vivo mouse organ model.

FIG. **30** shows longitudinal bone growth of 2-3 day-old wild-type mouse tibias treated with CNP22, CNP37 or PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) every two days. Results are normalized to measurements prior to treatment (day 0). Data is represented as means±SEM (n=8).

FIG. **31** shows longitudinal bone growth of 2-3 day old achondroplastic $FGFR3^{ach}$ mouse tibias treated with

CNP22, CNP37 or PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) every two days. Results are normalized to measurements prior to treatment (day 0). Data is represented as means±SEM (n=7-8).

FIG. **32** shows longitudinal bone growth of 2-3 day-old wild-type mouse femurs treated with CNP22, CNP37 or PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) every two days. Results are normalized to measurements prior to treatment (day 0). Data is represented as means±SEM (n=8).

FIG. **33** shows longitudinal bone growth of 2-3 day-old FGFR3^{ach} mouse femurs treated with CNP22, CNP37 or PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) every two days. Results are normalized to measurements prior to treatment (day 0). Data is represented as means±SEM (n=3-7).

FIGS. **34**A-I depict CNP37 biodistribution in FGFR3^{ach} mouse femurs treated ex vivo every two days. Panels A-C illustrate distribution in distal femurs, panels D-F illustrate distribution in articular chondrocytes and panels G-I illustrate distribution in hypertrophic chondrocytes.

FIGS. **35**A-C depict the cellularity of proliferating columns in growth plates after treatment of wild-type and FGFR3^{ach} mouse femurs with CNP22 or CNP37 every two days for 8 days. (A) no treatment, (B) cell numbers per column after treatment, (C) morphological studies after treatment. The panels in FIGS. **35**C(i) to C(vi). correspond to the sample order set out in FIG. **35**B. Data is represented as means±SEM (n=4-8).

FIGS. **36**A-C depict chondrocyte hypertrophy after ex vivo treatment of wild-type and FGFR3^{ach} mouse femurs with CNP22 or CNP37 every two days for 8 days. (A) no treatment, (B) cell size after treatment, (C) morphological studies after treatment. The panels in FIGS. **36**C(i) to C(vi) correspond to the sample order set out in FIG. **36**B. Data is represented as means±SEM (n32 4-9).

FIGS. **37**A-I depict the biodistribution of CNP37 in FGFR3^{ach} mouse tibias treated in vivo. Panels A-C show distribution in distal femurs, panels D-F illustrate distribution in articular chondrocytes and panels G-I illustrate distribution in hypertrophic chondrocytes.

FIGS. **38**A-C illustrate the in vivo effects of CNP37 on FGFR3^{ach} mouse tibia growth plate: (A) total growth plate thickness, (B) proliferating zone thickness, and (C) hypertrophic zone thickness. Data is represented as means±SEM (n=7-15).

FIG. **39** shows cGMP levels in conditioned media from wild-type mouse femurs treated ex vivo with CNP22, CNP37 or PEO24-GANRR-CNP22(K4R) ("CNP27-PEO24") (SEQ ID NO: 36) ($p<0.01$).

FIG. **40** shows cGMP levels in conditioned media from FGFR3^{ach} mouse femurs treated ex vivo with CNP22, CNP37 or PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) ($p<0.01$).

FIG. **41** depicts cGMP levels in conditioned media from wild-type mouse tibias treated ex vivo with CNP22, CNP37 or PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) ($p<0.01$).

FIG. **42** shows cGMP levels in conditioned media from FGFR3^{ach} mouse tibias treated ex vivo with CNP22, CNP37 or PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) ($p<0.01$).

FIG. **43** demonstrates that ex vivo exposure of wild-type and FGFR3^{ach} mouse femurs to CNP22, CNP37 or PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) increased the levels of cleaved collagen type II in the conditioned media ($p<0.05$).

FIG. **44** depicts the hypertrophic region of femoral bones isolated from wild-type mice and FGFR3^{Y367C} mice (a mouse model of severe achondroplasia) and treated ex vivo with vehicle or 1 uM Pro-Gly-CNP37 ("ProCNP38") for 6 days, demonstrating that Pro-Gly-CNP37 treatment resulted in increase in bone growth and expansion in the growth plate.

FIG. **45** shows that CNP37 and PEO24-GANRR-CNP22 (K4R) (SEQ ID NO: 36) intravenously (i.v.) administered to rats have a much longer half-life and a much greater bioavailability in the plasma than CNP22.

FIG. **46** illustrates that PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) subcutaneously (s.c.) administered to rats also has a much longer half-life and a much greater bioavailability in the plasma than CNP22.

FIG. **47** demonstrates that i.v. administered CNP37 and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) stimulate a much greater level of cGMP production in rats than CNP22.

FIG. **48** shows that s.c. administered PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) and, to a lesser extent, CNP37 are substantially more effective in stimulating cGMP production in rats than CNP22.

FIG. **49** shows body weight measurements of wild-type mice treated with Gly-CNP37 or PEO12-GANRR-CNP22 (K4R) (SEQ ID NO: 36).

FIG. **50** shows tail length measurements of wild-type mice treated with Gly-CNP37 or PEO12-GANRR-CNP22 (K4R) (SEQ ID NO: 36).

FIG. **51** illustrates the effect of treatment of FGFR3^{ach} mice with CNP22, CNP37 or PEO24-GANRR-CNP22 (K4R) (SEQ ID NO: 36) on body length ($p=0.02$, 1-tailed t-test, unequal variance).

FIG. **52** shows the effect of CNP22, CNP37 and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) on tail length in FGFR3^{ach} mice.

FIGS. **53**A and B show the effect of CNP22, CNP37 and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) on the length of distal long bones (A, ulna; B, tibia) in FGFR3^{ach} mice ($p<0.01$, one-tailed t-test, unequal variance).

FIGS. **54**A and B show the effect of CNP22, CNP37 and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) on the length of proximal bones (A, humerus; B, femur) in FGFR3^{ach} mice ($p<0.01$, one-tailed t-test, unequal variance).

FIG. **55** illustrates that CNP37 administration corrects rhizomelia (disproportion of the length of the proximal limbs) as assessed by the femur:tibia ratio observed in FGFR3^{ach} mice ($p<0.01$, one-tailed t-test, unequal variance).

FIG. **56** shows the effect of CNP22, CNP37 and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) on head length in FGFR3^{ach} mice ($p<0.01$ one-tailed t-test, unequal variance).

FIG. **57** shows that treatment of FGFR3^{ach} mice with CNP37 increases the size of the external auditory meatus (EAM) ($P=0.03$, one-tailed t-test, unequal variance).

FIG. **58** shows the effect of CNP22, CNP37 and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) on spinal length in achondroplasic mice, expressed as extension of vertebral bodies (e.g., lumbar vertebra 5).

FIG. **59** shows that treatment of FGFR3^{ach} mice with CNP37 or PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) results in increased cGMP plasma levels 15-min post-dose.

FIG. **60** illustrates the serum levels of cleaved collagen type II in FGFR3^{ach} mice treated 5 weeks with CNP22, CNP37 or PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36).

FIG. **61** shows the serum levels of osteocalcin in FGFR3^{ach} mice treated 5 weeks with CNP22, CNP37 or PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36).

FIG. **62** shows cGMP plasma levels 15-min post-dose from wild-type mice treated with Gly-CNP37 or PEO12-GANRR-CNP22(K4R) (SEQ ID NO: 36) ($p<0.05$).

FIG. **63** shows the serum levels of cleaved collagen type II in wild-type mice treated 5 weeks with Gly-CNP37 or PEO12-GANRR-CNP22(K4R) (SEQ ID NO: 36).

FIGS. **64-66** display the levels of cGMP, cleaved collagen type II and alkaline phosphatase after administration of vehicle or 20 nmol/kg or 70 nmol/kg Pro-Gly-CNP37 ("Pro-CNP38") to wild-type mice.

FIGS. **67** and **68** depict cleaved collagen type II and total alkaline phosphatase levels after administration of vehicle or Pro-Gly-CNP37 ("Pro-CNP38") under different dosing regimens.

FIG. **69** illustrates the relative increase in body length of Pro-Gly-CNP37 ("Pro-CNP38") and vehicle treated animals in two separate studies (S1 and S2) at Day 37 vs Day 1.

FIGS. **70**A and B show changes in bone mineral density (A) and bone mineral content (B) after administration of Pro-Gly-CNP37 ("Pro-CNP38").

FIG. **71** shows plasma cGMP levels 15 min after the last subcutaneous dose of CNP variant, Day 36, where Gly-wtCNP37 is "CNP38", Pro-Gly-wtCNP37 is "Pro-CNP38", and GHKSEVAHRFK-wtCNP27 (SEQ ID NO: 144) is "HSA-CNP27" in FIGS. **71-73**.

FIG. **72** shows serum levels of cleaved collagen type II from mice treated with Gly-CNP37, Pro-Gly-CNP37 or GHKSEVAHRFK-CNP27 (SEQ ID NO: 144).

FIG. **73** shows serum levels of alkaline phosphatase from mice treated with Gly-CNP37, Pro-Gly-CNP37 or GHKSEVAHRFK-CNP27 (SEQ ID NO: 144).

FIGS. **74**A and B depict desensitization of the cGMP response after acute (A) or chronic (B) treatment with 1 uM Gly-CNP37.

FIG. **75**A demonstrates that daily treatment of wild-type mice with 200 nmol/kg of Pro-Gly-CNP37 ("Pro-CNP38") for 8 days did not desensitize the cGMP response. FIG. **75**B shows that treatment of the mice with 200 nmol/kg of Pro-Gly-CNP37 once a day for two consecutive days potentiated the cGMP response.

FIGS. **76**A-D show that treatment of wild-type mice with 200 nmol/kg of Gly-CNP37 stimulated cGMP secretion in distal femurs (cartilage and bone) (A), femoral cortices (bone) (B), ear pinna (cartilage) (C), and kidney (D). FIGS. **76**E-H show that liver (E), heart (F), lung (G) and brain (H) tissues did not exhibit appreciable cGMP secretion in response to Gly-CNP37 relative to vehicle control at the studied time points.

FIGS. **77-82** show results from an on-going study in normal juvenile cynomolgus monkeys subcutaneously injected daily with vehicle or 10 or 36 ug/kg of Pro-Gly-CNP37. Both doses of Pro-Gly-CNP37 have increased growth plate width (FIG. **77**), increased right and left tibia lengths (FIGS. **78**A and B), increased leg length (FIG. **79**), increased arm length (FIG. **80**), increased body length (FIG. **81**), and increased the serum level of alkaline phosphatase (FIG. **82**).

FIG. **83** depicts the observed plot of degradation rate constant (K_{obs}) vs. pH at pH 3-8 and 5° C., 25° C. and 40° C. for Gly-CNP37 formulations.

DETAILED DESCRIPTION OF THE DISCLOSURE

The present disclosure relates to novel variants of CNP having reduced affinity to NEP and/or NPR-C, and reduced susceptibility to cleavage by NEP and/or clearance by NPR-C, pharmaceutical compositions comprising such CNP variants, and methods of using such

CNP variants to treat disorders responsive to CNP, including but not limited to bone-related disorders such as achondroplasia and disorders associated with vascular smooth muscle cells and tissues.

A. Definitions

Unless otherwise stated, the following terms used in this application, including the specification and claims, have the definitions given below.

As used in the specification and the appended claims, the indefinite articles "a" and "an" and the definite article "the" include plural as well as singular referents unless the context clearly dictates otherwise.

The term "about" or "approximately" means an acceptable error for a particular value as determined by one of ordinary skill in the art, which depends in part on how the value is measured or determined. In certain embodiments, the term "about" or "approximately" means within 1, 2, 3, or 4 standard deviations. In certain embodiments, the term "about" or "approximately" means within 30%, 25%, 20%, 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, or 0.05% of a given value or range. Whenever the term "about" or "approximately" precedes the first numerical value in a series of two or more numerical values, it is understood that the term "about" or "approximately" applies to each one of the numerical values in that series.

The terms "ambient temperature" and "room temperature" are used interchangeably herein and refer to the temperature of the surrounding environment (e.g., the room in which a reaction is conducted or a composition is stored). In certain embodiments, ambient temperature or room temperature is a range from about 15° C. to about 28° C., or from about 15° C. to about 25° C., or from about 20° C. to about 28° C., or from about 20° C. to about 25° C., or from about 22° C. to about 28° C., or from about 22° C. to about 25° C. In other embodiments, ambient temperature or room temperature is about 15° C., 16° C., 17° C., 18° C., 19° C., 20° C., 21° C., 22° C., 23° C., 24° C., 25° C., 26° C., 27° C. or 28° C.

Definition of standard chemistry terms may be found in reference works, including Carey and Sundberg, Advanced Organic Chemistry, 3rd Edition, Vols. A and B (Plenum Press, New York 1992). The practice of the present disclosure may employ, unless otherwise indicated, certain conventional methods of synthetic organic chemistry, mass spectrometry, preparative and analytical chromatography, protein chemistry, biochemistry, recombinant DNA technology and pharmacology, within the skill of the art. See, e.g., T. E. Creighton, Proteins: Structures and Molecular Properties (W.H. Freeman and Company, 1993); A. L. Lehninger, Biochemistry (Worth Publishers, Inc., 4th Edition, 2004); Sambrook, et al., Molecular Cloning: A Laboratory Manual (2nd Edition, 1989); Methods In Enzymology (S. Colowick and N. Kaplan eds., Academic Press, Inc.); Remington's Pharmaceutical Sciences, 18th Edition (Easton, Pa.: Mack Publishing Company, 1990).

All publications, patents and patent applications cited herein, whether supra or infra, are hereby incorporated by reference in their entirety.

The following amino acid abbreviations are used throughout the text:

Alanine: Ala (A)	Arginine: Arg (R)
Asparagine: Asn (N)	Aspartic acid: Asp (D)
Cysteine: Cys (C)	Glutamine: Gln (Q)
Glutamic acid: Glu (E)	Glycine: Gly (G)
Histidine: His (H)	Isoleucine: Ile (I)
Leucine: Leu (L)	Lysine: Lys (K)
Methionine: Met (M)	Phenylalanine: Phe (F)
Proline: Pro (P)	Serine: Ser (S)
Threonine: Thr (T)	Tryptophan: Trp (W)
Tyrosine: Tyr (Y)	Valine: Val (V)

"Polypeptide" and "protein" refer to a polymer composed of amino acid residues, related naturally occurring structural variants, and synthetic non-naturally occurring analogs thereof, linked via peptide bonds or peptide bond isosteres. Synthetic polypeptides can be synthesized, for example, using an automated polypeptide synthesizer. The terms "polypeptide" and "protein" are not limited to a minimum length of the product. The term "protein" typically refers to large polypeptides. The term "peptide" typically refers to short polypeptides. Thus, peptides, oligopeptides, dimers, multimers, and the like, are included within the definition. Both full-length proteins and fragments thereof are encompassed by the definition. The terms "polypeptide" and "protein" also include postexpression modifications of the polypeptide or protein, for example, glycosylation, acetylation, phosphorylation and the like. Furthermore, for purposes of the present disclosure, a "polypeptide" can include "modifications," such as deletions, additions, substitutions (which may be conservative in nature or may include substitutions with any of the 20 amino acids that are commonly present in human proteins, or any other naturally or non-naturally-occurring or atypical amino acids), and chemical modifications (e.g., addition of or substitution with peptidomimetics), to the native sequence. These modifications may be deliberate, as through site-directed mutagenesis, or through chemical modification of amino acids to remove or attach chemical moieties, or may be accidental, such as through mutations arising with hosts that produce the proteins or through errors due to PCR amplification.

Conventional notation is used herein to portray polypeptide sequences: the left-hand end of a polypeptide sequence is the amino-terminus; the right-hand end of a polypeptide sequence is the carboxyl-terminus.

"Conservative substitution" refers to substitution of an amino acid in a polypeptide with a functionally, structurally or chemically similar natural or unnatural amino acid. In one embodiment, the following groups each contain natural amino acids that are conservative substitutions for one another:

(1), Alanine (A) Serine (S), Threonine (T);

(2) Aspartic acid (D), Glutamic acid (E);

(3) Asparagine (N), Glutamine (Q);

(4) Arginine (R), Lysine (K);

(5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); and

(6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W).

In another embodiment, the following groups each contain natural amino acids that are conservative substitutions for one another:

(1) Glycine (G), Alanine (A);

(2) Aspartic acid (D), Glutamic acid (E);

(3) Asparagine (N), Glutamine (Q);

(4) Arginine (R), Lysine (K);

(5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V), Alanine (A);

(6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W); and

(7) Serine (S), Threonine (T), Cysteine (C).

In a further embodiment, amino acids may be grouped as set out below.

(1) hydrophobic: Met, Ala, Val, Leu, Ile, Phe, Trp;

(2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;

(3) acidic: Asp, Glu;

(4) basic: His, Lys, Arg;

(5) residues that influence backbone orientation: Gly, Pro; and

(6) aromatic: Trp, Tyr, Phe, His.

In one embodiment, the peptides or polypeptides described herein are generated via recombinant means, using a polynucleotide encoding a CNP variant. The disclosure thus encompasses polynucleotides encoding any of the CNP variants described herein, host cells or vectors comprising such polynucleotides, optionally linked to expression control sequences, and methods of using such polynucleotides, vectors or host cells to produce CNP variants of the disclosure. CNP variants expressed by such polynucleotides may be produced by methods including growing host cells in culture medium under conditions suitable for expression of the polynucleotide encoding a CNP variant, and isolating the expression product from the host cells or culture medium. Actual expression products may vary slightly from the encoded protein product depending on any post-translational processing.

"Polynucleotide" refers to a polymer composed of nucleotide units. Polynucleotides include naturally occurring nucleic acids, such as deoxyribonucleic acid ("DNA") and ribonucleic acid ("RNA") as well as nucleic acid analogs. The term "nucleic acid" typically refers to large polynucleotides. The term "oligonucleotide" typically refers to short polynucleotides, generally no greater than about 50 nucleotides. It will be understood that when a nucleotide sequence is represented by a DNA sequence (i.e., A, T, G, C), the nucleotide sequence also encompasses an RNA sequence (i.e., A, U, G, C) in which "U" replaces "T." "cDNA" refers to a DNA that is complementary or identical to an mRNA, in either single stranded or double stranded form.

"Expression control sequence" refers to a nucleotide sequence that regulates the expression of a nucleotide sequence operatively linked thereto. "Operatively linked" refers to a functional relationship between two parts in which the activity of one part (e.g., the ability to regulate transcription) results in an action on the other part (e.g., transcription of the sequence). Expression control sequences can include, for example and without limitation, sequences of promoters (e.g., inducible or constitutive), enhancers, transcription terminators, a start codon (i.e., ATG), splicing signals for introns, and stop codons.

"Recombinant polynucleotide" refers to a polynucleotide having sequences that are not naturally joined together. An amplified or assembled recombinant polynucleotide may be included in a suitable vector, and the vector can be used to transform a suitable host cell. A host cell that comprises the recombinant polynucleotide is referred to as a "recombinant host cell." The gene is then expressed in the recombinant host cell to produce, e.g., a "recombinant polypeptide." A recombinant polynucleotide may serve a non-coding function (e.g., promoter, origin of replication, ribosome-binding site, etc.) as well.

"Chimera" as used herein refers to a polynucleotide or polypeptide comprising at least two heterologous polynucleotide or polypeptide sequences (i.e. derived from different sources or not associated with each other as a naturally-occurring sequence) which are directly or indirectly attached or linked together using techniques commonly known in the

art, e.g., recombinant expression or chemical crosslinking. In one embodiment, the heterologous sequence can comprise a protein or peptide directly or indirectly linked to a CNP peptide or variant, including proteins or peptides which are cleavable from the CNP peptide or variant. In a related embodiment, CNP variants are chimera as described herein.

In certain embodiments, chimeras include CNP fusion proteins comprising a cleavable carrier protein or peptide tag. The term "cleavable carrier protein" or "cleavable peptide tag" refers to a peptide or polypeptide sequence that may be fused, directly or indirectly via a linker, to a heterologous polypeptide sequence, and is removable from the heterologous sequence using an agent that cleaves or separates the cleavable peptide or polypeptide from the heterologous polypeptide or protein. In some embodiments, the cleavable carrier protein or peptide tag improves generation, purification and/or detection of the fusion protein or the heterologous polypeptide. Exemplary cleavable carrier proteins and peptide tags include, but are not limited to, human transcription factor TAF12 (TAF12), ketosteroid isomerase (KSI), maltose-binding protein (MBP), β-galactosidase (β-Gal), glutathione-S-transferase (GST), thioredoxin (Trx), chitin-binding domain (CBD), BMP-2 mutation (BMPM), SUMO, CAT, TrpE, staphylococcal protein A, streptococcal proteins, starch-binding protein, cellulose-binding domain of endoglucanase A, cellulose-binding domain of exoglucanase Cex, biotin-binding domain, recA, Flag, c-Myc, poly(His), poly (Arg), poly(Asp), poly(Gln), poly(Phe), poly(Cys), green fluorescent protein, red fluorescent protein, yellow fluorescent protein, cyan fluorescent protein, biotin, avidin, streptavidin, antibody epitopes, and fragments thereof.

A "cleaving agent" is an agent that is useful to cleave or separate, e.g., a cleavable peptide or polypeptide from a heterologous polypeptide or protein. Exemplary cleaving agents include, but are not limited to, palladium, cyanogen bromide (CNBr), formic acid, hydroxylamine, clostripain, thrombin, chymotrypsin, trypsin, trypsin-like proteases, carboxypeptidase, enterokinase (enteropeptidase), Kex 2 protease, Omp T protease, Factor Xa protease, subtilisin, pro-TEV, SUMO protease, V8 protease, HIV protease, rhinovirus protease, furilisin protease, IgA proteases, human Pace protease, collagenase, Nia protease, poliovirus 2Apro protease, poliovirus 3C protease, genenase, furin, elastase, Proteinase K, pepsin, rennin (chymosin), microbial aspartic proteases, papain, calpain, chymopapain, ficin (ficain), bromelain (bromelase), cathespisin B, caspases, thermolysin, Endoprotease Arg-C, Endoprotease Glu-C, Endoprotease Lys-C, kallikrein, and plasmin.

The terms "identical" and percent "identity", in the context of two or more polynucleotide or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of nucleotides or amino acid residues that are the same, when compared and aligned for maximum correspondence, as measured using one of the following sequence comparison algorithms or by visual inspection.

The phrase "substantially homologous" or "substantially identical", in the context of two nucleic acids or polypeptides, generally refers to two or more sequences or subsequences that have at least 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 98% nucleotide or amino acid residue identity, when compared and aligned for maximum correspondence, as measured using one of the following sequence comparison algorithms or by visual inspection. In certain embodiments, the substantial homology or identity exists over regions of the sequences that are at least about 25, 50, 100 or 150 residues in length. In another embodiment, the sequences are substantially homologous or identical over the entire length of either or both comparison biopolymers.

For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are inputted into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, Adv. Appl. Math., 2: 482 (1981), by the homology alignment algorithm of Needleman & Wunsch, J. Mol. Biol., 48: 443 (1970), by the search for similarity method of Pearson & Lipman, Proc. Natl. Acad. Sci. USA, 85: 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis.), or by visual inspection. One example of a useful algorithm is PILEUP, which uses a simplification of the progressive alignment method of Feng & Doolittle, J. Mol. Evol., 35: 351-360 (1987) and is similar to the method described by Higgins & Sharp, CABIOS, 5: 151-153 (1989). Another algorithm useful for generating multiple alignments of sequences is Clustal W (Thompson et al., Nucleic Acids Research, 22: 4673-4680 (1994)). An example of an algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm (Altschul et al., J. Mol. Biol., 215: 403-410 (1990); Henikoff & Henikoff, Proc. Natl. Acad. Sci. USA, 89: 10915 (1989); Karlin & Altschul, Proc. Natl. Acad. Sci. USA, 90: 5873-5787 (1993)). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information.

A further indication that two nucleic acid sequences or polypeptides are substantially homologous or identical is that the polypeptide encoded by the first nucleic acid is immunologically cross reactive with the polypeptide encoded by the second nucleic acid, as described below. Thus, a polypeptide is typically substantially identical to a second polypeptide, for example, where the two polypeptides differ only by conservative substitutions. Another indication that two nucleic acid sequences are substantially identical is that the two molecules hybridize to each other under stringent conditions, as described herein.

"Substantially pure" or "isolated" means an object species is the predominant species present (i.e., on a molar basis, more abundant than any other individual macromolecular species in the composition), and a substantially purified fraction is a composition wherein the object species comprises at least about 50% (on a molar basis) of all macromolecular species present. In one embodiment, a substantially pure composition means that the species of interest comprises at least about 70%, 75%, 80%, 85%, 90%, 95%, 98% or more of the macromolecular species present in the composition on a molar or weight basis. The object species is purified to essential homogeneity (contaminant species cannot be detected in the composition by conventional detection methods) if the composition consists essentially of a single macromolecular species. Solvent species, small molecules (<500 Daltons), stabilizers (e.g., BSA), and elemental ion species are not considered macromolecular

species for purposes of this definition. In an embodiment, the compounds of the disclosure are substantially pure or isolated. In another embodiment, the compounds of the disclosure are substantially pure or isolated with respect to the macromolecular starting materials used in their production. In yet another embodiment, the pharmaceutical compositions of the disclosure comprise a substantially pure or isolated CNP variant admixed with one or more pharmaceutically acceptable excipients, carriers or diluents, and optionally with another biologically active agent.

"Naturally occurring" as applied to an object refers to the fact that the object can be found in nature. For example, a polypeptide or polynucleotide sequence that is present in an organism (including viruses) and which has not been intentionally modified by man in the laboratory is naturally occurring. In one embodiment, a "naturally occurring" substance is of human origin.

"Wild-type" (wt) is a term referring to the natural form, including sequence, of a polynucleotide, polypeptide or protein in a species. A wild-type form is distinguished from a mutant form of a polynucleotide, polypeptide or protein arising from genetic mutation(s).

In one embodiment, a first polypeptide that is an "analog" or "variant" or "derivative" of a second polypeptide is a polypeptide having at least about 50%, 60% or 70% sequence homology, but less than 100% sequence homology, with the second polypeptide. Such analogs, variants or derivatives may be comprised of non-naturally occurring amino acid residues, including without limitation, homoarginine, ornithine, penicillamine, and norvaline, as well as naturally occurring amino acid residues. Such analogs, variants or derivatives may also be composed of one or a plurality of D-amino acid residues, and may also contain peptidomimetics or peptide bond isosteres such as non-peptide linkages between two or more amino acid or peptidomimetic residues. In another embodiment, a first polypeptide is an "analog", "variant" or "derivative" of a second polypeptide if the first polypeptide is not a known cleavage product of the second polypeptide or is not a known precursor of the second polypeptide, even if the first polypeptide has 100% sequence homology to the second polypeptide or has a wild-type sequence.

In an embodiment, the term "derived from" as used herein refers to a polypeptide or peptide sequence that is based on a wild type or naturally occurring polypeptide or peptide sequence and can have one or more deletions, additions, and/or substitutions with natural amino acids, unnatural amino acids or peptidomimetics. In one embodiment, the derivative sequence shares at least about 40%, 50%, 60% or 70%, but less than 100%, sequence similarity to the wild-type or naturally occurring sequence. In another embodiment, the derivative may be a fragment of a polypeptide, wherein the fragment is substantially homologous (e.g., at least about 70%, 75%, 80%, 85%, 90%, or 95% homologous) to the wild-type polypeptide over a length of at least about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 amino acids. In still another embodiment, a polypeptide is "derived from" a wild-type polypeptide if it has a moiety (e.g., a polymer such as, e.g., PEG) directly or indirectly attached to it which is not present on the wild-type polypeptide, even if both polypeptides share 100% homology in their amino acid sequence.

As used herein, an "NPPC-derived" polypeptide refers to a polypeptide derived from the natriuretic peptide precursor C(NPPC) polypeptide, which is a single chain 126-amino acid pre-pro polypeptide, and which upon cleavage ultimately results in wtCNP22. Removal of the signal peptide from NPPC yields pro-CNP, and further cleavage by the endoprotease furin generates an active 53-amino acid peptide (CNP-53), which is secreted and cleaved again by an unknown enzyme to produce the mature 22-amino acid peptide (CNP, or CNP-22). Therefore, CNP22 itself is an "NPPC-derived" polypeptide by virtue of being derived from NPPC. In one embodiment, an NPPC-derived polypeptide is at least about 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, or 95% homologous to the wild type NPPC over the same number of amino acid residues. It is further contemplated that an NPPC-derived peptide may comprise from about 1 to about 53, or 1 to 37, or 1 to 35, or 1 to 31, or 1 to 27, or 1 to 22, or to 35, or about 15 to about 37 residues of the NPPC polypeptide. In one embodiment, an NPPC-derived peptide may comprise a sequence of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, or 53 amino acids derived from the NPPC polypeptide.

The term "effective amount" means a dosage sufficient to produce a desired result on a health condition, pathology, or disease of a subject or for a diagnostic purpose. The desired result may comprise a subjective or objective improvement in the recipient of the dosage. "Therapeutically effective amount" refers to that amount of an agent effective to produce the intended beneficial effect on health. An appropriate "effective" amount in any individual case may be determined by one of ordinary skill in the art using routine experimentation. It will be understood that the specific dose level and frequency of dosage for any particular patient may be varied and will depend upon a variety of factors, including the activity of the specific compound employed; the bioavailability, metabolic stability, rate of excretion and length of action of that compound; the mode and time of administration of the compound; the age, body weight, general health, sex, and diet of the patient; and the severity of the particular condition.

"Treatment" refers to prophylactic treatment or therapeutic treatment or diagnostic treatment. In certain embodiments, "treatment" refers to administration of a compound or composition to a subject for therapeutic, prophylactic or diagnostic purposes.

A "prophylactic" treatment is a treatment administered to a subject who does not exhibit signs of a disease or exhibits only early signs of the disease, for the purpose of decreasing the risk of developing pathology. The compounds or compositions of the disclosure may be given as a prophylactic treatment to reduce the likelihood of developing a pathology or to minimize the severity of the pathology, if developed.

A "therapeutic" treatment is a treatment administered to a subject who exhibits signs or symptoms of pathology for the purpose of diminishing or eliminating those signs or symptoms. The signs or symptoms may be biochemical, cellular, histological, functional or physical, subjective or objective. The compounds of the disclosure may also be given as a therapeutic treatment or for diagnosis.

"Diagnostic" means identifying the presence, extent and/or nature of a pathologic condition. Diagnostic methods differ in their specificity and selectivity. While a particular diagnostic method may not provide a definitive diagnosis of a condition, it suffices if the method provides a positive indication that aids in diagnosis.

"Bone- or cartilage-associated biomarker" or "bone- or cartilage-associated marker" refers to a growth factor, enzyme, protein, or other detectable biological substance or moiety whose level is increased or decreased in association with, e.g., cartilage turnover, cartilage formation, cartilage growth, bone resorption, bone formation, bone growth, or

combinations thereof. Such biomarkers may be measured before, during and/or after administration of a CNP variant as described herein. Exemplary bone- or cartilage-associated biomarkers include, but are not limited to, CNP, cGMP, propeptides of collagen type II and fragments thereof, collagen type II and fragments thereof, propeptides of collagen type I and fragments thereof, collagen type 1 and fragments thereof, osteocalcin, proliferating cell nuclear antigen (PCNA), aggrecan chondroitin sulfate, and alkaline phosphatase. Cartilage- and bone-associated biomarkers can be measured in any appropriate biological sample, including but not limited to tissues, blood, serum, plasma, cerebrospinal fluid, synovial fluid and urine. In some embodiments, the biomarkers are measured in blood, plasma or serum from animals undergoing efficacy/pharmacodynamic in vivo studies and/or from the conditioned media of ex vivo studies.

In certain embodiments, the level of at least one bone- or cartilage-associated biomarker is measured and the amount or frequency of administration of CNP variant administered to a subject can be adjusted according to the level of the biomarker measured. In some embodiments, the level of biomarker is "below a target level" or "above a target level." A target level of a biomarker is a level or range of levels of the biomarker at which a therapeutic effect is observed in the subject receiving the CNP variant. In certain embodiments, the target level of a biomarker for a subject having a CNP-responsive disorder or condition is the level or range of levels of the biomarker observed in a normal, non-affected subject. In other embodiments, to indicate a therapeutic effect, the target level of a biomarker need not be equivalent to the level or range of levels of the biomarker observed in a normal subject, but can be within, e.g., 100%, 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10% or 5% of the "normal" level or range of levels of the biomarker observed in a non-affected subject.

For example, if the level of a biomarker increases in association with bone or cartilage formation or growth, the target level of the biomarker indicating a therapeutic effect may be higher than the level of the biomarker in patients suffering from a CNP-responsive disorder who have not been administered a CNP variant, and may optionally be lower than the "normal" level(s), at about the "normal" level(s), or above the "normal" level(s) of the biomarker in subjects not suffering from that disorder. In one embodiment, if the level of a biomarker is below a target level, it indicates an inadequate therapeutic effect, which may require an increase in the amount or frequency of administration of CNP variant administered. In a related embodiment, if the biomarker is above a target level, it indicates that more CNP variant than necessary has been administered, which may require a decrease in the amount or frequency of administration of the CNP variant administered.

As another example, if the level of a biomarker decreases in association with bone or cartilage formation or growth, the target level of the biomarker indicating a therapeutic effect may be lower than the level of the biomarker in patients suffering from a CNP-responsive disorder who have not been administered a CNP variant, and may optionally be higher than the "normal" level(s), at about the "normal" level(s), or below the "normal" level(s) of the biomarker in subjects not suffering from that disorder. In such a case, the converse of the above adjustments in CNP variant amount and frequency of administration may apply.

"Pharmaceutical composition" refers to a composition suitable for pharmaceutical use in subject animal, including humans and mammals. A pharmaceutical composition comprises a therapeutically effective amount of a CNP variant, optionally another biologically active agent, and optionally a pharmaceutically acceptable excipient, carrier or diluent. In an embodiment, a pharmaceutical composition encompasses a composition comprising the active ingredient(s), and the inert ingredient(s) that make up the carrier, as well as any product that results, directly or indirectly, from combination, complexation or aggregation of any two or more of the ingredients, or from dissociation of one or more of the ingredients, or from other types of reactions or interactions of one or more of the ingredients. Accordingly, the pharmaceutical compositions of the present disclosure encompass any composition made by admixing a compound of the disclosure and a pharmaceutically acceptable excipient, carrier or diluent.

"Pharmaceutically acceptable carrier" refers to any of the standard pharmaceutical carriers, buffers, and the like, such as a phosphate buffered saline solution, 5% aqueous solution of dextrose, and emulsions (e.g., an oil/water or water/oil emulsion). Non-limiting examples of excipients include adjuvants, binders, fillers, diluents, disintegrants, emulsifying agents, wetting agents, lubricants, glidants, sweetening agents, flavoring agents, and coloring agents. Suitable pharmaceutical carriers, excipients and diluents are described in Remington's Pharmaceutical Sciences, 19th Ed. (Mack Publishing Co., Easton, 1995). Preferred pharmaceutical carriers depend upon the intended mode of administration of the active agent. Typical modes of administration include enteral (e.g., oral) or parenteral (e.g., subcutaneous, intramuscular, intravenous or intraperitoneal injection; or topical, transdermal, or transmucosal administration).

A "pharmaceutically acceptable salt" is a salt that can be formulated into a compound for pharmaceutical use, including but not limited to metal salts (e.g., sodium, potassium, magnesium, calcium, etc.) and salts of ammonia or organic amines.

By "pharmaceutically acceptable" or "pharmacologically acceptable" is meant a material that is not biologically or otherwise undesirable, i.e., the material may be administered to an individual without causing any undesirable biological effects or without interacting in a deleterious manner with any of the components of the composition in which it is contained or with any components present on or in the body of the individual.

The term "unit dosage form" refers to physically discrete units suitable as unitary dosages for human and animal subjects, each unit containing a predetermined quantity of a compound of the disclosure calculated in an amount sufficient to produce the desired effect, optionally in association with a pharmaceutically acceptable excipient, diluent, carrier or vehicle. The specifications for the novel unit dosage forms of the present disclosure depend on the particular compound employed and the effect to be achieved, and the pharmacodynamics associated with each compound in the host.

"Physiological conditions" refer to conditions in the body of an animal (e.g., a human). Physiological conditions include, but are not limited to, body temperature and an aqueous environment of physiologic ionic strength, pH and enzymes. Physiological conditions also encompass conditions in the body of a particular subject which differ from the "normal" conditions present in the majority of subjects, e.g., which differ from the normal human body temperature of approximately 37° C. or differ from the normal human blood pH of approximately 7.4.

By "physiological pH" or a "pH in a physiological range" is meant a pH in the range of approximately 7.0 to 8.0 inclusive, more typically in the range of approximately 7.2 to 7.6 inclusive.

As used herein, the term "subject" encompasses mammals and non-mammals. Examples of mammals include, but are not limited to, any member of the mammalian class: humans, non-human primates such as chimpanzees, and other apes and monkey species; farm animals such as cattle, horses, sheep, goats, swine; domestic animals such as rabbits, dogs, and cats; laboratory animals including rodents, such as rats, mice and guinea pigs, and the like. Examples of non-mammals include, but are not limited to, birds, fish, and the like. The term does not denote a particular age or gender.

The terms "polyethylene glycol", "PEG", "polyethylene oxide" and "PEO" are used interchangeahly herein unless indicated otherwise. A CNP peptide (CNP22 or a variant thereof) conjugated via an amino group to a "PEOn" polymer associated with the number n, in general has the formula: CH_3—[—O—CH_2CH_2—]$_n$—C(=O)—NHR, where n is the number of ethylene oxide units and R denotes the rest of the peptide. The "PEOn" polymer can optionally have an alkylene group, $(CH_2)_m$, where m is an integer from 1 to 5, between the carbonyl carbon and the repeating ethylene oxide units. Such a "PEOn" (e.g., PEO12 or PEO24) polymer is monodispersed, i.e., is a single discrete polymer of a particular molecular weight. Similarly, a CNP peptide conjugated via an amino group to a "PEGnK" polymer associated with the number nK, in general has the formula: CH_3—[—O—CH_2CH_2—]$_p$—C(=O)—NHR, where p is an integer greater than 1. The "PEGnK" polymer also can optionally have an alkylene group, $(CH_2)_m$, where m is an integer from 1 to 5, between the carbonyl carbon and the repeating ethylene oxide units. However, such a "PEGnK" (e.g., PEG1K, PEG2K, PEG5K or PEG20K) polymer is polydispersed, i.e., contains a mixture of polymers having a distribution of molecular weights, where the number nK denotes the polymer number-average molecular weight (M_n) in kilo Daltons. For example, "PEG2K" conjugated to a CNP peptide denotes a polydispersed PEG polymer having a polymer number-average molecular weight of around 2 kDa.

When a range of the mass of a polymer (e.g., PEG) is given (e.g., in units of kDa), the range refers to a range of polymer number-average molecular weights, not to a range of molecular weights of multiple polymers in a polydispersed mixture, unless expressly indicated otherwise.

The term "halogen", "halide" or "halo" refers to fluorine, chlorine, bromine, and/or iodine.

The term "alkyl" refers to a linear or branched saturated monovalent hydrocarbon radical, wherein the alkyl may optionally be substituted with one or more substituents Q as described herein. In certain embodiments, the alkyl is a linear saturated monovalent hydrocarbon radical that has 1 to 20 (C_{1-20}), 1 to 15 (C_{1-15}), 1 to 12 (C_{1-12}), 1 to 10 (C_{1-10}), or 1 to 6 (C_{1-6}) carbon atoms, or a branched saturated monovalent hydrocarbon radical of 3 to 20 (C_{3-20}), 3 to 15 (C_{3-15}), 3 to 12 (C_{3-12}), 3 to 10 (C_{3-10}), or 3 to 6 (C_{3-6}) carbon atoms. As used herein, linear C_{1-6} and branched C_{3-6} alkyl groups are also referred as "lower alkyl." Examples of alkyl groups include, but are not limited to, methyl, ethyl, propyl (including all isomeric forms, including n-propyl and isopropyl), butyl (including all isomeric forms, including n-butyl, isobutyl, sec-butyl and tert-butyl), pentyl (including all isomeric forms), and hexyl (including all isomeric forms). For example, C_{1-6} alkyl refers to a linear saturated monovalent hydrocarbon radical of 1 to 6 carbon atoms or a branched saturated monovalent hydrocarbon radical of 3 to 6 carbon atoms.

The term "alkoxy" refers to an —O-alkyl group. In certain embodiments, an alkoxy group may optionally be substituted with one or more substituents Q as described herein.

The term "haloalkyl" refers to an alkyl group that is substituted with one or more halide atoms. In certain embodiments, a haloalkyl group is substituted with one, two, three, four, five or six halide atoms. In certain embodiments, a haloalkyl group may optionally be substituted with one or more additional substituents Q as described herein.

The term "cycloalkyl" refers to a cyclic saturated bridged and/or non-bridged monovalent hydrocarbon radical, which may be optionally substituted with one or more substituents Q as described herein. In certain embodiments, the cycloalkyl has from 3 to 20 (C_{3-20}), from 3 to 15 (C_{3-15}), from 3 to 12 (C_{3-12}), from 3 to 10 (C_{3-10}), or from 3 to 7 (C_{3-7}) carbon atoms. Examples of cycloalkyl groups include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, decalinyl, and adamantyl.

The term "heterocyclyl" or "heterocyclic" refers to a monocyclic non-aromatic ring system or a multicyclic ring system that contains at least one non-aromatic ring, wherein one or more of the non-aromatic ring atoms are heteroatoms independently selected from O, S, or N, and the remaining non-aromatic ring atoms are carbon atoms. In certain embodiments, the heterocyclyl or heterocyclic group has from 3 to 20, from 3 to 15, from 3 to 10, from 3 to 8, from 4 to 7, or from 5 to 6 ring atoms. In certain embodiments, the heterocyclyl is a monocyclic, bicyclic, tricyclic, or tetracyclic ring system, which may include a fused or bridged ring system, and in which the nitrogen or sulfur atoms may be optionally oxidized, the nitrogen atoms may be optionally quaternized, and some rings may be partially or fully saturated, or aromatic. The heterocyclyl may be attached to the main structure at any heteroatom or carbon atom which results in the creation of a stable compound. Examples of heterocyclic groups include, but are not limited to, acridinyl, azepinyl, benzimidazolyl, benzindolyl, benzoisoxazolyl, benzisoxazinyl, benzodioxanyl, benzodioxolyl, benzofuranonyl, benzofuranyl, benzonaphthofuranyl, benzopyranonyl, benzopyranyl, benzotetrahydrofuranyl, benzotetrahydrothienyl, benzothiadiazolyl, benzothiazolyl, benzothiophenyl, benzotriazolyl, benzothiopyranyl, benzoxazinyl, benzoxazolyl, benzothiazolyl, β-carbolinyl, carbazolyl, chromanyl, chromonyl, cinnolinyl, coumarinyl, decahydroisoquinolinyl, dibenzofuranyl, dihydrobenzisothiazinyl, dihydrobenzisoxazinyl, dihydrofuryl, dihydropyranyl, dioxolanyl, dihydropyrazinyl, dihydropyridinyl, dihydropyrazolyl, dihydropyrimidinyl, dihydropyrrolyl, dioxolanyl, 1,4-dithianyl, furanonyl, furanyl, imidazolidinyl, imidazolinyl, imidazolyl, imidazopyridinyl, imidazothiazolyl, indazolyl, indolinyl, indolizinyl, indolyl, isobenzotetrahydrofuranyl, isobenzotetrahydrothienyl, isobenzothienyl, isochromanyl, isocoumarinyl, isoindolinyl, isoindolyl, isoquinolinyl, isothiazolidinyl, isothiazolyl, isoxazolidinyl, isoxazolyl, morpholinyl, naphthyridinyl, octahydroindolyl, octahydroisoindolyl, oxadiazolyl, oxazolidinonyl, oxazolidinyl, oxazolopyridinyl, oxazolyl, oxiranyl, perimidinyl, phenanthridinyl, phenathrolinyl, phenarsazinyl, phenazinyl, phenothiazinyl, phenoxazinyl, phthalazinyl, piperazinyl, piperidinyl, 4-piperidonyl, pteridinyl, purinyl, pyrazinyl, pyrazolidinyl, pyrazolyl, pyridazinyl, pyridinyl, pyridopyridinyl, pyrimidinyl, pyrrolidinyl, pyrrolinyl, pyrrolyl, quinazolinyl, quinolinyl, quinoxalinyl, quinuclidinyl, tetrahydrofuryl, tetrahydrofuranyl, tetrahydroisoquinolinyl, tet-

rahydropyranyl, tetrahydrothienyl, tetrazolyl, thiadiazolopyrimidinyl, thiadiazolyl, thiamorpholinyl, thiazolidinyl, thiazolyl, thienyl, triazinyl, triazolyl, and 1,3,5-trithianyl. In certain embodiments, a heterocyclic group may optionally be substituted with one or more substituents Q as described herein.

The term "aryl" refers to a monocyclic aromatic group or a multicyclic monovalent aromatic group that contain at least one aromatic hydrocarbon ring. In certain embodiments, the aryl has from 6 to 20 (C_{6-20}), from 6 to 15 (C_{6-15}), or from 6 to 10 (C_{6-10}) ring atoms. Examples of aryl groups include, but are not limited to, phenyl, naphthyl, fluorenyl, azulenyl, anthryl, phenanthryl, pyrenyl, biphenyl, and terphenyl. Aryl also refers to bicyclic or tricyclic carbon rings, where at least one of the rings is aromatic and the others may be saturated, partially unsaturated, or aromatic, for example, dihydronaphthyl, indenyl, indanyl, and tetrahydronaphthyl (tetralinyl). In certain embodiments, an aryl group may optionally be substituted with one or more substituents Q as described herein.

The term "heteroaryl" refers to a monocyclic aromatic group or a multicyclic aromatic group that contain at least one aromatic ring, wherein at least one aromatic ring contains one or more heteroatoms independently selected from O, S, and N. Each ring of a heteroaryl group can contain one or two O atoms, one or two S atoms, and/or one to four N atoms, provided that the total number of heteroatoms in each ring is four or less and each ring contains at least one carbon atom. The heteroaryl may be attached to the main structure at any heteroatom or carbon atom which results in the creation of a stable compound. In certain embodiments, the heteroaryl has from 5 to 20, from 5 to 15, or from 5 to 10 ring atoms. Examples of monocyclic heteroaryl groups include, but are not limited to, pyrrolyl, pyrazolyl, pyrazolinyl, imidazolyl, oxazolyl, isoxazolyl, thiazolyl, thiadiazolyl, isothiazolyl, furanyl, thienyl, oxadiazolyl, pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, and triazinyl. Examples of bicyclic heteroaryl groups include, but are not limited to, indolyl, benzothiazolyl, benzoxazolyl, benzothienyl, quinolinyl, tetrahydroisoquinolinyl, isoquinolinyl, benzimidazolyl, benzopyranyl, indolizinyl, benzofuranyl, isobenzofuranyl, chromonyl, coumarinyl, cinnolinyl, quinoxalinyl, indazolyl, purinyl, pyrrolopyridinyl, furopyridinyl, thienopyridinyl, dihydroisoindolyl, and tetrahydroquinolinyl. Examples of tricyclic heteroaryl groups include, but are not limited to, carbazolyl, benzindolyl, phenanthrollinyl, acridinyl, phenanthridinyl, and xanthenyl. In certain embodiments, a heteroaryl group may optionally be substituted with one or more substituents Q as described herein.

The term "optionally substituted" is intended to mean that a group, including alkyl, alkoxy, haloalkyl, cycloalkyl, heterocyclyl, aryl and heteroaryl, may be substituted with one or more substituents Q (in one embodiment, one, two, three or four substituents Q), where each Q is independently selected from the group consisting of cyano, halo, oxo, nitro, C_{1-6} alkyl, C_{1-6} alkoxy, halo-C_{1-6} alkyl, C_{3-7} cycloalkyl, heterocyclyl, C_{6-14} aryl, heteroaryl, —$C(O)R^e$, —$C(O)OR^e$, —$C(O)NR^fR^g$, —$C(NR^e)NR^fR^g$, —OR^e, —$OC(O)R^e$, —$OC(O)OR^e$, —$OC(O)NR^fR^g$, —$OC(=NR^e)NR^fR^g$, —$OS(O)R^e$, —$OS(O)_2R^e$, —$OS(O)NR^fR^g$, —$OS(O)_2NR^fR^g$, —NR^fR^g, —$NR^eC(O)R^f$, —$NR^eC(O)OR^f$, —$NR^eC(O)NR^fR^g$, —$NR^eC(=NR^h)NR^fR^g$, —$NR^eS(O)R^f$, —$NR^eS(O)_2R^f$, —$NR^eS(O)NR^fR^g$, —$NR^eS(O)_2NR^fR^g$, —SR^e, —$S(O)R^e$, —$S(O)_2R^e$, and —$S(O)_2NR^fR^g$, wherein each R^e, R^f, R^g, and R^h is independently hydrogen, C_{1-6} alkyl, C_{3-7} cycloalkyl, heterocyclyl, C_{6-14} aryl, or heteroaryl; or R^f and R^g, together with the N atom to which they are attached, form heterocyclyl.

B. CNP Variants

The use of CNP22 as a therapeutic is limited by its short half-life in plasma (J. Clin. Endocrinol. Metab., 78: 1428-35 (1994)). In human plasma, the concentration of CNP22 typically is less than five picomolar. CNP22 is degraded and cleared from circulation by NEP and NPR-C in humans (Growth Hormone & IGF Res., 16: S6-S14). In all human and animal studies using systemically administered CNP22, continuous infusion has been used to increase the CNP22 concentration in the subjects. A CNP peptide having a longer half-life and at least a similar level of functionality would be beneficial to a CNP-based therapeutic strategy. CNP variants are also disclosed in related International Application No. PCT/US08/84270, specifically incorporated herein by reference.

The present disclosure provides CNP variants which have reduced affinity to NEP and/or NPR-C, and reduced susceptibility to cleavage by NEP and/or clearance by NPR-C, but which have substantially similar or better functionality than wild-type CNP22. Reduced susceptibility of CNP variants to cleavage by NEP and/or clearance by NPR-C would increase the plasma or serum half-life of the variants, thereby increasing the opportunity for the variants to distribute to the target tissues and sites and effectuate the desired pharmacological effects. In certain embodiments, the CNP variants described herein have reduced susceptibility to cleavage by NEP and/or clearance by NPR-C in vitro or in vivo by at least about 1.5-fold, 2-fold, 2.5-fold, 3-fold, 3.5-fold, 4-fold, 4.5-fold, or 5-fold compared to wtCNP22, and have increased plasma or serum half-life in vivo by at least about 1.5-fold, 2-fold, 2.5-fold, 3-fold, 3.5-fold, 4-fold, 4.5-fold, or 5-fold compared to wtCNP22, while retaining at least about 50%, 60%, 70%, 80%, 90% or 100% of the functionality of wtCNP22, or having at least about 1.5-fold, 2-fold, 2.5-fold, 3-fold, 3.5-fold, 4-fold, 4.5-fold, or 5-fold greater functionality than wtCNP22. CNP functionality can be evaluated in terms of, e.g., the level of one or more biomarkers (e.g., cGMP) associated with cartilage or bone formation or growth in an in vitro or in vivo study, the length of particular bones in an ex vivo or in vivo study, etc.

Natural substrates of NEP are small and natriuretic peptides (about 2.2 to about 3.2 kDa) are the largest of the natural substrates. According to X-ray crystallographic analyses, the NEP active-site is buried deep inside a central cavity, effectively restricting the size of substrate molecules to no more than about 3 kDa (Oefner et al., J. Mol. Biol., 296: 341-349 (2000)). Based on NPR-B signaling studies, variants of CNP-22, such as CNP-17 (retaining only the cyclic domain, Cys6-Cys22, of CNP22) and CNP-53 (CNP-22 with a 31-amino acid extension at the N-terminus), can still bind and activate NPR-B similarly to the 2.2 kDa wtCNP-22. Accordingly, the disclosure encompasses CNP variants conjugated to a natural (e.g., peptide) and/or synthetic (e.g., PEG) polymer at the N-terminus and/or C-terminus of CNP22 or variants thereof, which exhibit increased NEP resistance but retain the ability to bind and activate the NPR-B signaling receptor.

In one embodiment, the disclosure encompasses CNP variants represented by the general formula:

(x)-Cys_6-Phe_7-Gly_8-Leu_9-Lys_{10}-Leu_{11}-Asp_{12}-Arg_{13}-Ile_{14}-Gly_{15}-Ser_{16}-Met_{17}-Ser_{18}-Gly_{19}-Leu_{20}-Gly_{21}-Cys_{22}-(z) (SEQ ID NO:139), or

(x)-Gly_1-Leu_2-Ser_3-Lys_4-Gly_5 -Cys_6-Phe_7-Gly_8-Leu_9-Lys_{10}-Leu_{11}-Asp_{12}-Arg_{13}-Ile_{14}-Gly_{15}-Ser_{16}-Met_{17}-Ser_{18}-Gly_{19}-Leu_{20}-Gly_{21}-Cys_{22}-(z) (SEQ ID NO:140), wherein:

(x) and (z) each independently are a natural polymer (e.g., a peptide sequence containing at least one amino acid) and/or a synthetic polymer (e.g., PEG) as described herein, such that the total mass of the CNP variant is characterized by the ranges described generally herein, e.g., in the range from about 2.6 kDa or 2.8 kDa to about 6 or 7 kDa. In one embodiment, the residues from Cys6 to Cys22 form a cyclic portion. In an embodiment, (x) and/or (z) comprise an amino acid extension derived from NPPC or a non-CNP polypeptide (e.g., ANP, BNP, IgG, etc.), wherein the extension contains 1 to 40, 1 to 35, 1 to 31, 5 to 35, 5 to 31 or 5 to 15 amino acids. In another embodiment, the CNP variants comprise one or more modifications and/or substitutions with another natural amino acid, an unnatural amino acid, a peptidomimetic and/or a peptide bond isostere at one or more of the following positions of CNP22: Gly1, Lys4, Gly5, Cys6, Phe7, Gly8, Leu9, Lys10, Leu11, Ile14, Gly15, Ser16, Met17, Gly19, Leu20 and Gly21.

In another embodiment, CNP variants having a total mass characterized by the ranges described generally herein, e.g., from about 2.6 kDa or 2.8 kDa to about 6 or 7 kDa, designed for increased resistance to NEP degradation, are represented by the general formula:

(x)-Cys_6-Phe_7-Gly_8-Leu_9-$(h)_{10}$-Leu_{11}-Asp_{12}-Arg_{13}-Ile_{14}-Gly_{15}-Ser_{16}-Met_{17}-Ser_{18}-Gly_{19}-Leu_{20}-Gly_{21}-Cys_{22}-(z) (SEQ ID NO: 141), or

(x)-Gly_1-Leu_2-Ser_3-$(b)_4$-Gly_5 -Cys_6-Phe_7-Gly_8-Leu_9-$(h)_{10}$-Leu_{11}-Asp_{12}-Arg_{13}-Ile_{14}-Gly_{15}-Ser_{16}-Met_{17}-Ser_{18}-Gly_{19}-Leu_{20}-Gly_{21}-Cys_{22}-(z) (SEQ ID NO: 6), wherein:

(x) is a synthetic or natural polymeric group, or a combination thereof, wherein a non-limiting example of a synthetic polymeric group is PEG (or PEO), and a non-limiting example of a natural polymeric group is an amino acid sequence containing from 1 to 35 amino acids and derived from NPPC or variants thereof with substitutions and/or deletions, ANP, BNP, or other non-CNP (poly)peptides such as, e.g., serum albumin, IgG, histidine-rich glycoproteins, fibronectin, fibrinogen, zinc finger-containing polypeptides, osteocrin or fibroblast growth factor 2 (FGF2);

(z) may be absent or may be a synthetic or natural polymeric group, or a combination thereof, wherein a non-limiting example of a synthetic polymeric group is PEG, and a non-limiting example of a natural polymeric group is an amino acid sequence derived from a natriuretic polypeptide (e.g., NPPC, CNP, ANP or BNP) or non-natriuretic polypeptide (e.g., serum albumin or IgG); and

(b) and (h) independently may be the wild type Lys at that position or may be replaced with a conservative amino acid substitution or any natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amine on a side chain, including but not limited to Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Glu or Ser. In one embodiment, (b) is Arg. In another embodiment, for improved NEP resistance, (b) is not Gly. In yet another embodiment, (h) is not Arg.

Non-limiting examples of amino acid sequences derived from NPPC or variants thereof include:

Arg,

Glu-Arg,

Gly-Ala-Asn-Lys-Lys, (SEQ ID NO: 7)

-continued

Gly-Ala-Asn-Arg-Arg, (SEQ ID NO: 8)

Gly-Ala-Asn-Pro-Arg, (SEQ ID NO: 9)

Gly-Ala-Asn-Gln-Gln, (SEQ ID NO: 10)

Gly-Ala-Asn-Ser-Ser, (SEQ ID NO: 11)

Gly-Ala-Asn-Arg-Gln, (SEQ ID NO: 12)

Gly-Ala-Asn-Arg-Met, (SEQ ID NO: 13)

Gly-Ala-Asn-Arg-Thr, (SEQ ID NO: 14)

Gly-Ala-Asn-Arg-Ser, (SEQ ID NO: 15)

Ala-Ala-Trp-Ala-Arg-Leu-Leu-Gln-Glu-His-Pro-Asn-Ala, (SEQ ID NO: 16)

Ala-Ala-Trp-Ala-Arg-Leu-Leu-Gln-Glu-His-Pro-Asn-Ala-Arg, (SEQ ID NO: 17)

Asp-Leu-Arg-Val-Asp-Thr-Lys-Ser-Arg-Ala-Ala-Trp-Ala-Arg, (SEQ ID NO: 18)

Gln-Glu-His-Pro-Asn-Ala-Arg-Lys-Tyr-Lys-Gly-Ala-Asn-Lys-Lys, (SEQ ID NO: 19)

Gln-Glu-His-Pro-Asn-Ala-Arg-Lys-Tyr-Lys-Gly-Ala-Asn-Arg-Arg, (SEQ ID NO: 20)

Asp-Leu-Arg-Val-Asp-Thr-Lys-Ser-Arg-Ala-Ala-Trp-Ala-Arg-Leu-Leu-Gln-Glu-His-Pro-Asn-Ala-Arg-Lys-Tyr-Lys-Gly-Ala-Asn-Lys-Lys, (SEQ ID NO: 21)

and

Asp-Leu-Arg-Val-Asp-Thr-Lys-Ser-Arg-Ala-Ala-Trp-Ala-Arg-Leu-Leu-Gln-Glu-His-Pro-Asn-Ala-Arg-Lys-Tyr-Lys-Gly-Ala-Asn-Arg-Arg. (SEQ ID NO: 22)

Non-limiting examples of amino acid sequences derived from non-CNP polypeptides such as, e.g., ANP, BNP, serum albumin and IgG include:

Ser-Leu-Arg-Arg-Ser-Ser; (SEQ ID NO: 23)

Asn-Ser-Phe-Arg-Tyr; (SEQ ID NO: 24)

Ser-Pro-Lys-Met-Val-Gln-Gly-Ser-Gly; (SEQ ID NO: 25)

Met-Val-Gln-Gly-Ser-Gly; (SEQ ID NO: 26)

Lys-Val-Leu-Arg-Arg-Tyr; (SEQ ID NO: 27)

Lys-Val-Leu-Arg-Arg-His; (SEQ ID NO: 28)

-continued

(SEQ ID NO: 29)
Gly-Gln-His-Lys-Asp-Asp-Asn-Pro-Asn-Leu-Pro-Arg;

(SEQ ID NO: 30)
Gly-Val-Pro-Gln-Val-Ser-Thr-Ser-Thr;

(SEQ ID NO: 31)
Gly-Glu-Arg-Ala-Phe-Lys-Ala-Trp-Ala-Val-Ala-Arg-Leu-Ser-Gln;

(SEQ ID NO: 32)
Gly-Gln-Pro-Arg-Glu-Pro-Gln-Val-Tyr-Thr-Leu-Pro-Pro-Ser.

In an embodiment, the N-terminal (x) group and/or the C-terminal (z) group of any of the CNP variants having an (x) and/or (z) group, as described herein, independently comprise an amino acid sequence that contains a small number of, if any, acidic natural or unnatural amino acids (e.g., Asp or Glu). In another embodiment, (x) and/or (z) are enriched in basic natural or unnatural amino acids (e.g., Lys, Arg or His) to maintain an alkaline pI similar to the pI of CNP22 (pI 8.9). In one embodiment, the pI of the CNP variants is in the range from about 8 to about 10.5, designed so that the CNP variants can diffuse more readily through the extracellular matrix surrounding chondrocytes of bone growth plates. In narrower embodiments, the pI of the CNP variants is from about 8.5 to about 10.5, or from about 8.5 to about 10, or from about 9 to about 10.

In yet another embodiment, (x) and/or (z) are enriched in polar natural or unnatural amino acids, designed for increased aqueous solubility. In still another embodiment, (x) and/or (z) contain a small number of, if any, hydrophobic natural or unnatural amino acids (e.g., Ala, Val, Leu, Ile or Met).

In a further embodiment, the N-terminus of the CNP variants terminates in at least one glycine residue, designed for increased serum half-life. In a related embodiment, to prevent pyroglutamine formation, the N-terminus of CNP variants terminates in a glycine residue if it would otherwise terminate in glutamine. In one embodiment, the (x) group contains an amino acid extension whose N-terminus terminates in at least one glycine residue. In another embodiment, (x) and/or (z) do not contain two adjacent basic natural or unnatural amino acids (e.g., Lys-Lys or Arg-Arg), designed to reduce susceptibility to cleavage by the protease furin. In an embodiment, (x) does not contain two adjacent basic amino acids immediately preceding the position corresponding to Gly1 of CNP22.

In still another embodiment, the (x) group and/or the (z) group of the CNP variants comprise an amino acid sequence derived from NPPC (e.g., derived from CNP53). In an embodiment, (x) comprises an amino acid sequence derived from the N-terminal tail of ANP or BNP. In another embodiment, (z) comprises an amino acid sequence derived from the C-terminal tail of ANP or BNP. In a further embodiment, (x) and/or (z) comprise an amino acid sequence derived from a non-natriuretic polypeptide such as, e.g., IgG, human serum albumin (HSA), histidine-rich glycoproteins, fibronectin, fibrinogen, zinc finger-containing polypeptides, FGF-2, and bone-targeting proteins (e.g., osteocrin, osteopontin, osteocalcin, and sialoprotein).

In any embodiment described herein in which CNP22 or a variant thereof can have an N-terminal (x) group and/or a C-terminal (z) group, (x) and/or (z) independently can contain an amino acid sequence derived from the functional domain of a bone morphogenetic protein (BMP). An N-terminal and/or C-terminal amino acid extension derived from the functional domain of a BMP can increase the NEP resistance, and hence the serum half-life of the CNP variant, by increasing the total mass of the CNP variant to characterized by the ranges described generally herein, e.g., a range from about 2.6 kDa or 2.8 kDa to about 6 or 7 kDa. In addition, since certain BMPs are growth factors and cytokines that induce the formation of bone and cartilage, a fragment derived from the functional domain of a BMP can promote chondrocyte, cartilage or bone growth by a mechanism distinct from activation of the guanylyl cyclase function of NPR-B by the cyclic domain of CNP22 or a variant thereof. Non-limiting examples of BMPs that promote bone formation and development, cartilage formation and development, and/or osteoblast differentiation include BMP1, BMP2, BMP3, BMP5, BMP7 and BMP8a. In an embodiment, the N-terminus and/or C-terminus of CNP22 or a variant thereof independently are conjugated to an amino acid sequence derived from the last 140 amino acids in the C-terminal portion of BMP1, BMP2, BMP3, BMP5, BMP7 or BMP8a.

In one embodiment, the CNP variants contain an amino acid extension at the N-terminus and/or C-terminus of CNP22 or CNP17, including but not limited to:

(SEQ ID NO: 4)
DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMS GLGC (CNP-53);

(SEQ ID NO: 60)
QEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-37, Analog BL);

(SEQ ID NO: 61)
AAWARLLQEHPNAGLSKGCFGLKLDRIGSMSGLGC (Analog CA);

(SEQ ID NO: 62)
AAWARLLQEHPNARGLSKGCFGLKLDRIGSMSGLGC (Analog CB);

(SEQ ID NO: 63)
DLRVDTKSRAAWARGLSKGCFGLKLDRIGSMSGLGC (Analog CC);

(SEQ ID NO: 40)
RGLSKGCFGLKLDRIGSMSGLGC;

(SEQ ID NO: 38)
ERGLSKGCFGLKLDRIGSMSGLGC;

(SEQ ID NO: 64)
GANQQGLSKGCFGLKLDRIGSMSGLGC;

(SEQ ID NO: 65)
GANRRGLSKGCFGLKLDRIGSMSGLGC;

(SEQ ID NO: 66)
GANPRGLSKGCFGLKLDRIGSMSGLGC;

(SEQ ID NO: 67)
GANSSGLSKGCFGLKLDRIGSMSGLGC;

(SEQ ID NO: 144)
GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC;
and

(SEQ ID NO: 68)
SPKMVQGSG-CNP17-KVLARH (Analog CD) (CNP17 having N-terminal and C-terminal tails derived from BNP).

In another embodiment, the CNP variants have a K4R substitution at position 4 of CNP22. Non-limiting examples of CNP(K4R) variants include:

(SEQ ID NO: 36)
GANRRGLSRGCFGLKLDRIGSMSGLGC (Analog AY);

-continued

(SEQ ID NO: 37)
GANPRGLSRGCFGLKLDRIGSMSGLGC (Analog CI);

(SEQ ID NO: 41)
RGLSRGCFGLKLDRIGSMSGLGC (Analog AZ);

(SEQ ID NO: 39)
ERGLSRGCFGLKLDRIGSMSGLGC (Analog BA);

(SEQ ID NO: 69)
GANQQGLSRGCFGLKLDRIGSMSGLGC (Analog CH);
and

(SEQ ID NO: 70)
GANSSGLSRGCFGLKLDRIGSMSGLGC (Analog CG).

In further embodiments, the CNP variants are chimeras comprising CNP22, or a variant thereof having amino acid addition(s), deletion(s) and/or substitution(s), and a peptide fragment derived from a polypeptide or protein other than CNP, or the whole non-CNP polypeptide or protein, to the N-terminus of the CNP peptide, wherein CNP22 or the variant thereof may optionally have an N-terminal amino acid extension of one or more amino acid residues. In certain embodiments, the CNP chimeras comprise CNP22 or a variant thereof that has an N-terminal amino acid extension of one or more amino acid residues. In certain embodiments, the CNP chimeras contain lysine-lysine (KK) residues or GANKK residues immediately preceding the first position of CNP22 (Gly in the case of CNP22) or a variant thereof. In other embodiments, the CNP chimeras contain one or two residues different from lysine-lysine immediately preceding the first position of CNP22 or a variant thereof. Non-limiting examples of residues that can immediately precede the first position of CNP22 or a variant thereof include KP, PK, PR, PQ, QK, QQ, RR, SS, GANKP(SEQ ID NO: 200), GANPK (SEQ ID NO: 201), GANPR (SEQ ID NO: 9), GANPQ (SEQ ID NO: 202), GANQK (SEQ ID NO: 203), GANQQ (SEQ ID NO: 10), GANRR (SEQ ID NO: 8), and GANSS (SEQ ID NO: 11).

In another embodiment, the CNP variants are chimera comprising CNP22 and an N-terminal peptide fragment, including but not limited to:

(SEQ ID NO: 76)
GHHSHEQHPHGANQQGLSKGCFGLKLDRIGSMSGLGC (Analog CQ) (histidine-rich glycoprotein (HRGP) fragment-CNP22 chimera);

(SEQ ID NO: 77)
GAHHPHEHDTHGANQQGLSKGCFGLKLDRIGSMSGLGC (Analog CR) (HRGP fragment-CNP22 chimera);

(SEQ ID NO: 78)
GHHSHEQHPHGANPRGLSKGCFGLKLDRIGSMSGLGC (Analog CX) (HRGP fragment-CNP22 chimera);

(SEQ ID NO: 79)
GQPREPQVYTLPPSGLSKGCFGLKLDRIGSMSGLGC (Analog CF) (IgG_1(F) fragment-CNP22 chimera);

(SEQ ID NO: 80)
GQHKDDNPNLPRGANPRGLSKGCFGLKLDRIGSMSGLGC (Analog CY) (human serum albumin (HSA) fragment-CNP22 chimera);

(SEQ ID NO: 81)
GERAFKAWAVARLSQGLSKGCFGLKLDRIGSMSGLGC (Analog CE) (HSA fragment-CNP22 chimera);

(SEQ ID NO: 82)
FGIPMDRIGRNPRGLSKGCFGLKLDRIGSMSGLGC (Analog CZ) (osteocrin "NPR C inhibitor" fragment-CNP22 chimera);
and

(SEQ ID NO: 83)
GKRTGQYKLGSKTGPGPKGLSKGCFGLKLDRIGSMSGLGC (Analog DA) (FGF2 "heparin-binding domain" fragment-CNP22 chimera).

In yet another embodiment, the CNP variants are chimera comprising an N-terminal peptide fragment and CNP22 in which arginine is substituted for Lys4 of CNP22 ("CNP22 (K4R)"), including but not limited to:

(SEQ ID NO: 84)
GQPREPQVYTGANQQGLSRGCFGLKLDRIGSMSGLGC (Analog CK) (IgG_1(F) fragment-CNP22(K4R) chimera);

(SEQ ID NO: 85)
GVPQVSTSTGANQQGLSRGCFGLKLDRIGSMSGLGC (Analog CL) (HSA fragment-CNP22(K4R) chimera);

(SEQ ID NO: 86)
GQPSSSSQSTGANQQGLSRGCFGLKLDRIGSMSGLGC (Analog CM) (fibronectin fragment-CNP22(K4R) chimera);

(SEQ ID NO: 87)
GQTHSSGTQSGANQQGLSRGCFGLKLDRIGSMSGLGC (Analog CN) (fibrinogen fragment-CNP22(K4R) chimera);

(SEQ ID NO: 88)
GSTGQWHSESGANQQGLSRGCFGLKLDRIGSMSGLGC (Analog CO) (fibrinogen fragment-CNP22(K4R) chimera);
and

(SEQ ID NO: 89)
GSSSSSSSSSGANQQGLSRGCFGLKLDRIGSMSGLGC (Analog CP) (zinc finger fragment-CNP22(K4R) chimera).

Chimera comprising IgG and CNP22 or a variant thereof are designed for, inter alia, increased resistance to NEP degradation and reduced binding to serum albumin. CNP chimera comprising a surface fragment of HSA are designed for, inter alia, reduced immunogenicity and reduced binding to serum albumin. HRGP-CNP22 and HRGP-CNP22(K4R) chimera containing a cationic, histidine-rich, non-lysine, non-arginine sequence at the N-terminus are designed for, inter alia, increased stability to proteases. Chimera containing an osteocrin fragment are designed to release, upon protease (e.g., furin) cleavage, the osteocrin fragment at bone growth plates, where the fragment would inhibit the clearance receptor NPR-C. With respect to chimera comprising an FGF2 heparin-binding fragment, heparin binding to the fragment is designed to protect the chimera from degradation, thereby providing a longer serum half-life. Chimera containing a fibronectin, fibrinogen, or zinc-finger fragment are designed for reduced binding to serum albumin, among other features.

Not intending to be bound by theory, a CNP variant of molecular weight from about 2.6 or 2.8 kDa to about 6 or 7 kDa which has increased resistance to NEP degradation and has similar or improved functionality (e.g., binding to NPR-B and stimulation of cGMP signaling) as compared to wtCNP22, may be more effective if it does not bind tightly to plasma proteins such as serum albumin. A CNP variant that does not bind tightly to plasma proteins (e.g., serum albumin) may be more effective in diffusing through cartilage, getting to chondrocytes of bone growth plates, and binding to and activating NPR-B for cGMP signaling. In one embodiment, CNP variants designed for reduced binding to

plasma proteins (e.g., serum albumin) are chimeras comprising CNP22 or a variant thereof and a peptide fragment from IgG. In another embodiment, CNP variants designed for reduced binding to plasma proteins are chimeras comprising CNP22 or CNP22(K4R) and a fragment from a polypeptide (e.g., IgG, HSA, fibronectin, fibrinogen, a zinc finger-containing polypeptide, etc.). In yet another embodiment, CNP variants designed for reduced binding to plasma proteins comprise CNP22 or a variant thereof conjugated to a hydrophilic or water-soluble polymer. In one embodiment, the hydrophilic or water-soluble polymer is PEG (or PEO). In another embodiment, the hydrophilic or water-soluble polymer (e.g., PEG) is functionalized with one or more functional groups that impart a negative charge to the polymer under physiological conditions, such as. e.g, carboxyl, sulfate or phosphate groups, or a combination thereof.

In a further embodiment, CNP variants of the disclosure include truncated CNP peptides ranging from human CNP-17 (hCNP-17) to human CNP-53 (hCNP-53), and having wild-type amino acid sequences derived from hCNP-53. Such truncated CNP peptides include:

(SEQ ID NO: 4)
DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSG
LGC (CNP-53);

(SEQ ID NO: 146)
LAVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGL
GC (CNP-52);

(SEQ ID NO: 147)
RVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGL
GC (CNP-51);

(SEQ ID NO: 148)
VDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC
(CNP-50);

(SEQ ID NO: 149)
DTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC
(CNP-49);

(SEQ ID NO: 150)
TKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC
(CNP-48);

(SEQ ID NO: 151)
KSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC
(CNP-47);

(SEQ ID NO: 152)
SRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC
(CNP-46);

(SEQ ID NO: 153)
RAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC
(CNP-45);

(SEQ ID NO: 154)
AAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC
(CNP-44);

(SEQ ID NO: 155)
AWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC
(CNP-43);

(SEQ ID NO: 156)
WARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC
(CNP-42);

(SEQ ID NO: 157)
ARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC
(CNP-41);

-continued

(SEQ ID NO: 158)
RLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-40);

(SEQ ID NO: 159)
LLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-39);

(SEQ ID NO: 160)
LQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-38);

(SEQ ID NO: 60)
QEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-37);

(SEQ ID NO: 161)
EHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-36);

(SEQ ID NO: 162)
HPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-35);

(SEQ ID NO: 163)
PNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-34);

(SEQ ID NO: 164)
NARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-33);

(SEQ ID NO: 165)
ARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-32);

(SEQ ID NO: 166)
RKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-31);

(SEQ ID NO: 167)
KYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-30);

(SEQ ID NO: 168)
YKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-29);

(SEQ ID NO: 169)
KGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-28);

(SEQ ID NO: 170)
GANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-27);

(SEQ ID NO: 171)
ANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-26);

(SEQ ID NO: 172)
NKKGLSKGCFGLKLDRIGSMSGLGC (CNP-25);

(SEQ ID NO: 173)
KKGLSKGCFGLKLDRIGSMSGLGC (CNP-24);

(SEQ ID NO: 174)
KGLSKGCFGLKLDRIGSMSGLGC (CNP-23);

(SEQ ID NO: 1)
GLSKGCFGLKLDRIGSMSGLGC (CNP-22);

(SEQ ID NO: 175)
LSKGCFGLKLDRIGSMSGLGC (CNP-21);

(SEQ ID NO: 176)
SKGCFGLKLDRIGSMSGLGC (CNP-20);

(SEQ ID NO: 177)
KGCFGLKLDRIGSMSGLGC (CNP-19);

(SEQ ID NO: 178)
GCFGLKLDRIGSMSGLGC (CNP-18);
and

(SEQ ID NO: 2)
CFGLKLDRIGSMSGLGC (CNP-17).

In certain embodiments, CNP variants do not include CNP-17, CNP-22 or CNP-53.

In another embodiment, the truncated CNP peptides ranging from hCNP-17 to hCNP-53 can contain amino acid addition(s), deletion(s) and/or substitution(s) with natural or unnatural amino acid(s) or peptidomimetic(s) (e.g., peptide bond isostere(s)), as described herein, at any one or more of

the amino acid positions of the particular truncated CNP peptides. In yet another embodiment, the truncated CNP peptides having wild-type sequences or amino acid addition(s), deletion(s) and/or substitution(s), can be conjugated at the N-terminus, C-terminus and/or internal site(s) to any of the moieties described herein, including but not limited to bone- or cartilage-targeting moieties (e.g., bisphosphonates, bone- or cartilage-targeting peptide sequences (e.g., polyAsp, poly-Glu), peptide sequences derived from bone-targeting domains of bone proteins (e.g., osteopontin, osteocalcin, sialoprotein)), peptide sequences derived from the functional domains of bone morphogenetic proteins (e.g., BMP2, BMP3, BMP5, BMP7, BMP8a), peptide sequences derived from natriuretic polypeptides (e.g., NPPC, ANP, BNP), peptide sequences derived from polypeptides of non-natriuretic origin (e.g., serum albumin, IgG, histidine-rich glycoproteins, fibronectin, fibrinogen, zinc finger-containing polypeptides, FGF-2, osteocrin), moieties that reduce renal clearance (e.g., negatively charged PEG moieties), hydrophilic polymers (e.g., PEG), carbohydrates (e.g., carbohydrates recognized by receptors on the surface of cells at bone growth plates), hydrophobic acids (e.g., C_5-C_{12} carboxylic acids, natural fatty acids), phospholipids, and combinations thereof. In an embodiment, the truncated CNP peptides having wild-type sequences or amino acid addition(s), deletion(s) and/or substitution(s), and optionally conjugated to one or more moieties at the N-terminus, C-terminus and/or internal site(s), have a total mass characterized by the ranges described generally herein, e.g., from about 2.6 kDa or 2.8 kDa to about 6 or 7 kDa.

In a further embodiment, the CNP variants are derivatives of CNP37, which is QEHPNARKYKGANKK-CNP22 (SEQ ID NO: 60). The CNP37 variants can contain amino acid addition(s), deletion(s), and/or substitution(s) with natural or unnatural amino acid(s) or peptidomimetic(s) (e.g., peptide bond isostere(s)) at any one or more of the 37 positions of CNP37. Non-limiting examples of substitutions that can be made in CNP37, based on the numbering of CNP22, include K4R, G5S, G5R, G8S, K10R, G15S, S16Q, M17N, G19R, and combinations thereof. In an embodiment, the CNP37 derivatives contain a substitution of Met17 to a natural (e.g., asparagine) or unnatural amino acid or peptidomimetic, designed in part to avoid oxidation of the sulfur atom of methionine. In another embodiment, the CNP37 variants contain substitution(s) of Lys8, Lys10, Lys14 and/or Lys15 (based on numbering from the N-terminus of CNP37) to non-basic natural or unnatural amino acid(s) or peptiomimetic(s), designed in part to reduce albumin binding.

In addition or alternatively to amino acid addition(s), deletion(s) and/or substitution(s), the CNP37 derivatives can be conjugated at the N-terminus, C-terminus, and/or an internal site to any of the moieties described herein, including but not limited to bone- or cartilage-targeting moieties (e.g., bone-targeting peptide domains), moieties that reduce renal clearance (e.g., negatively charged PEG moieties), hydrophilic polymers (e.g., PEG), amino acid sequences comprising one or more amino acids (e.g., osteocrin "NPR-C inhibitor" fragment), carbohydrates (e.g., carbohydrates recognized by receptors on the surface of cells at bone growth plates), hydrophobic acids (e.g., C_5-C_{12} carboxylic acids and natural fatty acids), and combinations thereof.

In one embodiment, the CNP variants are modified CNP37 peptides having mutation(s)/substitution(s) at the furin cleavage site (underlined), designed to improve in vivo resistance to the furin protease, and/or containing glycine (underlined) at the N-terminus, designed to improve plasma stability and prevent pyroglutamine formation. Such CNP37 variants include but are not limited to:

(SEQ ID NO: 71)
GQEHPNARKYKGANPKGLSKGCFGLKLDRIGSMSGLGC (An. CS);

(SEQ ID NO: 72)
GQEHPNARKYKGANQKGLSKGCFGLKLDRIGSMSGLGC (An. CT);

(SEQ ID NO: 73)
GQEHPNARKYKGANQQGLSKGCFGLKLDRIGSMSGLGC (An. CU);

(SEQ ID NO: 74)
GQEHPNARKYKGANKPGLSKGCFGLKLDRIGSMSGLGC (An. CW);

(SEQ ID NO: 75)
GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC
(Gly-CNP37, An. DB);
and

(SEQ ID NO: 145)
PGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC
(Pro-Gly-CNP37)

In a further embodiment, the CNP variants of the disclosure include CNP peptides and variants thereof that can be produced by the fusion protein process described herein. Non-limiting examples of CNP variants that can be produced by the fusion protein process described herein, using chemical or proteolytic cleavage or protein self-cleavage, include:

(SEQ ID NO: 179)
GDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMS
GLGC (Gly-wtCNP53);

(SEQ ID NO: 75)
GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC
(Gly-wtCNP37);

(SEQ ID NO: 60)
QEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (wtCNP37);

(SEQ ID NO: 144)
GHKSEVAHRFK-GANKKGLSKGCFGLKLDRIGSMSGLGC
(HSA fragment-wtCNP27);

(SEQ ID NO: 36)
GANRRGLSRGCFGLKLDRIGSMSGLGC [CNP27(K4,5,9R)];

(SEQ ID NO: 180)
DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSG
LGC [CNP53(M48N)];

(SEQ ID NO: 181)
GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC
[Gly-CNP37(M32N)];

(SEQ ID NO: 182)
QEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC
[CNP37(M32N)];

(SEQ ID NO: 183)
GHKSEVAHRFK-GANKKGLSKGCFGLKLDRIGSNSGLGC
[HSA-CNP27(M22N)];

(SEQ ID NO: 184)
GANRRGLSRGCFGLKLDRIGSNSGLGC [CNP27(K4,5,9R,
M22N)];

(SEQ ID NO: 185)
PDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMS
GLGC (Pro-wtCNP53);

(SEQ ID NO: 145)
PGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC
(Pro-Gly-wtCNP37);

-continued

(SEQ ID NO: 186)
PQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC
(Pro-wtCNP37);

(SEQ ID NO: 187)
PNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (wtCNP34);

(SEQ ID NO: 188)
P-GHKSEVAHRFK-GANKKGLSKGCFGLKLDRIGSMSGLGC
(Pro-HSA-wtCNP27);

(SEQ ID NO: 189)
PGANRRGLSRGCFGLKLDRIGSMSGLGC [Pro-CNP27(K4,5,9R)];

(SEQ ID NO: 190)
MDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMS
GLGC (Met-wtCNP53);

(SEQ ID NO: 191)
MGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC
(Met-Gly-wtCNP37);

(SEQ ID NO: 192)
MQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC
(Met-wtCNP37);

(SEQ ID NO: 193)
M-GHKSEVAHRFK-GANKKGLSKGCFGLKLDRIGSMSGLGC
(Met-HSA-wtCNP27);

(SEQ ID NO: 194)
MGANRRGLSRGCFGLKLDRIGSMSGLGC [Met-CNP27(K4,5,9R)].

Other CNP variants, including truncated CNP peptides ranging from hCNP-17 to hCNP-53 and having wild-type sequences or amino acid addition(s), deletion(s) and/or substitution(s), can also be produced by the fusion protein process described herein, so long as the intended site of chemical or proteolytic cleavage of the fusion protein is not present within the amino acid sequence of the target CNP variant itself. As a non-limiting example, the fusion protein process described herein can be employed to produce truncated wtCNP34 using formic acid cleavage.

In additional embodiments, for any of the CNP peptides and CNP variants described herein that have asparagine (Asn/N) residue(s) and/or glutamine (Gln/Q) residue(s), whether they have a wild-type sequence or a non-natural amino acid sequence, any Asn residue(s) and/or any Gln residue(s) can independently be substituted with any other natural or unnatural amino acids, including conservative substitutions such as Asn to Gln. Such substitution(s) are designed in part to minimize or avoid any potential deamidation of asparagine and/or glutamine. Non-limiting examples of CNP peptides and variants in which any Asn residue(s) and/or any Gln residue(s) can independently be substituted with any other natural or unnatural amino acids, including conservative substitutions such as Asn to Gln, include wtCNP34, wtCNP37, Gly-wtCNP37, Pro-wtCNP37, Pro-Gly-wtCNP37, GHKSE-VAHRFK-wtCNP27 (SEQ ID NO: 144), Pro-GHKSE-VAHRFK-wtCNP27 (SEQ ID NO: 188), PEO12-GANRR-CNP22 (K4R) (SEQ ID NO: 36), and PEO24-GANRR-CNP22 (K4R) (SEQ ID NO: 36). In certain embodiments, an asparagine residue of the CNP peptides and CNP variants described herein is not substituted with glutamine, aspartic acid or glutamic acid. In certain embodiments, a glutamine residue of the CNP peptides and CNP variants described herein is not substituted with asparagine, aspartic acid or glutamic acid. As a non-limiting example, asparagine residues 7 and/or 15 of Pro-Gly-wtCNP37 (PGQEHP NARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC) SEQ ID NO: 145) can independently be substituted with any other natural or unnatural amino acids, including glutamine, to avoid any potential deamidation of the asparagine residue(s) to aspartic acid or isoaspartic acid. In certain embodiments, asparagine residues 7 and/or 15 of Pro-Gly-wtCNP37 are not substituted with glutamine, aspartic acid or glutamic acid.

It is understood, however, that the present disclosure encompasses CNP variants in which any one or more, up to all, residues susceptible to deamidation or a deamidation-like reaction (e.g., isomerization) may be converted to other residue(s) via deamidation or a deamidation-like reaction to any extent, up to 100% conversion per converted residue. In certain embodiments, the disclosure encompasses CNP variants in which:

(1) any one or more, up to all, asparagine (Asn/N) residues may be converted to aspartic acid or aspartate, and/or to isoaspartic acid or isoaspartate, via deamidation up to about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% conversion per converted residue; or

(2) any one or more, up to all, glutamine (Gln/Q) residues may be converted to glutamic acid or glutamate, and/or to isoglutamic acid or isoglutamate, via deamidation up to about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% conversion per converted residue; or

(3) any one or more, up to all, aspartic acid or aspartate (Asp/D) residues may be converted to isoaspartic acid or isoaspartate via a deamidation-like reaction (also called isomerization) up to about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% conversion per converted residue; or

(4) any one or more, up to all, glutamic acid or glutamate (Glu/E) residues may be converted to isoglutamic acid or isoglutamate via a deamidation-like reaction (also called isomerization) up to about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% conversion per converted residue; or

(5) a combination of the above.

As a non-limiting example, the disclosure encompasses CNP variants in which any one or more, up to all, asparagine, glutamine, aspartic acid, and/or glutamic acid residues of Pro-Gly-wtCNP37 [PGQEHPNARKYKGA NKKGLSKGCFGLKLDRIGSMSGLGC] (SEQ ID NO: 145) may be converted to (1) aspartic acid/aspartate and/or isoaspartic acid/isoaspartate, (2) glutamic acid/glutamate and/or isoglutamic acid/isoglutamate, (3) isoaspartic acid/isoaspartate, and/or (4) isoglutamic acid/isoglutamate, respectively, via deamidation or a deamidation-like reaction up to about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% conversion per converted residue, as described above.

As a further example, the disclosure encompasses CNP variants in which any one or more, up to all, asparagine and/or aspartic acid residues of Pro-Gly-wtCNP37 [PGQEHPNARKYKGANKKGLSKGCFGLKL DRIGSMSGLGC] (SEQ ID NO: 145) may be converted to (1) aspartic acid/aspartate and/or isoaspartic acid/isoaspartate, and/or (2) isoaspartic acid/isoaspartate, respectively, via deamidation or a deamidation-like reaction up to about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% conversion per converted residue.

As another example, the present disclosure encompasses CNP variants in which any one or more, up to all, asparagine, glutamine, aspartic acid, and/or glutamic acid residues of Gly-wtCNP37 [GQEHPNARKYKGA NKKGLSKGCFGLKLDRIGSMSGLGC (SEQ ID NO: 75)] may be converted to (1) aspartic acid/aspartate and/or isoaspartic acid/isoaspartate, (2) glutamic acid/glutamate

and/or isoglutamic acid/isoglutamate, (3) isoaspartic acid/isoaspartate, and/or (4) isoglutamic acid/isoglutamate, respectively, via deamidation or a deamidation-like reaction up to about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% conversion per converted residue.

As yet another example, the disclosure encompasses CNP variants in which any one or more, up to all, asparagine, glutamine, aspartic acid, and/or glutamic acid residues of wtCNP37 [QEHPNARKYKGANKKGLSKGCFGLKL DRIGSMSGLGC (SEQ ID NO: 60)] may be converted to (1) aspartic acid/aspartate and/or isoaspartic acid/isoaspartate, (2) glutamic acid/glutamate and/or isoglutamic acid/isoglutamate, (3) isoaspartic acid/isoaspartate, and/or (4) isoglutamic acid/isoglutamate, respectively, via deamidation or a deamidation-like reaction up to about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% conversion per converted residue.

As a further example, the present disclosure encompasses CNP variants in which any one or more, up to all, asparagine, aspartic acid, and/or glutamic acid residues of an HSA-wtCNP27 chimera, GHKSEVAHRFKGA NKKGLSKGCFGLKLDRIGSMSGLGC (SEQ ID NO: 144), may be converted to (1) aspartic acid/aspartate and/or isoaspartic acid/isoaspartate, (2) isoaspartic acid/isoaspartate, and/or (3) isoglutamic acid/isoglutamate, respectively, via deamidation or a deamidation-like reaction up to about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% conversion per converted residue.

As a still further example, the disclosure encompasses CNP variants in which any one or more, up to all, asparagine, aspartic acid, and/or glutamic acid residues of a Pro-HSA-wtCNP27 chimera, PGHKSEVAHRFKGA NKKGLSKGCFGLKLDRIGSMSGLGC (SEQ ID NO: 188), may be converted to (1) aspartic acid/aspartate and/or isoaspartic acid/isoaspartate, (2) isoaspartic acid/isoaspartate, and/or (3) isoglutamic acid/isoglutamate, respectively, via deamidation or a deamidation-like reaction up to about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% conversion per converted residue.

In addition, the present disclosure encompasses CNP variants in which any one or more, up to all, methionine (Met/M) residues may be oxidized to any chemically feasible oxidized form (e.g., sulfoxide and/or sulfone) up to about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% transformation per oxidized residue.

In another embodiment, the CNP variants comprise CNP22 or variants thereof conjugated at the N-terminus and/or C-terminus to moiet(ies) that facilitate translocation of the variants across a cell membrane or cell barrier. In one embodiment, the CNP variants are conjugated at the N-terminus and/or C-terminus to peptide sequence(s) that facilitate transport of the variants across a cell membrane or cell barrier, including via active peptide transporters.

In a further embodiment, the N-terminus and/or C-terminus of CNP22 or a variant thereof are conjugated to chemical moieties such as, e.g., natural and/or synthetic polymers, to increase the total mass of the modified CNP peptide to the ranges described generally herein, e.g., a range from about 2.6 or 2.8 kDa to about 6 or 7 kDa. In one embodiment, the chemical moieties are biocompatible hydrophilic or water-soluble natural (e.g., peptides, carbohydrates) or synthetic (e.g., PEG (or PEO)) polymers.

In a particular embodiment, the N-terminus and/or C-terminus of CNP22 or a variant thereof are conjugated to PEG (or PEO) polymers to result in a total mass characterized by the ranges described generally herein, e.g., from about 2.6 or 2.8 kDa to about 6 or 7 kDa. Pegylation of CNP22 or a variant thereof is designed, inter alia, to reduce immunogenicity and improve half-life by reducing renal clearance and increasing protease resistance. A PEG moiety can be attached to the N- and/or C-terminus of CNP22 or any variant described herein, including but not limited to CNP-17 (the Cys6-Cys22 cyclized portion of CNP22), CNP37, and variants of CNP17, CNP22 or CNP37 having N- and/or C-terminal amino acid extension(s), amino acid substitution(s) and/or amino acid deletion(s). In an embodiment, the Lys4 and/or Lys10 residues of CNP17, CNP22 or CNP37, or variants thereof, are substituted with a natural or unnatural amino acid (e.g., Arg, Gly, Ser, Gln, Glu or Cit) or peptidomimetic that does not contain a reactive primary amine on a side chain, to preclude any potential PEGylation of these lysine residues. In one embodiment, the Lys4 and/or Lys10 residues of the CNP peptides are substituted with Arg. In another embodiment, the Lys10 residue is not substituted with Arg.

In a further embodiment, CNP variants (including CNP22 and variants thereof) having a PEG (or PEO) moiety and an amino acid extension at the N-terminus contain arginine at the position immediately preceding the position corresponding to Gly1 of CNP22. Such PEGylated CNP variants are designed for increased resistance to NEP degradation, reduced binding to serum albumin, and enhanced CNP functional activity (e.g., activation of cGMP signaling). Non-limiting examples of PEGylated CNP variants include PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36), PEO12-GANRR-CNP22(K4R) (SEQ ID NO: 36), PEO24-GANRR-CNP22(SEQ ID NO: 36), PEO12-GANRR-CNP22(SEQ ID NO: 36), PEO24-GANPR-CNP22(K4R) (SEQ ID NO: 37), PEO12-GANPR-CNP22(K4R) (SEQ ID NO: 37), PEO24-GANPR-CNP22(SEQ ID NO: 37), PEO12-GANPR-CNP22 (SEQ ID NO: 37), PEO24-GANQQ-CNP22(SEQ ID NO: 64), PEO12-GANQQ-CNP22(SEQ ID NO: 64), PEO24-ER-CNP22(K4R) (SEQ ID NO: 39), PEO12-ER-CNP22 (K4R) (SEQ ID NO: 39), PEO24-ER-CNP22(SEQ ID NO: 39), PEO12-ER-CNP22(SEQ ID NO: 39), PEO24-R-CNP22(K4R) (SEQ ID NO: 41), PEO12-R-CNP22(K4R) (SEQ ID NO: 41), PEO24-R-CNP22(SEQ ID NO: 41), and PEO12-R-CNP22(SEQ ID NO: 41), wherein PEO24 is a monodispersed 1.2 kDa PEG polymer and PEO12 is a monodispersed 0.6 kDa PEG polymer. In one embodiment, the PEG (or PEO) polymer is conjugated to the N-terminus of the CNP variants.

The disclosure contemplates use of hydrophilic or water soluble polymers (e.g., PEG) that can vary in type (e.g., homopolymer or copolymer; random, alternating or block copolymer; linear or branched; monodispersed or polydispersed), linkage (e.g., hydrolysable or stable linkage such as, e.g., amide, imine, aminal, alkylene, or ester bond), conjugation site (e.g., at the N-terminus and/or C-terminus, preferably not at any of the residues in the cyclized region of CNP (corresponding to residues 6-22 of CNP22)), and length (e.g., from about 0.2, 0.4 or 0.6 kDa to about 2, 3, 4 or 5 kDa). The hydrophilic or water-soluble polymer can be conjugated to the CNP peptide by means of N-hydroxy succinimide (NHS)- or aldehyde-based chemistry or other chemistry, as is known in the art. Such CNP variants can be generated using, e.g., wtCNP22 (2.2 kDa), CNP17 retaining only the cyclized region (residues 6-22) of wtCNP22, CNP variants having an amino acid extension at the N-terminus and/or C-terminus of CNP22 or CNP17, or variants having amino acid substitutions, additions and/or deletions such as, e.g., GANRR-CNP22(K4R) (SEQ ID NO: 36), GANPR-CNP22(K4R) (SEQ ID NO: 37), R-CNP22(SEQ ID NO: 40), R-CNP22(K4R) (SEQ ID NO: 41), ER-CNP22(SEQ ID

NO: 38) and ER-CNP22(K4R) (SEQ ID NO: 39). In an embodiment, the PEG-CNP variants having a total mass characterized by the ranges described generally herein, e.g., from about 2.6 or 2.8 kDa to about 6 or 7 kDa, contain a monodispersed, linear PEG (or PEO) moiety conjugated at the N-terminus and/or C-terminus via NHS- or aldehyde-based chemistry, or a two-arm or three-arm branched PEG moiety conjugated at the N-terminus and/or C-terminus via NHS-based chemistry. The disclosure also encompasses negatively charged PEG-CNP variants designed for reduced renal clearance, including but not limited to carboxylated, sulfated and phosphorylated compounds (Caliceti, Adv. Drug Deliv. Rev., 55: 1261-77 (2003); Perlman, J. Clin. Endo. Metab., 88: 3227-35 (2003); Pitkin, Antimicrob. Ag. Chemo., 29: 440-444 (1986); Vehaskari, Kidney Int'l, 22: 127-135 (1982)). In one embodiment, the PEG (or PEO) moiety contains carboxyl group(s), sulfate group(s), and/or phosphate group(s).

In another embodiment, the PEG (or PEO) moieties conjugated to the N-terminus, C-terminus and/or internal site(s) of CNP variants described herein contain one or more functional groups that are positively charged under physiological conditions. Such PEG moieties are designed, inter alia, to improve distribution of such PEGylated CNP variants to cartilage tissues. In one embodiment, such PEG moieties contain one or more primary, secondary or tertiary amino groups, quaternary ammonium groups, and/or other amine-containing (e.g., urea) groups.

In an embodiment, the disclosure encompasses CNP22 or variants thereof conjugated via NHS- or aldehyde-based chemistry to PEG (or PEO) of the formula $(CH_2CH_2O)_n$, wherein n is an integer from about 6 to about 100, and the PEG polymer is from about 0.3 kDa to about 5 kDa. In another embodiment, n is an integer from about 12 to about 50, and the PEG polymer is from about 0.6 kDa to about 2.5 kDa. In yet another embodiment, n is from about 12 to about 24, and the PEG polymer is from about 0.6 kDa to about 1.2 kDa. In still another embodiment, the terminal hydroxyl group of the PEG polymer is capped with a non-reactive group. In a particular embodiment, the end-capping group is an alkyl group, e.g., a lower alkyl group such as methyl.

In a further embodiment, the disclosure provides CNP variants having one or more peptide bonds or peptide bond isosteres that have reduced susceptibility to cleavage by peptidases including neutral endopeptidase (NEP). NEP is a membrane-bound zinc-dependent endopeptidase that cleaves a substrate peptide bond at the amino end of large hydrophobic residues. Thus, modification of a peptide bond at a cleavage site for NEP to an unnatural peptide or non-peptide bond may preclude or decrease the efficiency of NEP cleavage.

For ANP and CNP, NEP cleavage is reported to occur first at the Cys6-Phe7 bond within the cyclized region, then elsewhere throughout the remainder of the structures. For BNP, cleavage is reported to occur first at the peptide N-terminus, then within the cyclic structure. Although the primary NEP cleavage site on CNP is reported to be the Cys6-Phe7 bond, when wtCNP22 was exposed to NEP digestion for 2.5 minutes in vitro, all possible sites were unexpectedly hydrolyzed, with the Cys6-Phe7 and Gly8-Leu9 peptide bonds being slightly most labile, as described in Example 2.

Substrate specificity of NEP is primarily determined by two substrate-binding subsites, S1' and S2' (Oefner et al., J. Mol. Biol. 296:341-349 (2000)). The S1' site accepts a large hydrophobic P1' residue of which the N-terminal peptide bond is subjected to hydrolysis (e.g., Phe, Leu, Be and Met). The S2' site generally prefers a smaller residue, termed P2' (e.g., Gly or Ser). In the case of CNP, Phe7 is reported to be the preferred P1' residue for the NEP S1' site, while Gly8 is the preferred P2' residue for the S2' site. Because these two subsites can together accommodate only a certain total side chain size, any increase in the total size of the P1'-P2' residues of CNP can potentially disrupt NEP binding. For example, addition of a chloride atom at the 3-position of the P1' Phe7 aromatic ring (i.e., 3-Cl-Phe7) can potentially modify (e.g., destabilize) interactions between CNP and the NEP cleavage sites, for example at the S1' subsite. Addition of a tertiary butyl group to the smaller P2' residue Gly8 (i.e., tBu-Gly8) can potentially disrupt the interaction between CNP and the S2' subsite.

Accordingly, in one embodiment, CNP variants of the disclosure include CNP having an increase in the size of the P1'-P2' residues, such as Phe7-Gly8, to interfere with substrate recognition at the active site, thereby reducing susceptibility to NEP cleavage. Natural amino acids, unnatural amino acids and/or peptidomimetic moieties are substituted for one or more large P1' hydrophobic residues, including but not limited to Phe7, Leu9, Leu11, Ile14, Met17 and Leu20, and/or for one or more smaller P2' residues, including but not limited to Cys6, Gly8, Gly15, Ser16 and Gly19.

The disclosure encompasses CNP variants comprising at least one modified amino acid and/or at least one modified peptide bond, at at least one residue involved in substrate recognition and/or cleavage by NEP, wherein the modified amino acids and modified peptide bonds can be natural amino acids, unnatural amino acids, peptidomimetics and/or peptide bond isosteres. In one embodiment, the NEP cleavage site on CNP between Cys6 and Phe7 is modified. In a related embodiment, the peptide bond (—C(═O)—NH—) between Cys6 and Phe7 is replaced with one of the following peptide bond isosteres:

—CH_2—NH—,

—C(═O)—N(R)—, where the amide group is alkylated with any of the following R groups: methyl, ethyl, n-propyl, isopropyl, cyclopropyl, n-butyl, isobutyl, sec-butyl or tert-butyl,

—C(═O)—NH—CH_2—,

—CH_2—S—,

—CH_2—S(O)$_n$—, where n is 1 or 2,

—CH_2—CH_2—,

—CH═CH—,

—C(═O)—CH_2—,

—CH(CN)—NH—,

—CH(OH)—CH_2—,

—O—C(═O)—NH—, or

—NHC(═O)NH—.

In another embodiment, the CNP variants are represented by the formula:

(x)-Gly_1-Leu_2-Ser_3-Lys_4-Gly_5-(b)$_6$-(c)$_7$-(d)$_8$-Leu_9-Lys_{10}-Leu_{11}-Asp_{12}-Arg_{13}-Ile_{14}-Gly_{15}-Ser_{16}-Met_{17}-Ser_{18}-Gly_{19}-Leu_{20}-Gly_{21}-Cys_{22}-(z) (SEQ ID NO: 90), wherein:

(x) and (z) independently may be absent or may be selected from the group consisting of synthetic bone-targeting compounds such as, e.g., bisphosphonates; amino acid sequences useful in bone or cartilage targeting such as, e.g., polyAsp and polyGlu; amino acid sequences derived from bone-targeting domains of bone proteins such as, e.g., osteopontin, osteocalcin, and sialoprotein (Wang et al., Adv. Drug Delivery Rev., 57: 1049-76 (2005)); polymeric and non-polymeric molecules that reduce renal clearance such as, e.g., negatively charged PEGs; and natural polymers (e.g., those containing amino acids, fatty acids and/or carbohydrates) and synthetic polymers (e.g., PEGs) that increase

resistance of the CNP variant to NEP degradation by increasing the total mass of the CNP variant to the ranges described generally herein, e.g., from about 2.6 or 2.8 kDa to about 6 or 7 kDa;

(b) and (c) may be the wild-type Cys6 and Phe7, another natural amino acid or an unnatural amino acid, or may contain a peptide bond isostere as described herein to increase resistance to NEP cleavage; and

(d) may be the wild-type Gly8, or may be a larger natural or unnatural (e.g., t-Bu-Gly) amino acid or peptidomimetic to reduce binding to NEP.

In one embodiment, such CNP variants contain at least one modified amino acid at (b), (c) and/or (d).

Other peptide bonds within CNP may be cleaved even if CNP22 or a variant thereof has an NEP-resistant peptide bond or peptide bond isostere at Cys6-Phe7, including the Gly8-Leu9, Lys10-Leu11, Arg13-Ile14, Ser16-Met17, and Gly19-Leu20 bonds. Therefore, the disclosure encompasses CNP variants having peptide bond isostere(s) at one or more other NEP cleavages sites in addition to the Cys6-Phe7 bond, wherein the peptide bond isosteres include those described herein.

In another embodiment, the disclosure encompasses CNP variants having a cysteine analog at Cys6 and/or Cys22, including but not limited to homocysteine, penicillamine, 2-mercaptopropionic acid, and 3-mercaptopropionic acid. In an embodiment, such CNP variants have a cyclic domain formed by a disulfide bond between the wild-type Cys6 or analog and Cys22 or analog.

In yet another embodiment, one or more residues of CNP22 or a variant thereof, up to all the residues, are substituted with a D-amino acid. Substitution of an L-amino acid with a D-amino acid essentially moves the side chain about 120 degrees from its original position, thereby potentially disrupting the binding of the CNP peptide to NEP. In a specific embodiment, L-Phe at Phe7 is substituted with its D-enantiomer, D-Phe.

In still another embodiment, a beta amino acid such as, e.g., 3-amino-2-phenylpropionic acid (or 2-phenyl-beta-alanine), replaces the wild-type alpha-amino acid Phe7. Use of a beta-amino acid effectively increases the peptide backbone length by one methylene unit. Protease resistance can result from the change in substrate conformation or the increased distance between amino acid side chains.

Non-limiting examples of variants of CNP22 having an unnatural alpha-amino acid, a beta-amino acid or a peptide bond isostere include:

(SEQ ID NO: 56)
GLSKGC(CH_2NH)FGLKLDRIGSMSGLGC (Analog A),

(SEQ ID NO: 57)
GLSKGC-(N-Me—Phe)-GLKLDRIGSMSGLGC (Analog B),

(SEQ ID NO: 136)
GLSKGC-(D-Phe)-GLKLDRIGSMSGLGC (Analog E),

(SEQ ID NO: 58)
GLSKGCF-(tBu—Gly)-LKLDRIGSMSGLGC (Analog F),

(SEQ ID NO: 137)
GLSKGC-(3-Cl-Phe)-GLKLDRIGSMSGLGC (Analog G), and

(SEQ ID NO: 59)
GLSKGC-[NHCH_2CH(Ph)CO]-GLKLDRIGSMSGLGC (Analog H, formed using 3-amino-2-phenylpropionic acid).

In a further embodiment, the CNP variants have a total mass characterized by the ranges described generally herein, e.g., from about 2.6 or 2.8 kDa to about 6 or 7 kDa, designed for increased resistance to NEP degradation, and are represented by the formula:

(x)-Gly_1-Leu_2-Ser_3-(a)$_4$-Gly_5-(b)$_6$-(c)$_7$-(d)$_8$-(e)$_9$-(f)$_{10}$-(g)$_{11}$-Asp_{12}-Arg_{13}-(h)$_{14}$-Gly_{15}-Ser_{16}-(i)$_{17}$-Ser_{18}-Gly_{19}-(j)$_{20}$-Gly_{21}-Cys_{22}-(z) (SEQ ID NO: 46), wherein:

(x) and (z) independently may be absent or may be selected from the group consisting of synthetic bone-targeting compounds such as, e.g., bisphosphonates; amino acid sequences useful in bone or cartilage targeting such as, e.g., polyAsp and polyGlu; amino acid sequences derived from bone-targeting domains of bone proteins such as, e.g., osteopontin, osteocalcin, and sialoprotein; polymeric and non-polymeric moieties that reduce renal clearance such as, e.g., negatively charged PEGs; polymers containing, e.g., amino acids, hydrophobic acids, and/or carbohydrates; and synthetic hydrophilic polymers such as, e.g., PEGs;

(a) may be the wild-type Lys at that position or may be replaced with a conservative amino acid substitution or a natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amine on a side chain, including but not limited to Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu, wherein in one embodiment (a) is Arg;

(b) is selected from the group consisting of Cys and peptide-bond isosteres between Cys6 and Phe7 such as, e.g., Cys-CH_2—NH;

(c) is selected from the group consisting of L-Phe; D-Phe; 3-amino-2-phenylpropionic acid; N-alkylated derivatives of Phe, wherein the N-alkyl group is methyl, ethyl, n-propyl, isopropyl, cyclopropyl, n-butyl, isobutyl, sec-butyl or tert-butyl; and Phe analogs, wherein one or more ortho-, meta-, and/or para-positions of the benzene ring of the Phe analog are substituted with one or more substituents selected from the group consisting of halogen, hydroxyl, cyano, straight or branched C_{1-6} alkyl, straight or branched C_{1-6} alkoxy, straight or branched halo-C_{1-6} alkyl, C_{3-10} cycloalkyl, heterocyclyl, C_{6-14} aryl and heteroaryl (examples include, but are not limited to, tyrosine, 3-chlorophenylalanine, 2,3-chloro-phenylalanine, 3-chloro-5-fluoro-phenylalanine, 2-chloro-6-fluoro-3-methyl-phenylalanine), or wherein the benzene ring of the Phe analog can be replaced with another aryl group (non-limiting examples include 1- and 2-naphthylalanine) or with a heteroaryl group (non-limiting examples include pyridylalanine, thienylalanine and furylalanine);

(d) is selected from the group consisting of Gly, tert-butyl-Gly (tBu-Gly), Thr, Ser, Val and Asn;

(e) is selected from the group consisting of Leu, Ser, Thr and peptide-bond isosteres such as, e.g., N-Me-Leu;

(f) may be the wild type Lys at that position or may be replaced with a conservative amino acid substitution or a natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amine on a side chain, including but not limited to Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu, wherein in one embodiment (f) is not Arg;

(g) is selected from the group consisting of Leu and peptide-bond isosteres such as, e.g., N-Me-Leu;

(h) is selected from the group consisting of Ile, tBu-Gly, and peptide-bond isosteres such as, e.g., N-Me-Ile;

(i) is selected from the group consisting of Met, Val, Asn, beta-Cl-Ala, 2-aminobutyric acid (Abu) and 2-amino-isobutyric acid (Aib); and

(j) is selected from the group consisting of Leu, norleucine (Nle), homoleucine (Hleu), Val, tert-butyl-Ala (tBu-Ala), Ser, Thr, Arg, and peptide-bond isosteres such as, e.g., N-Me-Leu.

In another embodiment, the CNP variants have a total mass characterized by the ranges described generally herein, e.g., from about 2.6 or 2.8 kDa to about 6 or 7 kDa, designed for increased resistance to NEP cleavage, and are represented by the formula:

(x)-Gly_1-Leu_2-Ser_3-$(a)_4$-Gly_5-$(b)_6$-$(c)_7$-$(d)_8$-$(e)_9$-$(f)_{10}$-$(g)_{11}$-Asp_{12}-Arg_{13}-$(h)_{14}$-$(i)_{15}$-Ser_{16}-$(j)_{17}$-Ser_{18}-Gly_{19}-$(k)_{20}$-Gly_{21}-Cys_{22}-(z) (SEQ ID NO:143), wherein:

(x) and (z) independently may be absent or may be selected from the group consisting of synthetic bone-targeting compounds such as, e.g., bisphosphonates; amino acid sequences useful in bone or cartilage targeting such as, e.g., polyAsp and polyGlu; amino acid sequences derived from bone-targeting domains of bone proteins and derivatives thereof, such as, e.g., fusion proteins or peptide sequences of osteopontin, osteocalcin, sialoprotein, etc.; moieties that reduce renal clearance, including but not limited to hydrophilic or water-soluble polymers such as, e.g., charged PEG molecules; and moieities comprising, e.g., PEGs, amino acids, carbohydrates, and/or hydrophobic acids;

(a) may be the wild type Lys at that position or may be replaced with a conservative amino acid substitution or a natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amine on a side chain, including but not limited to Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu, wherein in one embodiment (a) is Arg;

(b) is selected from the group consisting of Cys and peptide bond isosteres between Cys6 and Phe7 such as, e.g., Cys-CH_2—NH;

(c) is selected from the group consisting of L-Phe; D-Phe; 3-amino-2-phenylpropionic acid; N-alkylated derivatives of Phe, wherein the N-alkyl group is methyl, ethyl, n-propyl, isopropyl, cyclopropyl, n-butyl, isobutyl, sec-butyl or tert-butyl; and Phe analogs, wherein one or more ortho-, meta-, and/or para-positions of the benzene ring of the Phe analog are substituted with one or more substituents selected from the group consisting of halogen, hydroxyl, cyano, straight or branched C_{1-6} alkyl, straight or branched C_{1-6} alkoxy, straight or branched halo-C_{1-6} alkyl, C_{3-10} cycloalkyl, C_{6-14} aryl, heterocyclyl and heteroaryl (examples include, but are not limited to, tyrosine, 3-chlorophenylalanine, 2,3-chlorophenylalanine, 3-chloro-5-fluoro-phenylalanine, 2-chloro-6-fluoro-3-methyl-phenylalanine), or wherein the benzene ring of the Phe analog can be replaced with another aryl group (non-limiting examples include 1- and 2-naphthylalanine) or with a heteroaryl group (non-limiting examples include pyridylalanine, thienylalanine and furylalanine);

(d) is selected from the group consisting of Gly, tert-butyl-Gly, Thr, Ser, Val and Asn;

(e) is selected from the group consisting of Leu, Ser, Thr, and peptide bond isosteres such as, e.g., N-Me-Leu;

(f) is selected from the group consisting of Lys, Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln and Ser;

(g) is selected from the group consisting of Leu, Asn, and peptide bond isosteres such as, e.g., N-Me-Leu;

(h) is selected from the group consisting of Ile, tert-butyl-Gly (tBu-Gly), Asn, and peptide bond isosteres such as, e.g., N-Me-Ile;

(i) is selected from the group consisting of Gly, Arg, Ser and Asn;

(j) is selected from the group consisting of Met, Val, Asn, beta-Cl-Ala, 2-aminobutyric acid (Abu) and 2-amino-isobutyric acid (Aib); and

(k) is selected from the group consisting of Leu, norleucine (Nle), homoleucine (Hleu), Val, tert-butyl-Ala (tBu-Ala), Arg, Thr, Ser, and peptide bond isosteres such as, e.g., N-Me-Leu.

To improve the delivery of the CNP variants to the target sites of bone-related disorders (e.g., skeletal dysplasias), the CNP variants can be attached (e.g., at the N-terminus and/or C-terminus) to bone- or cartilage-targeting moieties. Non-limiting examples of bone- or cartilage-targeting moieties include bisphosphonates; hydroxyapatite; glucosamine; collagen (e.g., collagen type X); polyAsp; polyGlu; and amino acid sequences derived from bone-targeting domains of bone proteins such as, e.g., osteocrin, osteopontin, osteocalcin, and sialoprotein.

In addition to being less susceptible to NEP cleavage, the CNP variants potentially have reduced affinity to the NPR-C clearance receptor, while retaining CNP functionality. Besides NEP-mediated degradation, the half-life of CNP22 is influenced by the clearance receptor, NPR-C, which shares 58% sequence homology with the extracellular peptide-binding domain of NPR-B. CNP22 binds tightly to not only NPR-B (7-30 pM affinity), but also NPR-C (11-140 pM) (Bennett, B. D. et al., J. Biol. Chem., 266: 23060-67 (1991); Koller K. J. & Goeddel, D. V., Circulation, 86: 1081-88 (1992); Suga, S. et al., Endocrinology, 130: 229-39 (1992)). Even though the NPR-B crystal structure has yet to be reported, sequence homology as well as similarities between the NPR-C and NPR-A crystal structures (He, X.-L. et al., Science, 293(5535): 1657-62 (2001); Ogawa, H. et al., J. Biol. Chem., 279(27): 28625-31 (2004); He, X.-L., J. Mol. Biol., 361(4): 698-714 (2006)) suggest that NPR-B likely assumes a similar overall structural fold.

Therefore, an NPR-B homology model was built based on structure-based sequence alignment and crystallographic structures of the following related systems: CNP bound to NPR-C, ANP bound to NPR-A, and ANP bound to NPR-C (He, X.-L. et al., Science, 293(5535): 1657-62 (2001); Ogawa, H. et al., J. Biol. Chem., 279(27): 28625-31 (2004); He, X.-L., J. Mol. Biol., 361(4): 698-714 (2006)). Based on observations that the receptor appears to determine the bound peptide conformation, and that NPR-B most closely resembles NPR-A with respect to primary structure and functional properties, the NPR-B/CNP homology model was built with the NPR-A/ANP crystal structure as a model. Published signaling data of CNP variants (U.S. Pat. No. 5,434,133 and US Patent Application Publication No. 2004/0138134 A1), and of functional ANP variants that no longer bind to NPR-C (Cunningham, EMBO 13(11) 2508-15, 1994) were used to refine and interpret the NPR-B/CNP model.

The present disclosure encompasses CNP variants designed for improved NPR-B selectivity based on a homology-based structural model of the NPR-B/CNP complex. Combining the experimental and computational structure data of natriuretic peptides bound to the various receptors with the published functional data, CNP variants were generated that continue to bind to NPR-B, but can potentially have reduced affinity to the NPR-C clearance receptor. For example, NPR-C has a unique insertion in a loop structure in the peptide-binding site, placing its loop residues closer to such peptide residues as CNP Gly8 (or ANP Gly9), compared to respective loop residues in NPR-A and NPR-B. Earlier studies indicated that the G9T mutation in ANP contributes to reduce affinity to NPR-C, thereby improving NPR-A selectivity (Cunningham, EMBO J., 13(11): 2508-15 (1994)). Accordingly, CNP variants were generated to replace the corresponding Gly8 residue with a larger residue (Ser, Val, Thr or Asn) to disrupt the CNP binding to NPR-C without affecting its binding to NPR-B. Further, one or more mutations were introduced at the C-terminal end of CNP, encompassing Gly15 to Gly21,

which is predicted to interact with receptor-specific residues, based on the detailed structural analyses of the receptor/peptide complexes. For example, a G19R mutation in CNP22 does not result in a significant loss of NPR-B signaling activity. This mutation, however, cannot be modeled into the available crystal structure of NPR-C/CNP without altering the conformations of neighboring residues. These observations suggest that the G19R mutation may selectively disrupt the binding of CNP to a particular receptor, such as NPR-C.

In an embodiment, the CNP variants have substitution(s) at one or more Gly sites at positions 1, 5, 8, 15, 19 and 21, to reduce conformational flexibility and thereby increase receptor specificity. Comparative analyses of crystal structures of ANP bound to NPR-C and NPR-A (Ogawa, H. et al., J. Biol. Chem., 279: 28625-31 (2004); He, X.-L., J. Mol. Biol., 361: 698-714 (2006)) indicate that the conformational flexibility of ANP may play an important role in determining the receptor selectivity.

In one embodiment, functional CNP variants with potentially reduced affinity to NPR-C have one or more of the following amino acid substitutions: G1R, G1E, G5R, G5Q, G5S, F7Y, G8T, G8S, G8V, G8N, L9S, L9T, K10Cit, K10Q, K10S, 114N, G15R, G15S, G15N, G15Cit, S16Q, M17V, M17N, G19S, G19R, G19N, L20V, L20R, L20I, L20S, G21S, G21T and G21R. In an embodiment, the CNP variants have multipoint substitutions at positions 1, 5, 7, 8, 9, 10, 14, 15, 16, 17, 19, 20 and/or 21, and may optionally have modifications at any of the other positions in the peptide sequence of the variant.

In a further embodiment, the CNP variants described herein may be conjugated to moieties, up to a total mass characterized by the ranges described generally herein, e.g., from about 2.6 or 2.8 kDa to about 6 or 7 kDa, at the N-terminus, the C-terminus and/or an internal site, to facilitate bone/cartilage targeting, reduce NPR-C and renal clearance, increase resistance to NEP degradation, and/or improve CNP functionality. In one embodiment, the CNP variants are not conjugated to a polymeric moiety at a site within the cyclic region (corresponding to Cys6 to Cys22 of CNP22). Non-limiting examples of polymeric or non-polymeric moieties that can be conjugated to the CNP variants include synthetic bone-targeting compounds such as, e.g., bisphosphonates; bone/cartilage targeting peptide sequences such as, e.g., polyAsp and polyGlu; peptide sequences derived from bone-targeting domains of bone proteins such as, e.g., osteopontin, osteocalcin and sialoprotein; peptide sequences derived from the functional domains of bone morphogenetic proteins such as, e.g., BMP2, BMP3, BMP5, BMP7 and BMP8a; peptide sequences derived from polypeptides of natriuretic origin such as, e.g., NPPC, ANP and BNP; other natural polymeric or non-polymeric moieties such as, e.g., carbohydrates, fatty acids and phospholipids; biocompatible synthetic hydrophilic polymers such as, e.g., PEG (or PEO); hydrophobic polymeric or non-polymeric moieties such as, e.g., heptanoic acid and pentanoic acid; and combinations thereof.

The CNP variants described herein can have substantially similar or better functional activity than CNP22, e.g., with respect to stimulation of cGMP production and signaling. In one embodiment, the CNP variants in vitro or in vivo stimulate the production of at least about 50% of the cGMP level produced under the same concentration of wtCNP22 (e.g., 1 uM). In certain embodiments, the CNP variants retain at least about 50%, 60%, 70%, 80%, 90%, 95% or 100% of the cGMP-stimulation activity of wild-type CNP22 in vitro or in vivo. In another embodiment, the CNP variants have improved cGMP-stimulation activity compared to CNP22. In certain embodiments, the CNP variants in vitro or in vivo stimulate the production of at least about 110%, 120%, 130%, 140%, 150%, 200%, 250%, 300%, 350%, 400%, 450%, 500% or more of the cGMP level produced under the same concentration of wtCNP22 (e.g., 1 uM).

Optionally excluded from the present disclosure are any of the natriuretic (e.g., CNP) peptides, fragments and variants specifically disclosed, and any of the natriuretic (e.g., CNP) peptides, fragments and variants actually produced, in any of the prior publications referenced herein, including but not limited to, U.S. Pat. Nos. 5,434,133, 6,034,231, 6,020,168, 6,743,425, 7,276,481, WO 94/20534, WO 02/047871, WO 2005/098490, WO 2004/047871, EP 0497368, EP 0466174, and Furuya et al., Biochem. Biophys. Res. Comm. 183: 964-969 (1992)). All such documents are incorporated by reference herein in their entirety.

In one embodiment, the present disclosure optionally excludes all known wild-type CNP-53, wild-type CNP-22, wild-type CNP-17, wild-type BNP, and wild-type ANP of human origin and non-human origin. For example, in an embodiment the disclosure optionally excludes human CNP-17, human CNP-22, chicken CNP-22 (corresponding to hCNP-22(Leu9Val)), trout and eel CNP-22 (corresponding to hCNP-22(Leu2Trp, Ser3Asn, Lys4Arg)), frog CNP22-I (corresponding to hCNP-22(Leu2Tyr, Lys4Arg, Leu9Val, Ser16Ala, Met17Phe)), frog CNP22-II (corresponding to hCNP-22(Leu2Thr, Ser16Ala)), human CNP-53, and pig and rat CNP-53 (corresponding to hCNP-53 (Gln17His, Ala28Gly)). In another embodiment, the disclosure optionally excludes fragments of NPPC, proCNP and CNP-53 which are produced by proteolytic cleavage in vivo in humans and non-human animals. In yet another embodiment, optionally excluded from the disclosure are the following truncated fragments of wild-type human CNP-53: CNP-50, CNP-46, CNP-44, CNP-39, CNP-30, CNP-29, CNP-28, CNP-27 and CNP-26.

In a further embodiment, the present disclosure optionally excludes CNP peptides and fragments thereof isolated or sought from the shark species Triakis scyllia and Scyliorhinus canicula (see, e.g., M. Takano et al., Zool. Sci., 11: 451-454 (1994)), including:

(SEQ ID NO: 204)
RLLKDLSNNPLRFRGRSKKGPSRGCFGVKLDRIGAMSGLGC (CNP-41);

(SEQ ID NO: 205)
LKDLSNNPLRFRGRSKKGPSRGCFGVKLDRIGAMSGLGC (CRP-39);

(SEQ ID NO: 206)
KDLSNNPLRFRGRSKKGPSRGCFGVKLDRIGAMSGLGC (CNP-38); and

(SEQ ID NO: 207)
GPSRGCFGVKLDRIGAMSGLGC (CNP-22).

In another embodiment, optionally excluded from the disclosure are CNP peptides and fragments thereof isolated or sought from the shark species Lamna ditropis (see, e.g., M. Takano et al., Zool. Sci., 11: 451-454 (1994)), including:

(SEQ ID NO: 208)
RLLKDLSNNPLRFKGRSKKGPSRGCFGVKLDRIGAMSGLGC (CNP-41);

(SEQ ID NO: 209)
LKDLSNNPLRFKGRSKKGPSRGCFGVKLDRIGAMSGLGC (CRP-39);

-continued

(SEQ ID NO: 210)
KDLSNNPLRFKGRSKKGPSRGCFGVKLDRIGAMSGLGC (CNP-38);

(SEQ ID NO: 211)
FKGRSKKGPSRGCFGVKLDRIGAMSGLGC (CNP-29);
and

(SEQ ID NO: 212)
GPSRGCFGVKLDRIGAMSGLGC (CNP-22).

In still another embodiment, optionally excluded from the disclosure are CNP peptides and fragments thereof isolated from the shark species Squalus acanthias (see, e.g., M. Takano et al., Zool. Sci., 11: 451-454 (1994)), including:

(SEQ ID NO: 213)
RLLQDLSNNPLRFKGRSKKGPSRSCFGLKLDRIGAMSGLGC (CNP-41);
and

(SEQ ID NO: 214)
GPSRSCFGLKLDRIGAMSGLGC (CNP-22).

In a further embodiment, the present disclosure optionally excludes CNP peptides isolated from medaka and puffer fish, designated "CNP-1", "CNP-2", "CNP-3" and "CNP-4" in K. Inoue et al., Proc. Nat. Acad. Sci., 100(17): 10079-10084 (2003):

(SEQ ID NO: 215)
GWNRGCFGLKLDRIGSMSGLGC (medaka and puffer fish CNP-1);

(SEQ ID NO: 216)
PMVAGGGCFGMKMDRIGSISGLGC (medaka CNP-2);

(SEQ ID NO: 217)
GRSSMVGGRGCFGMKIDRIGSISGLGC (puffer fish CNP-2);

(SEQ ID NO: 218)
GGMRSCFGVRLERIGSFSGLGC (medaka CNP-3);

(SEQ ID NO: 219)
GGLASCFGVALARIGSFSGLGC (puffer fish CNP-3);

(SEQ ID NO: 220)
GGSTSRSGCFGHKMDRIGTISGMGC (medaka CNP-4);
and

(SEQ ID NO: 221)
GGSSRSGCFGHKMDRIGTISGMGC (puffer fish CNP-4).

In a still further embodiment, optionally excluded from the disclosure are CNP-39 isolated from platypus venom and the CNP-22 fragment thereof, designated "ovCNP-39" and "ovCNP-39(18-39)" in G. de Plater et al., Toxicon., 36(6): 847-857 (1998):

(SEQ ID NO: 222)
LLHDHPNPRKYKPANKKGLSKGCFGLKLDRIGSTSGLGC (ovCNP-39);
and

(SEQ ID NO: 223)
GLSKGCFGLKLDRIGSTSGLGC (ovCNP-39 (18-39).

In another embodiment, the present disclosure optionally excludes the following peptides as specifically disclosed in US 2007/0197434:

(SEQ ID NO: 224)
Gly-Leu-Ser-Lys-Gly-Cys-Phe-Gly-Leu-Lys-Leu-Asp-Arg-Ile-Gly-Ala-Met-Ser-Gly-Leu-Gly-Cys;

(SEQ ID NO: 225)
Gly-Leu-Ser-Lys-Gly-Cys-Phe-Gly-Leu-Lys-Leu-Asp-Arg-Ile-Gly-Ser-Gln-Ser-Gly-Leu-Gly-Cys;

(SEQ ID NO: 226)
Gly-Leu-Ser-Lys-Gly-Cys-Phe-Gly-Leu-Lys-Leu-Asp-Arg-Ile-Gly-Ser-Ala-Ser-Gly-Leu-Gly-Cys;

(SEQ ID NO: 227)
Ser-Leu-Arg-Arg-Ser-Ser-Cys-Phe-Gly-Leu-Lys-Leu-Asp-Arg-Ile-Gly-Ser-Met-Ser-Gly-Leu-Gly-Cys;

(SEQ ID NO: 228)
Gly-Leu-Ser-Lys-Gly-Cys-Phe-Gly-Leu-Lys-Leu-Asp-Arg-Ile-Gly-Ser-Met-Ser-Gly-Leu-Gly-Cys-Asn-Ser-Phe-Arg-Tyr;
and

(SEQ ID NO: 229)
Cys-Phe-Gly-Leu-Lys-Leu-Asp-Arg-Ile-Gly-Ser-Gln-Ser-Gly-Leu-Gly-Cys-Asn-Ser-Phe-Arg-Tyr.

In yet another embodiment, the disclosure optionally excludes peptides of SEQ ID NO:10, disclosed generically in US 2007/0197434, wherein such peptides are CNP-17 variants having certain natural amino acid substitution(s) at position(s) 4, 5, 6, 11, 12, 14 and/or 15. In still another embodiment, optionally excluded from the disclosure are peptides corresponding to hCNP-53(Ser47Ala), hCNP-53 (Met48Gln), hCNP-53(Met48Ala), and hCNP-53(C-term.)-Asn-Ser-Phe-Arg-Tyr.

In an embodiment, the present disclosure optionally excludes the peptides of SEQ ID NOs 1-4 and 6-71 as specifically disclosed in U.S. Pat. No. 7,276,481. In another embodiment, the disclosure optionally excludes peptides of SEQ ID NO 5, disclosed generically in U.S. Pat. No. 7,276,481, wherein such peptides are variants of CNP17 having at least one natural amino acid substitution at Leu9, Lys10, Leu11, Ser16, Met17, Gly19, and/or Leu20. In still another embodiment, optionally excluded are CNP17 variants in which CNP17 or variants thereof contain N-Me-Phe7, or N-Me-Phe7 and N-Me-Leu11. In a further embodiment, the disclosure optionally excludes CNP17 variants of SEQ ID NO 5, as disclosed in U.S. Pat. No. 7,276,481, which are fused or conjugated to growth hormone (GH), insulin-like growth factor 1 (IGF-1), or thyroid hormone (TH). In yet another embodiment, optionally excluded are CNP22 variants in which CNP22 is fused to GH, IGF-1 or TH, or attached to GH, IGF-1 or TH via a linker (e.g., a peptide linker). In still another embodiment, optionally excluded are CNP17 variants in which CNP17 or variants thereof are conjugated to biotin or fluorescein at the N-terminus or the C-terminus.

In a further embodiment, the present disclosure optionally excludes the peptides of Compound Nos. 1-27, and SEQ ID NOs 1-17, 22-24, 30, 31 and 40-42 as specifically disclosed in U.S. Pat. No. 5,434,133. In another embodiment, the disclosure optionally excludes peptides of SEQ ID NOs 18-21 and 25-29, disclosed generically in U.S. Pat. No. 5,434,133. In still another embodiment, the disclosure optionally excludes the peptides of SEQ ID NOs 1-4 and 9 as specifically disclosed in WO 94/20534.

In some embodiments, however, the disclosure still encompasses methods of use of the natriuretic (e.g., CNP) peptides, fragments and variants optionally excluded herein, as well as pharmaceutical compositions (including sterile

pharmaceutical compositions) comprising such natriuretic (e.g., CNP) peptides, fragments and variants.

C. Synthesis and Purification of CNP Variants

In some embodiments, the CNP variants described herein are produced by recombinant expression, using certain techniques known in the art in certain embodiments. See, e.g., Sambrook, Fritsch & Maniatis, Molecular Cloning: A Laboratory Manual, Second Edition. Cold Spring Harbor Laboratory Press (Cold Spring Harbor, N.Y. (1989)); DNA Cloning: A Practical Approach, Volumes I and II, D. N. Glover, Ed. (1985); and Current Protocols in Molecular Biology, John Wiley & Sons, Inc. (1994).

In certain embodiments, the CNP variants described herein are produced by a recombinant process that comprises culturing in a medium a host cell comprising a first polynucleotide encoding a CNP variant polypeptide linked to a second polynucleotide encoding a cleavable peptide or protein under conditions that result in expression of a fusion polypeptide encoded by the polynucleotides, wherein the fusion polypeptide comprises the CNP variant polypeptide directly linked to the cleavable peptide or protein or indirectly linked thereto via a linker. In some embodiments, the host cell is transformed with an expression vector comprising the polynucleotide encoding the CNP variant polypeptide linked to the polynucleotide encoding the cleavable peptide or protein. In certain embodiments, the fusion polypeptide is expressed as a soluble protein or as an inclusion body. The expressed fusion polypeptide can be isolated from the host cell or culture medium, and the isolated fusion polypeptide can be contacted with a cleaving agent to release the CNP variant.

Host cells used to produce CNP variants can be bacterial, yeast, insect, non-mammalian vertebrate, or mammalian cells. Bacterial cells include without limitation E. coli cell lines and strains. Non-limiting examples of E. coli cell lines and strains include BL21, BL21(DE3), BL21(DE3)pLysS, BL21(DE3)pGro7, ArcticExpress(DE3), C41 [also called C41(DE3)], C43 [also called C43(DE3)], Origami B(DE3), Origami B(DE3)pLysS, KRX, and Tuner(DE3). In an embodiment, CNP variants and CNP fusion proteins are produced using BL21(DE3) cells. Mammalian cells include, but are not limited to, hamster, monkey, chimpanzee, dog, cat, bovine, porcine, mouse, rat, rabbit, sheep and human cells. The host cells can be immortalized cells (a cell line) or non-immortalized (primary or secondary) cells and can be any of a wide variety of cell types, such as, but not limited to, fibroblasts, keratinocytes, epithelial cells (e.g., mammary epithelial cells, intestinal epithelial cells), ovary cells (e.g., Chinese hamster ovary or CHO cells), endothelial cells, glial cells, neural cells, formed elements of the blood (e.g., lymphocytes, bone marrow cells), chondrocytes and other bone-derived cells, and precursors of these somatic cell types. Host cells containing the CNP variant DNA or RNA are cultured under conditions appropriate for growth of the cells, expression of the DNA or RNA and identification/selection of cells expressing the CNP variant.

In some embodiments, the host cells are grown or cultured for a time period at a temperature from about 10° C. to about 40° C., or from about 20° C. to about 40° C., or from about 30° C. to about 40° C. In certain embodiments, the host cells are grown or cultured for a time period at about 20° C., 22° C., 25° C., 28° C., 30° C., 35° C. or 37° C. In certain embodiments, the host cells are grown or cultured for a time period at about 35° C. or 37° C.

Recombinant polynucleotides encoding CNP variant polypeptides (including CNP fusion proteins) are expressed in an expression vector comprising a recombinant polynucleotide comprising expression control sequences operatively linked to a nucleotide sequence to be expressed. An expression vector comprises sufficient cis-acting elements for expression; other elements for expression can be supplied by the host cell or in vitro expression system. Expression vectors include all those known in the art, including without limitation cosmids, plasmids (e.g., naked or contained in liposomes) and viruses that incorporate the recombinant polynucleotide. The expression vector is inserted into an appropriate host cell, via transformation or transfection, for expression of the polynucleotide encoding the polypeptide (see, e.g., Sambrook et al. (supra)).

Non-limiting examples of expression vectors contemplated for production of CNP variants, including cleavable CNP fusion proteins, include pJexpress, pJexpress401, pJexpress404, pET-15b, pET-21a, pET-22b, pET-31b, pET-32a, pET-41a, pMAL, pMAL-c2X, pQE-30, pET-SUMO, and pTYB11. Expression of particular constructs can generate soluble CNP variants (including CNP fusion proteins) or insoluble CNP variants (including CNP fusion proteins) in the form of inclusion bodies.

In some embodiments, expression of the polynucleotide(s) encoding a CNP variant or CNP fusion protein is enhanced using an isopropyl β-D-1-thiogalactopyranoside (IPTG)-inducible vector. In some embodiments, the host cells are grown or cultured for a time period at a temperature from about 10° C. to about 40° C., or from about 20° C. to about 40° C., or from about 30° C. to about 40° C., in the presence of IPTG. In certain embodiments, the host cells are grown or cultured for a time period at about 20° C., 22° C., 25° C., 28° C., 30° C., 35° C. or 37° C. in the presence of IPTG. In certain embodiments, the host cells are grown or cultured for a time period at about 35° C. or 37° C. in the presence of 1 mM IPTG.

In further embodiments, the host cells are cultured with IPTG at a concentration from about 0.4 mM to about 2 mM, or from about 0.4 mM to about 1.5 mM, or from about 0.4 mM to about 1 mM. In certain embodiments, the IPTG is at a concentration of about 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9 or 2 mM. In an embodiment, the concentration of IPTG is about 1 mM.

In certain embodiments, the CNP variants described herein are recombinantly expressed as fusion proteins comprising a CNP variant polypeptide and a cleavable carrier protein or cleavable tag (e.g., peptide tag), wherein the fusion protein comprises the CNP variant polypeptide directly linked to the cleavable carrier protein or tag or indirectly linked thereto via a linker. Use of a carrier protein or tag facilitates, e.g., detection, isolation and/or purification of the fusion protein. Cleavable carrier proteins and tags include, but are not limited to, histidine (e.g., hexa-His) tags; human transcription factor TAF12 (TAF12), TAF12 fragments, TAF12 histone fold domain, mutants of TAF12 and fragments thereof, TAF12(C/A), TAF12(D/E), TAF12(4D/4E), TAF12(6D/6E), TAF12(10D/10E), TAF12(C/A & D/E), TAF12(C/A & 4D/4E), TAF12(C/A & 6D/6E), TAF12(C/A & 10D/10E); ketosteroid isomerase (KSI); maltose-binding protein (MBP); β-galactosidase (β-Gal); glutathione-S-transferase (GST); thioredoxin (Trx); chitin binding domain (CBD); BMP-2, BMP-2 mutants, BMP-2 (C/A); SUMO; and mutants and fragments thereof.

An expression construct can express a fusion protein comprising a CNP variant and a carrier protein or tag. The tag can be an amino acid sequence that confers a useful

property to the fusion protein. In one embodiment, the tag is a ligand-binding domain that can be used to purify the fusion protein by applying the fusion protein to separation media containing the ligand. For example, a fusion protein comprising a glutathione-S-transferase (GST) domain can be applied to a chromatographic column containing glutathione-linked separation media. As another example, a fusion protein comprising maltose-binding protein (MBP) as a tag can be applied to separation media containing maltose. As a further example, a fusion protein comprising a polyhistidine tag may be applied to a nickel column, whereby chelation of the polyhistidine tag to the nickel column facilitates purification of the fusion protein. In another embodiment, the tag is a ligand. For example, a fusion protein can comprise glutathione as a tag and be applied to a chromatographic column containing glutathione-S-transferase-linked separation media. Non-limiting examples of carrier proteins and tags for use in fusion proteins include human transcription factor TAF12 (TAF12), ketosteroid isomerase (KSI), maltose-binding protein (MBP), β-galactosidase (β-Gal), glutathione-S-transferase (GST), thioredoxin (Trx), chitin-binding domain (CBD), BMP-2 mutation (BMPM), SUMO, CAT, TrpE, staphylococcal protein A, streptococcal proteins, starch-binding protein, cellulose-binding domain of endoglucanase A, cellulose-binding domain of exoglucanase Cex, biotin-binding domain, recA, Flag, poly(His), poly(Arg), poly(Asp), poly (Gln), poly (Phe), poly(Cys), green fluorescent protein, red fluorescent protein, yellow fluorescent protein, cyan fluorescent protein, biotin, avidin, streptavidin, antibody epitopes, and mutants and fragments thereof.

To generate the target CNP variant, the carrier protein or tag can be cleaved from the fusion protein by means of chemical cleavage, protease cleavage, or protein self-cleavage. Exemplary chemical and proteolytic cleavage agents (cleavage sites in parenthesis) include, but are not limited to, formic acid (Asp-Pro), cyanogen bromide (CNBr) (Met-X), hydroxylamine (Asn-Gly), Factor Xa (IEGR-X) (SEQ ID NO: 230), Enterokinase (DDDDK-X) (SEQ ID NO: 231), ProTEV (EXXYXQ-G) (SEQ ID NO: 232), and SUMO protease. Due to the nature of the particular kinds of chemical cleavage, cleavage using formic acid may generate Pro-CNP, CNBr may generate CNP having Met-to-Asn substitution, and hydroxylamine may generate Gly-CNP. Alternatively, chemical or protease cleavage may be avoided by using particular constructs (e.g., pET-21a-CNP) that express CNP variants not as fusion proteins. Expression of pET-21a-CNP may produce Met-CNP. Or certain fusion proteins (e.g., those containing intein-CBD) can undergo self-cleavage to generate CNP.

In further embodiments, a fusion protein comprises a cleavable peptide linker between a CNP variant and a carrier protein or tag (e.g., peptide tag). In certain embodiments, the cleavable peptide linker is selected from the group consisting of Asp-Pro, Asn-Gly, Met-X, Val-Asp-Asp-Arg (SEQ ID NO: 233), Gly-Ser-Asp-Arg (SEQ ID NO: 234), Ile-Thr-Asp-Arg (SEQ ID NO: 235), Pro-Gly-Asp-Arg (SEQ ID NO: 236), Ile-Glu-Gly-Arg-X (SEQ ID NO: 230), Asp-Asp-Asp-Asp-Lys-X (SEQ ID NO: 231), Glu-X-X-Tyr-X-Gln-Gly (SEQ ID NO: 232), Ala-Phe-Leu-Gly-Pro-Gly-Asp-Arg (SEQ ID NO: 237), and MGSSHHHHHHSSGLVPRG-SHTGD-DDDKHMD (pET-15b linker) (SEQ ID NO: 95), where X denotes an amino acid. In some embodiments, the cleavable peptide linker is cleaved by a cleaving agent selected from the group consisting of palladium, cyanogen bromide (CNBr), formic acid, hydroxylamine, clostripain, thrombin, chymotrypsin, trypsin, trypsin-like proteases, carboxypeptidase, enterokinase (enteropeptidase), Kex 2 protease, Omp T protease, Factor Xa protease, subtilisin, pro-TEV, SUMO protease, V8 protease, HIV protease, rhinovirus protease, furilisin protease, IgA proteases, human Pace protease, collagenase, Nia protease, poliovirus 2Apro protease, poliovirus 3C protease, genenase, furin, elastase, Proteinase K, pepsin, rennin (chymosin), microbial aspartic proteases, papain, calpain, chymopapain, ficin (ficain), bromelain (bromelase), cathespisin B, caspases, thermolysin, Endoprotease Arg-C, Endoprotease Glu-C, Endoprotease Lys-C, kallikrein, and plasmin.

In certain embodiments, the cleavable carrier protein, tag (e.g., peptide tag) or peptide linker is cleaved using formic acid to release the CNP variant from the fusion protein. In some embodiments, the formic acid is at a concentration from about 1% to about 20%, or from about 1% to about 15%, or from about 2% to about 15%, or from about 1% to about 10%, or from about 2% to about 10%, or from about 1% to about 5%, or from about 2% to about 5%. In certain embodiments, the formic acid is at a concentration of about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14% or 15%. In certain embodiments, the formic acid is at a concentration of about 2%, 5% or 10%.

In further embodiments, cleavage of the CNP fusion protein in the presence of formic acid is conducted at a temperature from about 20° C. to about 80° C., or from about 30° C. to about 75° C., or from about 40° C. to about 75° C., or from about 50° C. to about 75° C., or from about 50° C. to about 70° C., or from about 55° C. to about 70° C., or from about 50° C. to about 60° C. In some embodiments, cleavage in the presence of formic acid is conducted at about 20° C., 22° C., 25° C., 30° C., 35° C., 37° C., 40° C., 42° C., 45° C., 50° C., 55° C., 60° C., 65° C., 70° C., 75° C. or 80° C. In certain embodiments, cleavage in the presence of formic acid is conducted at about 50° C., 55° C., 60° C., 65° C. or 70° C. In certain embodiments, cleavage in the presence of formic acid is conducted at about 55° C. or 70° C.

In additional embodiments, cleavage of the CNP fusion protein in the presence of formic acid is performed for a time period from about 3 hr to about 48 hr, or from about 5 hr to about 48 hr, or from about 5 hr to about 36 hr, or from about 5 hr to about 24 hr, or from about 5 hr to about 18 hr, or from about 20 hr to about 24 hr, or for about 6 hr to about 10 hr. In certain embodiments, cleavage in the presence of formic acid is performed for about 5 hr, 6 hr, 12 hr, 15 hr, 18 hr, 20 hr or 24 hr.

In some embodiments, cleavage of the CNP fusion protein is conducted in the presence of about 2%, 5% or 10% formic acid at about 55° C. for about 20 hr to about 36 hr, or at about 60° C. for about 15 hr to about 24 hr, or at about 65° C. for about 10 hr to about 21 hr, or at about 70° C. for about 6 hr to about 18 hr. In certain embodiments, cleavage of the CNP fusion protein is conducted in the presence of about 2% formic acid at about 55° C. for about 20 hr to about 24 or 36 hr, or at about 60° C. for about 15 hr to about 24 hr, or at about 65° C. for about 10 hr to about 18 hr, or at about 70° C. for about 6 hr to about 10 hr.

The present disclosure provides mild conditions for cleavage of CNP fusion proteins using formic acid to afford high yields of CNP variants. The conditions described herein for fusion protein cleavage using formic acid are also suitable for cleavage of fusion proteins comprising polypeptides or proteins other than CNP, where the fusion proteins contain an Asp-Pro peptide bond.

In further embodiments, soluble CNP fusion proteins or CNP fusion protein inclusion bodies are treated with a buffer

and/or a detergent prior to chemical cleavage (e.g., using formic acid) or proteolytic cleavage of the CNP fusion protein. Non-limiting examples of buffers include B-PER II; diluted B-PER II (e.g., 1/20 dilution); B-PER; B-PER phosphate buffer; buffers containing Tris (e.g., 25 mM Tris, pH 7.5); buffers containing Tris and NaCl (e.g., 25 mM Tris, 150 mM NaCl, pH 7.9); and PBS. In an embodiment, the buffer is B-PER II. Non-limiting examples of detergents include octyl-sucrose, Triton X-100, Tween-20, NP-40, and CA-630. The detergent can be in a buffer (e.g., 1% detergent in 25 mM Tris buffer, pH 7.5). In certain embodiments, the detergent is Triton X-100 or CA-630.

It is understood that any of the methods and conditions described above may be used in combination with any of the other methods and conditions described above to generate a CNP variant disclosed herein.

In other embodiments, the CNP variants described herein are synthesized using a peptide synthesizer and purified according to methods known in the art, e.g., according to the methods of Atherton and Sheppard, Solid Phase Peptide Synthesis: a Practical Approach, IRL Press (Oxford, England (1989)).

Peptides can be synthesized based on, e.g., the following peptide sequence of CNP: G^1LS(K or R)$GC^6F^7G^8L$(K or R or Nle or 6-OH-Nle)$LDRIGSMSGLGC^{22}$.

Exemplary CNP variants include but are not limited to:

Analog A (GLSKGC(CH2NH)FGLKLDRIGSMSGLGC) (SEQ ID NO: 56) was made by converting the backbone "—C═O" group of C^6 to a "—CH_2" group;

Analog B (GLSKGC(N—Me—Phe)GLKLDRIGSMSGLGC) (SEQ ID NO: 57) was made by converting the backbone "—NH" group of F^7 to an "—N—CH_3" group;

Analog E (GLSKGC(D-Phe)GLKLDRIGSMSGLGC) (SEQ ID NO: 136) was made using D-Phe at F^7;

Analog F (GLSKGCF(tBu—Gly)LKLDRIGSMSGLGC) (SEQ ID NO: 58) was made using a tertiary-butyl-Gly at G^8;

Analog G (GLSKGC(3-Cl—Phe)GLKLDRIGSMSGLGC) (SEQ ID NO: 137) was made by adding a chloride atom to a meta position of the phenyl ring of F^7 (similar variants can be generated by making ortho, meta and/or para substitutions of the phenyl ring of Phe7 with Cl, F, Br, OH and/or CH_3); and

Analog H (GLSKGC[$NHCH_2$CH(Ph)CO]GLKLDRIGSMSGLGC) (SEQ ID NO: 59) was made using (±)-3-(amino)-2-phenylpropionic acid at F^7.

Examples of CNP variants having, e.g., amino acid extensions, substitutions with natural or unnatural amino acids or peptide bond isosteres, and/or conjugations to polymers or hydrophobic moieties, include without limitation:

Analog J (SEQ ID NO: 91)
C6-CH2-NH, N-Me-L9, N-Me-L20

Analog K (SEQ ID NO: 92)
N-Me-L9, N-Me-L20

Analog L (SEQ ID NO: 93)
N-Me-L9, N-Me-L11, N-Me-L20

-continued

Analog M (SEQ ID NO: 94)
N-Me-L9, N-Me-L11

Analog Z (SEQ ID NO: 95)
K4R, F7Y

Analog AA (SEQ ID NO: 96)
K4R, G8V

Analog AB (SEQ ID NO: 97)
K4R, GS

Analog AC (SEQ ID NO: 98)
K4R, GT

Analog AD (SEQ ID NO: 99)
K4R, L9T

Analog AE (SEQ ID NO: 100)
K4R, Gl5R

Analog AF (SEQ ID NO: 101)
K4R, Gl5Cit

Analog AG (SEQ ID NO: 102)
K4R, M17V

Analog AH (SEQ ID NO: 35)
K4R

Analog AJ (SEQ ID NO: 103)
K4R, L20V

Analog AK (SEQ ID NO: 104)
K4R, L20t-Bu-Ala

Analog AT (SEQ ID NO: 105)
GlE, K4E

Analog AV (SEQ ID NO: 106)
GlE, K4E- pentanoic acid (attached at the N-terminus)

Analog AW (SEQ ID NO: 107)
GlE, K4E - heptanoic acid (attached at the N-terminus)

Analog AX (SEQ ID NO: 2)
CNP17 (delta N-term)

Analog AY (SEQ ID NO: 36)
GANRR-CNP22(K4R)

Analog AZ (SEQ ID NO: 41)
R-CNP22(K4R)

Analog BB (SEQ ID NO: 108)
G1E-heptanoic acid (attached at the N-terminus)

-continued

Analog BC	
	(SEQ ID NO: 109)
G1E-pentanoic acid (attached at the N-terminus)	
Analog BF	
	(SEQ ID NO: 110)
K4R, K10Cit	
Analog BG	
	(SEQ ID NO: 111)
K4R, K10Q	
Analog BH	
	(SEQ ID NO: 112)
K4R, K10R	
Analog BJ	
	(SEQ ID NO: 113)
K4R, G15N	
Analog BK	
	(SEQ ID NO: 114)
K4R, G15S	
Analog BL	
	(SEQ ID NO: 60)
CNP-37	
	(SEQ ID NO: 4)
CNP-53	
Analog CA	
	(SEQ ID NO: 61)
AAWARLLQEHPNA-CNP22	
Analog CB	
	(SEQ ID NO: 62)
AAWARLLQEHPNAR-CNP22	
Analog CC	
	(SEQ ID NO: 63)
DLRVDTKSRAAWAR-CNP22	
Analog CD	
	(SEQ ID NO: 68)
SPKMVQGSG-CNP17-KVLARH (N- and C-terminal BNP tails)	
Analog CE	
	(SEQ ID NO: 81)
GERAFKAWAVARLSQ-CNP22 (HSA-CNP22)	
Analog CF	
	(SEQ ID NO: 79)
GQPREPQVYTLPPS-CNP22	
PEG(24K)-CNP22	
PEG(20K)-CNP22	
PEG(5K)-CNP22	
PEG(2K)-CNP22	
PEG(2K)-CNP17	
	(SEQ ID NO: 36)
PEG(1K)-GANRR-CNP22(K4R)	
PEG(1K)-CNP22	
PE04-(PE012)3(branched)-CNP22	
PE012-CNP22	
	(SEQ ID NO: 36)
PE012-GANRR-CNP22(K4R)	
	(SEQ ID NO: 36)
PE024-GANRR-CNP22(K4R); and SEQ ID NOs: 1 to 6 and 34 to 144, and variants thereof that comprise up to 1, 2, 3, 4, or 5 further modifications.	

In one embodiment, the CNP variants are cyclized via formation of a disulfide bond between Cys^6 and Cys^{22}. Cys^6 can be a cysteine analog such as, e.g., homocysteine or penicillamine. In a further embodiment, the CNP variants can be cyclized by a covalent bond formed head-to-tail, side chain-to-side chain, side chain-to-head, or side chain-to-tail. In an embodiment, the covalent bond is formed between an amino acid at or toward the N-terminus and an amino acid at or toward the C-terminus of the peptide (referred to as "terminal" amino acids in this context). In another embodiment, the covalent bond is formed between the side chains of the two terminal amino acids. In yet another embodiment, the covalent bond is formed between the side chain of one terminal amino acid and the terminal group of the other terminal amino acid, or between the terminal groups of the two terminal amino acids.

Head-to-tail cyclization of the terminal amine to the terminal carboxyl group can be carried out using a number of methods, e.g., using p-nitrophenyl ester, 2,4,5-trichlorophenyl ester, pentafluorophenyl ester, the azide method, the mixed anhydride method, HATU, a carbodimide (e.g., DIC, EDC or DCC) with a catalyst such as HOBt, HONSu or HOAt, or on-resin cyclization.

In addition, the cyclic structure can be formed via a bridging group involving the side chains of amino acid residues of the CNP variant and/or the terminal amino acid residues. A bridging group is a chemical moiety that allows cyclization of two portions of the peptide. Non-limiting examples of bridging groups include amides, thioethers, thioesters, disulfides, ureas, carbamates, sulfonamides, and the like. A variety of methods are known in the art for incorporation of units having such bridging groups. For example, a lactam bridge (i.e., a cyclic amide) can be formed between the N-terminal amino group or an amino group on a side chain and the C-terminal carboxylic acid or a carboxyl group on a side chain, e.g., the side chain of lysine or ornithine and the side chain of glutamic acid or aspartic acid. A thioester can be formed between the C-terminal carboxyl group or a carboxyl group on a side chain and the thiol group on the side chain of cysteine or a cysteine analog.

Alternatively, a cross link can be formed by incorporating a lanthionine (thio-dialanine) residue to link alanine residues that are covalently bonded together by a thioether bond. In another method, a cross-linking agent, such as a dicarboxylic acid (e.g., suberic acid (octanedioic acid)), can link the functional groups of amino acid side chains, such as free amino, hydroxyl, and thiol groups.

Enzyme-catalyzed cyclization can also be used. For example, it has been reported that the thioesterase domain of tyrocidine synthetase can be used to cyclize a thioester precursor, a subtilisin mutant can be utilized to cyclize peptide glycolate phenylalanylamide esters, and the antibody ligase 16G3 can be employed to cyclize a p-nitrophenylester. For a review of peptide cyclization, see Davies, J. Peptide Sci., 9: 471-501 (2003), incorporated herein by reference in its entirety.

In certain embodiments, the final cyclized product has a purity of at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or at least about 99%.

D. Chemically Modified CNP Variants

Chemical modification of CNP22 or variants thereof can potentially impart advantageous properties to the modified CNP peptides, such as increased stability and half-life and reduced immunogenicity (for a general discussion of chemical modification of therapeutic proteins, see Pharmazie, 57(1): 5-29 (2002)). For example, attaching natural or synthetic, polymeric or non-polymeric moieties (e.g., PEG)

to CNP peptides, to increase the total mass of the CNP peptides to the ranges described generally herein, e.g., a range from about 2.6 or 2.8 kDa to about 6 or 7 kDa, can reduce the susceptibility of the modified peptides to in vivo cleavage by exopeptidases and/or endopeptidases (e.g., NEP). In addition to PEGylation, glycosylation and other chemical derivatization procedures, e.g., modification by phosphorylation, amidation, carboxylation, acetylation, methylation, and creation of acid-addition salts, amides, esters and N-acyl derivatives, may also mask potentially immunogenic regions and/or proteolytically sensitive regions (Science, 303: 480-482 (2004)).

Examples of chemical modifications include, without limitation, the polymer addition method of Bednarsaki and the cross-linking method of Altus Corporation for improving stability and protease resistance and reducing immunogenicity. Bednarsaki showed that polymer addition can improve protein temperature stability (J. Am. Chem. Soc., 114(1): 378-380 (1992)), and Altus Corporation found that glutaraldehyde cross-linking can improve enzyme stability.

Chemical modification of polypeptides can be performed in a non-specific fashion (leading to mixtures of derivatized species) or in a site-specific fashion (e.g., based on wild-type macromolecule reactivity-directed derivatization and/or site-selective modification using a combination of site-directed mutagenesis and chemical modification) or, alternatively, using expressed protein ligation methods (Curr. Opin. Biotechnol., 13(4): 297-303 (2002)).

Pegylated CNP Variants

In one embodiment, for increased stability (e.g., resistance to NEP degradation), CNP22 or variants thereof (including those having amino acid additions, substitutions and/or deletions) are conjugated to hydrophilic, natural or synthetic polymers, to increase the total mass of the modified CNP peptides to a range from about 2.6 kDa or 2.8 kDa to about 4, 5, 6, 7 or higher kDa. In certain embodiments, the added hydrophilic polymers have a total mass of about 0.6, 0.8, 1, 1.2, 1.4, 1.6, 1.8, 2, 2.2, 2.4, 2.6, 2.8, 3, 3.2, 3.4, 3.6, 3.8, 4, 4.2, 4.4, 4.6, 4.8, or about 5 kDa.

In an embodiment, the hydrophilic polymers are water-soluble so that the CNP peptides conjugated thereto do not precipitate out in an aqueous (e.g., physiological) environment. Further, the hydrophilic polymers are biocompatible, i.e., do not cause injury, toxicity or an immunological reaction in vivo.

The hydrophilic polymers can be branched or unbranched. In one embodiment, the hydrophilic polymers are not branched.

Various sites of conjugation of CNP22 or variants thereof to a hydrophilic polymer are possible, including but not limited to: (1) only at the N-terminus; (2) only at the C-terminus; (3) only at an internal site (e.g., Lys4); (4) at both the N-terminus and the C-terminus; (5) at the N-terminus and an internal site; and (6) at the C-terminus and an internal site. In one embodiment, CNP22 or variants thereof are conjugated to a hydrophilic polymer only at the N-terminus. In another embodiment, conjugation is only at an internal site (e.g., Lys4). In yet another embodiment, conjugation is at the N-terminus and an internal site (e.g., Lys4). In still another embodiment, for better functionality the CNP peptides are not conjugated to a hydrophilic polymer at a site (e.g., Lys10) within the cyclic domain (corresponding to Cys6 to Cys22 of CNP22). If conjugation to a hydrophilic polymer is based on bond formation with a reactive primary amino group on the CNP peptide, conjugation at an internal site (e.g., Lys4 and/or Lys10) can be prevented by substitution of Lys4 and/or Lys10 with a natural or unnatural amino acid or peptidomimetic that does not contain a reactive primary amino group on a side chain, such as, e.g., Gly, Ser, Arg, Asn, Gln, Asp, Glu or citrulline (Cit). In a particular embodiment, Lys4 and/or Lys10 are replaced with Arg. In another embodiment, Lys10 is not replaced with Arg.

Non-limiting examples of hydrophilic polymers include polymers formed from carboxylic acid-bearing monomers (e.g., methacrylic acid (MA) and acrylic acid (AA)), polyvinyl alcohols, polymers formed from hydroxyl-bearing monomers (e.g., hydroxyethyl methacrylate (HEMA), hydroxypropyl methacrylate (HPMA), hydroxypropyl methacrylamide, and 3-trimethylsilylpropyl methacrylate (TMSPMA)), polyalkylene oxides, polyoxyethylated polyols (e.g., glycerol), poly(ethylene glycol) (PEG), poly(propylene glycol), mono-C_1-C_{10} alkoxy-PEGs (e.g., monomethoxy-PEG), tresyl monomethoxy-PEG, aryloxy-PEGs, PEG acrylate (PEGA), PEG methacrylate, PEG propionaldehyde, bis-succinimidyl carbonate PEG, copolymers of 2-methacryloyloxyethyl-phosphorylcholine (MPC) and N-vinyl pyrrolidone (VP), hydroxy functional poly(N-vinyl pyrrolidone) (PVP), SIS-PEG (SIS is polystyrene-poly-isobutylene-polystyrene block copolymer), polystyrene-PEG, polyisobutylene-PEG, PCL-PEG (PCL is polycaprolactone), PLA-PEG (PLA is polylactic acid), PMMA-PEG (PMMA is poly(methyl methacrylate)), PDMS-PEG (PDMS is poly-dimethyloxanone), PVDF-PEG (PVDF is polyvinylidene fluoride), PLURONIC™ surfactants (polypropylene oxide-co-polyethylene glycol), poly(tetramethylene glycol), poly (L-lysine-g-ethylene glycol) (PLL-g-PEG), poly (L-lysine-g-hyaluronic acid) (PLL-g-HA), poly(L-lysine-g-phosphoryl choline) (PLL-g-PC), poly(L-lysine-g-vinyl pyrrolidone) (PLL-g-PVP), poly(ethylimine-g-ethylene glycol) (PEI-g-PEG), poly(ethylimine-g-hyaluronic acid) (PEI-g-HA), poly (ethylimine-g-phosphoryl choline) (PEI-g-PC), poly(ethylimine-g-vinyl pyrrolidone) (PEI-g-PVP), PLL-co-HA, PLL-co-PC, PLL-co-PVP, PEI-co-PEG, PEI-co-HA, PEI-co-PC, PEI-co-PVP, cellulose and derivatives thereof (e.g., hydroxyethyl cellulose), dextran, dextrins, hyaluronic acid and derivatives thereof (e.g., sodium hyaluronate), elastin, chitosan, acrylic sulfate, acrylic sulfonate, acrylic sulfamate, methacrylic sulfate, methacrylic sulfonate, methacrylic sulfamate, polymers and copolymers thereof, and polymers and copolymers of combinations thereof.

In a particular embodiment, the hydrophilic polymer is poly(ethylene glycol) (PEG), also called poly(ethylene oxide) (PEO). As used herein, the term "PEG" or "PEO" encompasses all the forms of PEG, branched and unbranched, which can be used to derivatize polypeptides, including without limitation mono-(C_1-C_{10}) alkoxy-PEGs and aryloxy-PEGs.

In one embodiment, the PEG-CNP conjugates comprise a PEG (or PEO) polymer of the formula $(CH_2CH_2O)_n$, wherein n is an integer from about 6 to about 100, and the PEG polymer is from about 0.3 kDa to about 5 kDa. In another embodiment, n is an integer from about 12 to about 50, and the PEG polymer is from about 0.6 kDa to about 2.5 kDa. In yet another embodiment, n is from about 12 to about 24, and the PEG polymer is from about 0.6 kDa to about 1.2 kDa. In a further embodiment, the terminal hydroxyl group of the PEG polymer is capped with a non-reactive group. In a particular embodiment, the end-capping group is an alkyl group, e.g., a lower alkyl group such as methyl, so that the PEG polymer terminates in an alkoxy group. In an embodiment, the PEG polymer is not branched. In another embodiment, CNP22 or variants thereof are conjugated to a PEG polymer only at the N-terminus.

PEGs and PEOs potentially include molecules with a distribution of molecular weights, i.e., they are potentially poly-dispersed, depending on the manner in which they are prepared. The size/mass distribution of a polymeric preparation can be characterized statistically by its weight average molecular weight (M_w) and its number average molecular weight (M_n), the ratio of which is called the polydispersity index (M_w/M_n). M_w and M_n can be measured by mass spectroscopy. PEG-CNP variants conjugated to a PEG moiety larger than 1.5 kDa may exhibit a range of molecular weights due to the polydispersed nature of the parent PEG molecule. For example, in the case of mPEG2K (Sunbright ME-020HS, NOF Co.), the molecular masses of the PEG molecules are distributed over a range from about 1.5 kDa to about 3 kDa, with a polydispersity index of 1.036. By contrast, the PEGs conjugated to CNP22 or variants thereof using MS $(PEG)_n$ reagents (n=4, 8, 12 or 24, denoted as, e.g., "PEO12" or "PEO24") from Pierce Biotechnology (Rockford, Ill.) are monodispersed, having discrete chain length and defined molecular weight.

Methods for generating polypeptides comprising a PEG moiety are known in the art (see, e.g., U.S. Pat. No. 5,824,784). Methods for preparing PEGylated CNP peptides generally comprise the steps of (a) reacting CNP22 or a variant thereof with a PEGylation reagent under conditions suitable for attaching PEG to the CNP peptide (e.g., at the N-terminus), and (b) obtaining the reaction product(s). Because PEGylating a CNP peptide might significantly alter its binding to NPR-B, depending on the size of the PEG moiety and the location of PEGylation, different kinds of PEG and PEGylation reaction conditions can be explored. The chemistry that can be used for PEGylation of a CNP peptide includes acylation of reactive primary amine(s) of the peptide using the NHS-ester of methoxy-PEG (O—[(N-Succinimidyloxycarbonyl)-methyl]-O'-methylpolyethylene glycol). Acylation with methoxy-PEG-NHS or methoxy-PEG-SPA results in an amide linkage that eliminates any charge of the original primary amine. PEG-CNP peptides designated with the symbol "PEO12" or "PEO24", as well as those designated with the symbol "PEG1K", "PEG2K", "PEG5K" or "PEG20K", are PEGylated via reaction of a primary amino group on the peptide with an NHS ester-activated, methoxy-end capped PEG reagent. PEG-CNP variants can also be prepared by other methods, e.g., via reductive amination involving a primary amino group on the peptide and a PEG aldehyde, such as, e.g., PEG-propionaldehyde, or mono-C_1-C_{10} alkoxy or aryloxy derivatives thereof (see U.S. Pat. No. 5,252,714).

Unlike ribosome protein synthesis, synthetic peptide synthesis proceeds from the C-terminus to the N-terminus. Accordingly, Boc-PEG (containing tert-butyloxycarbonyl (Boc)) is one method to attach PEG to the C-terminus of a peptide (R. B. Merrifield, J. Am. Chem. Soc., 85(14): 2149-2154 (1963)). Alternatively, Fmoc (fluorenylmethoxycarbonyl) chemistry can be employed (E. Atherton and R. C. Sheppard, Solid Phase Peptide Synthesis: a Practical Approach, IRL Press (Oxford, England (1989)).

The present methods for preparing PEG-CNP variants provide a substantially homogenous mixture of polymer-protein conjugates. After purification, discrete PEG-CNP preparations are sufficiently pure for in vitro and in vivo testing of biological properties. As demonstrated herein, certain PEG-CNP variants exhibit reduced susceptibility to NEP cleavage and substantially similar or better functionality (e.g., stimulation of cGMP production).

As described herein, PEGylation reactions of CNP22 or variants thereof, using appropriate PEGylation reagent/CNP peptide ratios and reaction conditions, provide PEG-CNP derivatives. The nature and extent of PEGylation can be determined using, e.g., PAGE and HPLC analysis. In certain embodiments, at least about 50%, 60%, 70%, 80%, 90%, 95% or 99% of CNP22 or variants thereof are mono-PEGylated at the N-terminus. To optimize the beneficial effects of PEGylation on the biological properties of a CNP peptide, the polymer length, conformation (e.g., branched or linear), and/or functionalization (e.g., adding a negatively charged group) of a PEG moiety can be varied. PEGylated CNP variants are tested for NEP resistance, pharmacokinetics and bioactivity (e.g., the ability to bind to NPR-B and stimulate the generation of cGMP). PEGylated CNP variants that show improved NEP resistance and at least about 50% of the cGMP-stimulating activity of CNP22 can be further tested, e.g., in vitro in a rat chondrosarcoma cell-based achondroplasia model and in vivo in a murine achondroplasia animal model.

E. Methods of Using CNP Variants, Pharmaceutical Compositions of CNP Variants, and Routes of Administration

Methods of Using CNP Variants

Bone-Related Disorders

Fibroblast growth factors (FGFs) play important roles in bone formation, and mutations in FGF receptor genes (FGFR 1, 2 and 3) give rise to a variety of inherited skeletal malformations (Curr. Biol., 5: 500-507 (1995)). In particular, activating mutations in FGFR-3 are responsible for disorders of the long bones, including achondroplasia, the most common form of human genetic dwarfism (Nature, 371: 252-254 (1994); Cell, 78: 335-342 (1994)), the milder disorder hypochondroplasia (Ann. N.Y. Acad. Sci., 785: 182-187 (1996)), and the more severe and neonatal lethal thanatophoric dysplasia (TD) types I and II (Hum. Mol. Genet., 5: 509-512 (1996); Nat. Genet., 9: 321-328 (1995)). Mouse models over-expressing FGF-2, and consequentially activating FGFR-3, show shortened long bones and macrocephaly (Mol. Biol. Cell, 6: 1861-73 (1995)). Consistent with this model, mice deficient in FGFR-3 show remarkable skeletal overgrowth with wider growth plates (Nature Genet., 12: 390-397 (1996)).

Complementary experiments with CNP, NPR-B and NPR-C suggest a link between the peptide ligand, the corresponding receptors, and bone growth. Activation of NPR-B by elevated plasma concentrations of CNP in transgenic mice causes skeletal overgrowth (Nat. Med., 10: 80-86 (2004)) histologically similar to that of the growth plate cartilage of FGFR-3 knockout mice (Nat. Genet., 4: 390-397 (1996)). In NPR-C knockout mice, NPR-C-mediated clearance of CNP should be eliminated; consistent with this prediction, the knockout animals show elongated long bones and elongated vertebrae with kyphosis (Proc. Natl. Acad. Sci. USA 96: 7403-08 (1999)). Conversely, CNP knockout mice are dwarfed with shorter long bones and vertebrae, a phenotype histologically similar to that of achondroplasia, and have increased mortality as a result of malocclusion and pulmonary restriction from the small rib cage (Proc. Natl. Acad. Sci. USA, 98: 4016-4021 (2001)). Consistent with the proposed role of CNP as an activator of NPR-B, the NPR-B knockout mouse has the same dwarfed skeletal phenotype and increased mortality as the CNP knockout mouse (Proc. Natl. Acad. Sci. USA, 101: 17300-05 (2004)). Furthermore, in a mouse model of achondroplasia with activated FGFR-3 in the cartilage, targeted overexpression of CNP in chondrocytes counteracts dwarfism (Yasoda et al., Nat. Med., 10:

80-86 (2004)). Additionally, CNP has been show to play a role in regulating endochondral bone growth and chondrocyte activity, including but not limited to chondrocyte proliferation and differentiation, inhibition of the mitogen activated protein (MAP) kinase/MEK (Raf-1) kinase signaling pathway, and promotion of endochondral ossification (Yasoda et al., Nat. Med., 10: 80-86 (2004)). These results suggest that activation of the CNP/NPR-B system is a potential therapeutic strategy for treatment of human achondroplasia.

By stimulating matrix production, proliferation and differentiation of chondrocytes and increasing long bone growth, the CNP variants of the disclosure are useful for treating mammals, including humans, suffering from a bone-related disorder, such as a skeletal dysplasia. Non-limiting examples of CNP-responsive bone-related disorders and skeletal dysplasias inclnde achondroplasia, hypochondroplasia, short stature, dwarfism, osteochondrodysplasias, thanatophoric dysplasia, osteogenesis imperfecta, achondrogenesis, chondrodysplasia punctata, homozygous achondroplasia, chondrodysplasia punctata, camptomelic dysplasia, congenital lethal hypophosphatasia, perinatal lethal type of osteogenesis imperfecta, short-rib polydactyly syndromes, hypochondroplasia, rhizomelic type of chondrodysplasia punctata, Jansen-type metaphyseal dysplasia, spondyloepiphyseal dysplasia congenita, atelosteogenesis, diastrophic dysplasia, congenital short femur, Langer-type mesomelic dysplasia, Nievergelt-type mesomelic dysplasia, Robinow syndrome, Reinhardt syndrome, acrodysostosis, peripheral dysostosis, Kniest dysplasia, fibrochondrogenesis, Roberts syndrome, acromesomelic dysplasia, micromelia, Morquio syndrome, Kniest syndrome, metatrophic dysplasia, and spondyloepimetaphyseal dysplasia. Further, the CNP variants are useful as an adjunct or alternative to growth hormone for treating idiopathic short stature and other skeletal dysplasias.

In addition, the CNP variants are useful for treating other bone-related conditions and disorders, such as rickets, hypophosphatemic rickets [including X-linked hypophosphatemic rickets (also called vitamin D-resistant rickets) and autosomal dominant hypophosphatemic rickets], and osteomalacia [including tumor-induced osteomalacia (also called oncogenic osteomalacia or oncogenic hypophosphatemic osteomalacia)].

The CNP variants of the disclosure can also be used to treat osteoarthritis. Osteoarthritis is a degenerative disease of the articular cartilage and occurs frequently in the elderly. Osteoarthritis involves destruction of the cartilage and proliferative change in the bone and cartilage resulting from degeneration of articular components, with the change resulting in a secondary arthritis (e.g., synovitis). The extracellular matrix proteins, which are the functional entity of the cartilage, are reduced, and the number of chondrocytes decreases in osteoarthritis (Arth. Rheum. 46(8): 1986-1996 (2002)). By promoting the matrix production, growth and differentiation of chondrocytes, the CNP variants are useful for countering the undesired effects of FGF-2 and increasing matrix synthesis in subjects suffering from arthritis, including osteoarthritis, thereby treating arthritis, including osteoarthritis.

Vascular Smooth Muscle Disorders

CNP and other vasoactive peptides (including ANP, BNP and urodilatin) have vasodilator and diuretic properties and play an important role in cardiovascular homeostasis (J. Cardiovasc. Pharmacol., 117: 1600-06 (1998); Kidney Int., 49: 1732-37 (1996); Am. J. Physiol., 275: H1826-1833 (1998)). CNP is widely distributed in the cardiovascular system, especially in high concentration in vascular endothelial cells (J. Cardiovasc. Pharmacol., 117: 1600-06 (1998)). CNP is a potent relaxant of vascular smooth muscle, particularly in the coronary circulation (Biochem. Biophys. Res. Commun., 205: 765-771 (1994)), and is an inhibitor of smooth muscle cell proliferation (Biochem. Biophys. Res. Commun., 177: 927-931 (1991)). Although the vasodilator effect of CNP is less potent than that of ANP (about 1:100) (Hypertens. Res., 21: 7-13 (1998); Am. J. Physiol., 275: L645-L652 (1998)), CNP mRNA is increased in response to shear stress (FEBS Lett., 373: 108-110 (1995)) and plasma levels of CNP are elevated in inflammatory cardiovascular pathologies (Biochem. Biophys. Res. Commun., 198: 1177-1182 (1994)). CNP has been shown to suppress inflammation through inhibition of macrophage infiltration in injured carotid arteries of rabbits (Circ. Res., 91: 1063-1069 (2002)) and to directly inhihit cardiac fibrohlast proliferation through an NPR-B/cGMP-dependent pathway (Endocrinology, 144: 2279-2284 (2003)).

The cardiovascular actions of CNP are mediated via activation of the NPR subtypes, NPR-B and NPR-C (Endocrinology, 130: 229-239 (1992)), the latter accounting for 95% of NPRs expressed in vivo (Science, 293: 1657-1662 (2001)). The CNP/NPR-B pathway leads to elevation of cGMP, a well-established secondary messenger in the cardiovascular system. NPR-C's 37-amino acid portion from the C-terminus has a consensus sequence that interacts with the heterotrimeric G protein G_i (J. Biol. Chem., 274: 17587-17592 (1999)), which has been shown to regulate adenylate cyclase and phospholipase C activity (J. Biol. Chem., 276: 22064-70 (2001); Am. J. Physiol., 278: G974-980 (2000); J. Biol. Chem., 271: 19324-19329 (1996)). CNP mediates smooth muscle hyperpolarization and relaxation via activation of NPR-C and the opening of a G protein-regulated, inwardly rectifying K^+ channels (Proc. Natl. Acad. Sci. USA, 100: 1426-1431 (2003)). Likewise, CNP has important anti-proliferative effects in cardiac fibroblasts and, through interaction with NPR-C, regulates local blood flow and systemic blood pressure by hyperpolarizing smooth muscle cells (R. Rose and W. Giles, J. Physiol. 586: 353-366 (2008)).

By binding to NPR-B on vascular smooth muscle cells, CNP22 stimulates the production of cGMP, which acts as an intracellular secondary messenger to cause ultimately the relaxation of blood vessels. Based on the hypotensive actions of CNP, the CNP variants of the disclosure are useful for treating hypertension, congestive heart failure, cardiac edema, nephredema, hepatic edema, acute and chronic renal insufficiency, and so on. In addition, activation of cGMP signaling suppresses the growth of vascular smooth muscle cells. Accordingly, the CNP variants of the disclosure can be used to treat conditions or diseases caused by the abnormal growth of vascular smooth muscle cells, including but not limited to restenosis and arteriosclerosis.

The studies described above suggest that CNP may be a potential therapeutic candidate for vascular smooth muscle relaxation and remodeling. Pharmacological effects of CNP concerning certain disorders have been attributed, in part, to vasoprotective effects rather than to vasodilator activity (Am. J. Respir. Crit. Care Med., 170: 1204-1211 (2004)). Therefore, the CNP variants of the present disclosure are useful for treating conditions, e.g., vascular smooth muscle disorders, in which CNP may have a vasoprotective effect, including without limitation inducing smooth muscle relaxation and inhibiting infiltration of macrophages into cardiac tissue. In one embodiment, the CNP variants are used to treat heart failure, including but not limited to acute decompen-

sated heart failure and acute congestive heart failure. In another embodiment, the CNP variants are used to treat asthma, cardiomyopathy, and restenosis of coronary arteries (by increasing smooth muscle cell relaxation and decreasing proliferation of smooth muscle cells).

Pharmaceutical Compositions of CNP Variants

In additional embodiments, the disclosure provides pharmaceutical compositions comprising a CNP variant, and one or more pharmaceutically acceptable excipients, carriers and/or diluents. In certain embodiments, the compositions further comprise one or more other biologically active agents (e.g., inhibitors of proteases, receptor tyrosine kinases, and/or the clearance receptor NPR-C).

In some embodiments, the compositions comprise the desired CNP variant in at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% purity. In certain embodiments, the compositions contain less than about 10%, 5%, 4%, 3%, 2%, 1% or 0.5% of macromolecular contaminants, such as other mammalian (e.g., human) proteins and other CNP variants.

Non-limiting examples of excipients, carriers and diluents include vehicles, liquids, buffers, isotonicity agents, additives, stabilizers, preservatives, solubilizers, surfactants, emulsifiers, wetting agents, adjuvants, and so on. The compositions can contain liquids (e.g., water, ethanol); diluents of various buffer content (e.g., Tris-HCl, phosphate, acetate buffers, citrate buffers), pH and ionic strength; detergents and solubilizing agents (e.g., Polysorbate 20, Polysorbate 80); anti-oxidants (e.g., methionine, ascorbic acid, sodium metabisulfite); preservatives (e.g., Thimerosol, benzyl alcohol, m-cresol); and bulking substances (e.g., lactose, mannitol, sucrose). The use of excipients, diluents and carriers in the formulation of pharmaceutical compositions is known in the art; see, e.g., Remington's Pharmaceutical Sciences, 18th Edition, pages 1435-1712, Mack Publishing Co. (Easton, Pa. (1990)), which is incorporated herein by reference in its entirety.

For example, carriers include without limitation diluents, vehicles and adjuvants, as well as implant carriers, and inert, non-toxic solid or liquid fillers and encapsulating materials that do not react with the active ingredient(s). Non-limiting examples of carriers include phosphate buffered saline, physiological saline, water, and emulsions (e.g., oil/water emulsions). A carrier can be a solvent or dispersing medium containing, e.g., ethanol, a polyol (e.g., glycerol, propylene glycol, liquid polyethylene glycol, and the like), a vegetable oil, and mixtures thereof.

In some embodiments, the compositions are liquid formulations. In certain embodiments, the formulations comprise a CNP variant in a concentration range from about 0.1 mg/ml to about 20 mg/ml, or from about 0.5 mg/ml to about 20 mg/ml, or from about 1 mg/ml to about 20 mg/ml, or from about 0.1 mg/ml to about 10 mg/ml, or from about 0.5 mg/ml to about 10 mg/ml, or from about 1 mg/ml to about 10 mg/ml.

In further embodiments, the compositions comprise a buffer solution or buffering agent to maintain the pH of a CNP-containing solution or suspension within a desired range. Non-limiting examples of buffer solutions include phosphate buffered saline, Tris buffered saline, and Hank's buffered saline. Buffering agents include without limitation sodium acetate, sodium phosphate, and sodium citrate. Mixtures of buffering agents can also be used. In certain embodiments, the buffering agent is acetic acid/acetate or citric acid/citrate. The amount of buffering agent suitable in a composition depends in part on the particular buffer used and the desired pH of the solution or suspension. For example, acetate is a more efficient pH buffer at pH 5 than pH 6, so less acetate may be used in a solution at pH 5 than at pH 6. In some embodiments, the buffering agent has a concentration of about 10 mM±5 mM. In certain embodiments, the pH of a composition is from about pH 3 to about pH 7.5, or from about pH 3.5 to about pH 7, or from about pH 3.5 to about pH 6.5, or from about pH 4 to about pH 6, or from about pI I 4 to about pI I 5, or is at about pI I 5.0±1.0.

In other embodiments, the compositions contain an isotonicity-adjusting agent to render the solution or suspension isotonic and more compatible for injection. Non-limiting examples of isotonicity agents include NaCl, dextrose, glucose, glycerin, sorbitol, xylitol, and ethanol. In certain embodiments, the isotonicity agent is NaCl. In certain embodiments, NaCl is in a concentration of about 160±20 mM, or about 140 mM±20 mM, or about 120±20 mM, or about 100 mM±20 mM, or about 80 mM±20 mM, or about 60 mM±20 mM.

In yet other embodiments, the compositions comprise a preservative. Preservatives include, but are not limited to, m-cresol and benzyl alcohol. In certain embodiments, the preservative is in a concentration of about 0.4%±0.2%, or about 1%±0.5%, or about 1.5%±0.5%, or about 2.0%±0.5%.

In still other embodiments, the compositions contain an anti-adsorbent (e.g., to mitigate adsorption of a CNP variant to glass or plastic). Anti-adsorbents include without limitation benzyl alcohol, Polysorbate 20, and Polysorbate 80. In certain embodiments, the anti-adsorbent is in a concentration from about 0.001% to about 0.5%, or from about 0.01% to about 0.5%, or from about 0.1% to about 1%, or from about 0.5% to about 1%, or from about 0.5% to about 1.5%, or from about 0.5% to about 2%, or from about 1% to about 2%.

In additional embodiments, the compositions comprise a stabilizer. Non-limiting examples of stabilizers include glycerin, glycerol, thioglycerol, methionine, and ascorbic acid and salts thereof. In some embodiments, when the stabilizer is thioglycerol or ascorbic acid or a salt thereof, the stabilizer is in a concentration from about 0.1% to about 1%. In other embodiments, when the stabilizer is methionine, the stabilizer is in a concentration from about 0.01% to about 0.5%, or from about 0.01% to about 0.2%. In still other embodiments, when the stabilizer is glycerin, the stabilizer is in a concentration from about 5% to about 100% (neat).

In further embodiments, the compositions contain an anti-oxidant. Exemplary anti-oxidants include without limitation methionine and ascorbic acid. In certain embodiments, the molar ratio of antioxidant to CNP variant is from about 0.1:1 to about 15:1, or from about 1:1 to about 15:1, or from about 0.5:1 to about 10:1, or from about 1:1 to about 10:1 or from about 3:1 to about 10:1.

Pharmaceutically acceptable salts can be used in the compositions, including without limitation mineral acid salts (e.g., hydrochloride, hydrobromide, phosphate, sulfate), salts of organic acids (e.g., acetate, propionate, malonate, benzoate, mesylate, tosylate), and salts of amines (e.g., isopropylamine, trimethylamine, dicyclohexylamine, diethanolamine). A thorough discussion of pharmaceutically acceptable salts is found in Remington's Pharmaceutical Sciences, 18th Edition, Mack Publishing Company, (Easton, Pa. (1990)).

The pharmaceutical compositions can be administered in various forms, such as tablets, capsules, granules, powders, solutions, suspensions, emulsions, ointments, and transdermal patches. The dosage forms of the compositions can be tailored to the desired mode of administration of the compositions. For oral administration, the compositions can take

the form of, e.g., a tablet or capsule (including softgel capsule), or can be, e.g., an aqueous or nonaqueous solution, suspension or syrup. Tablets and capsules for oral administration can include one or more commonly used excipieints, diluents and carriers, such as mannitol, lactose, glucose, sucrose, starch, corn starch, sodium saccharin, talc, cellulose, magnesium carbonate, and lubricating agents (e.g., magnesium stearate, sodium stearyl fumarate). If desired, flavoring, coloring and/or sweetening agents can be added to the solid and liquid formulations. Other optional ingredients for oral formulations include without limitation preservatives, suspending agents, and thickening agents. Oral formulations can also have an enteric coating to protect the CNP variant from the acidic environment of the stomach. Methods of preparing solid and liquid dosage forms are known, or will be apparent, to those skilled in this art (see, e.g., Remington's Pharmaceutical Sciences, referenced above).

Formulations for parenteral administration can be prepared, e.g., as liquid solutions or suspensions, as solid forms suitable for solubilization or suspension in a liquid medium prior to injection, or as emulsions. For example, sterile injectable solutions and suspensions can be formulated according to techniques known in the art using suitable diluents, carriers, solvents (e.g., buffered aqueous solution, Ringer's solution, isotonic sodium chloride solution), dispersing agents, wetting agents, emulsifying agents, suspending agents, and the like. In addition, sterile fixed oils, fatty esters, polyols and/or other inactive ingredients can be used. As further examples, formulations for parenteral administration include aqueous sterile injectable solutions, which can contain anti-oxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient; and aqueous and nonaqueous sterile suspensions, which can contain suspending agents and thickening agents.

Compositions comprising a CNP variant can also be lyophilized formulations. In certain embodiments, the lyophilized formulations comprise a buffer and bulking agent, and optionally an antioxidant. Exemplary buffers include without limitation acetate buffers and citrate buffers. Exemplary bulking agents include without limitation mannitol, sucrose, dexran, lactose, trehalose, and povidone (PVP K24). In certain embodiments, mannitol is in an amount from about 3% to about 10%, or from about 4% to about 8%, or from about 4% to about 6%. In certain embodiments, sucrose is in an amount from about 6% to about 20%, or from about 6% to about 15%, or from about 8% to about 12%. Exemplary anti-oxidants include, but are not limited to, methionine and ascorbic acid.

The disclosure also provides kits containing, e.g., bottles, vials, ampoules, tubes, cartridges and/or syringes that comprise a liquid (e.g., sterile injectable) formulation or a solid (e.g., lyophilized) formulation. The kits can also contain pharmaceutically acceptable vehicles or carriers (e.g., solvents, solutions and/or buffers) for reconstituting a solid (e.g., lyophilized) formulation into a solution or suspension for administration (e.g., by injection), including without limitation reconstituting a lyophilized formulation in a syringe for injection or for diluting concentrate to a lower concentration. Furthermore, extemporaneous injection solutions and suspensions can be prepared from, e.g., sterile powder, granules, or tablets comprising a CNP-containing composition. The kits can also include dispensing devices, such as aerosol or injection dispensing devices, pen injectors, autoinjectors, needleless injectors, syringes, and/or needles.

As a non-limiting example, a kit can include syringes having a single chamber or dual chambers. For single-chamber syringes, the single chamber can contain a liquid CNP formulation ready for injection, or a solid (e.g., lyophilized) CNP formulation or a liquid formulation of a CNP variant in a relatively small amount of a suitable solvent system (e.g., glycerin) that can be reconstituted into a solution or suspension for injection. For dual-chamber syringes, one chamber can contain a pharmaceutically acceptable vehicle or carrier (e.g., solvent system, solution or buffer), and the other chamber can contain a solid (e.g., lyophilized) CNP formulation or a liquid formulation of a CNP variant in a relatively small amount of a suitable solvent system (e.g., glycerin) which can be reconstituted into a solution or suspension, using the vehicle or carrier from the first chamber, for injection.

As a further example, a kit can include one or more pen injector or autoinjector devices, and dual-chamber cartridges. One chamber of a cartridge can contain a pharmaceutically acceptable vehicle or carrier (e.g., solvent system, solution or buffer), and the other chamber can contain a solid (e.g., lyophilized) CNP formulation or a liquid formulation of a CNP variant in a relatively small amount of a suitable solvent system (e.g., glycerin) which can be reconstituted into a solution or suspension, using the vehicle or carrier from the first chamber, for injection. A cartridge can comprise an amount of the CNP variant that is sufficient for dosing over a desired time period (e.g., 1 day, 2 days, 3 days, 1 week, 2 weeks, 3 weeks, 4 weeks, etc.). The pen injector or autoinjector can be adjusted to administer a desired amount of the CNP formulation from a cartridge.

In addition, pharmaceutical compositions comprising a CNP variant can be formulated as a slow release, controlled release or sustained release system for maintaining a relatively constant level of dosage over a desired time period, such as 1 week, 2 weeks, 3 weeks, 1 month, 2 months, or 3 months. Slow release, controlled release and sustained release formulations can be prepared using, e.g., biodegradable polymeric systems {which can comprise, e.g., hydrophilic polymers [e.g., polylactide, polyglycolide, poly(lactide-glycolide)]}, and can take the form of, e.g., microparticles, microspheres or liposomes, as is known in the art.

Dosages and Frequency of Dosing

As used herein, the term "therapeutically effective amount" of an active agent (e.g., a CNP variant) refers to an amount that provides therapeutic benefit to a patient. The amount may vary from one individual to another and may depend upon a number of factors, including the overall physical condition of the patient. A therapeutically effective amount of a CNP variant can be readily ascertained by one skilled in the art, using publicly available materials and procedures. For example, the amount of a CNP variant used for therapy should give an acceptable rate of growth based on growth charts for children ages 0-17 years with achondroplasia (214 females and 189 males), which list height for age, head circumference, and segmental growth (Horton W A et al., Standard growth curves for achondroplasia, J. Pediatr., 93: 435-8 (1978)). CDC charts can be used to assess weight for age and weight for height or BMI for age. Secondary outcomes with courses that are more chronic in nature can also be measured.

Having a longer serum half-life than wild-type CNP22, the CNP variants can potentially be administered less frequently than CNP22. The dosing frequency for a particular individual may vary depending upon various factors, including the disorder being treated and the condition and response

of the individual to the therapy. In certain embodiments, a pharmaceutical composition containing a CNP variant is administered to a subject about one time per day, one time per two days, one time per three days, or one time per week. In one embodiment, for treatment of bone-related disorders (e.g., skeletal dysplasias, including achondroplasia), a daily or weekly dose of a CNP variant is administered to patients until and/or through adulthood.

The CNP variants described herein can be administered to patients at therapeutically effective doses to treat, ameliorate or prevent bone-related disorders (e.g., skeletal dysplasias, including achondroplasia) and conditions (e.g., vascular smooth muscle disorders) in which CNP can provide a vasoprotective effect. The safety and therapeutic efficacy of the CNP variants can be determined by standard pharmacological procedures in cell cultures or experimental animals, such as, for example, by determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50}. Active agents exhibiting a large therapeutic index are normally preferred.

Data obtained from cell culture assays and animal studies can be used to formulate a range of dosage for use in humans. The dosage normally lies within a range of circulating concentrations that include the ED_{50}, with little or minimal toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized. The therapeutically effective dose can be determined from cell culture assays and animal studies.

In certain embodiments, the CNP variants described herein are administered at a dose in the range from about 5 or 10 nmol/kg to about 300 nmol/kg, or from about 20 nmol/kg to about 200 nmol/kg. In some embodiments, the CNP variants are administered at a dose of about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 125, 130, 140, 150, 160, 170, 175, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 350, 400, 450, 500, 750, 1000, 1250, 1500, 1750 or 2000 nmol/kg or other dose deemed appropriate by the treating physician. In other embodiments, the CNP variants are administered at a dose of about 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950 or 1000 ug/kg, or about 1, 1.25, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5 or 10 mg/kg, or other dose deemed appropriate by the treating physician. The doses of CNP variants described herein can be administered according to the dosing frequency/frequency of administration described herein, including without limitation daily, 2 or 3 times per week, weekly, every 2 weeks, every 3 weeks, monthly, etc.

The frequency of dosing/administration of a CNP variant for a particular subject may vary depending upon various factors, including the disorder being treated and the condition and response of the subject to the therapy. The CNP variant can be administered in a single dose or in multiple doses per dosing. In certain embodiments, the CNP variant is administered, in a single dose or in multiple doses, daily, every other day, every 3 days, 2 times per week, 3 times per week, weekly, bi-weekly, every 3 weeks, monthly, every 6 weeks, every 2 months, every 3 months, or as deemed appropriate by the treating physician.

In some embodiments, a CNP variant is administered so as to allow for periods of growth (e.g., chondrogenesis), followed by a recovery period (e.g., osteogenesis). For example, the CNP variant may be administered intravenously, subcutaneously or by another mode daily or multiple times per week for a period of time, followed by a period of no treatment, then the cycle is repeated. In some embodiments, the initial period of treatment (e.g., administration of the CNP variant daily or multiple times per week) is for 3 days, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks or 12 weeks. In a related embodiment, the period of no treatment lasts for 3 days, 1 week, 2 weeks, 3 weeks or 4 weeks. In certain embodiments, the dosing regimen of the CNP variant is daily for 3 days followed by 3 days off; or daily or multiple times per week for 1 week followed by 3 days or 1 week off; or daily or multiple times per week for 2 weeks followed by 1 or 2 weeks off; or daily or multiple times per week for 3 weeks followed by 1, 2 or 3 weeks off; or daily or multiple times per week for 4, 5, 6, 7, 8, 9, 10, 11 or 12 weeks followed by 1, 2, 3 or 4 weeks off.

Modes of Administration

The CNP variants, or pharmaceutical compositions comprising them, can be administered to subjects in various ways such as, e.g., by injection subcutaneously, intravenously, intra-arterially, intraperitoneally, intramuscularly, intradermally, or intrathecally. In an embodiment, the CNP variants are administered by a single subcutaneous, intravenous, intra-arterial, intraperitoneal, intramuscular, intradermal or intrathecal injection once a day.

The CNP variants can also be administered by direct injection at or near the site of disease. Further, the CNP variants can be administered by implantation of a depot at the target site of action (e.g., an abnormal or dysplasic bone). Alternatively, the CNP variants can be administered sublingually under the tongue (e.g., sublingual tablet) or by inhalation into the lungs (e.g., inhaler or aerosol spray), by delivery into the nasal cavity (e.g., intranasal spray), by delivery into the eye (e.g., eye drop), or by transdermal delivery (e.g., by means of a patch on the skin). The CNP variants may also be administered orally in the form of microspheres, microcapsules, liposomes (uncharged or charged (e.g., cationic)), polymeric microparticles (e.g., polyamides, polylactide, polyglycolide, poly(lactide-glycolide)), microemulsions, and the like.

A further method of administration is by osmotic pump (e.g., an Alzet pump) or mini-pump (e.g., an Alzet mini-osmotic pump), which allows for controlled, continuous and/or slow-release delivery of the CNP variant or pharmaceutical composition over a pre-determined period. The osmotic pump or mini-pump can be implanted subcutaneously, or near the target site (e.g., the long bones of limbs, the epiphyses, etc).

As explained above, the CNP variants can be used to treat conditions or diseases caused by the abnormal growth of vascular smooth muscle cells, including but not limited to restenosis and arteriosclerosis. For local delivery of a CNP variant to the diseased bodily vessel (e.g., blood vessel), the CNP variant can be delivered by means of a medical device (e.g., a stent) implanted at the diseased site. In one embodiment, the CNP variant is impregnated in a polymeric matrix or polymeric coating disposed over a stent. In another embodiment, the CNP variant is contained in reservoirs or channels formed in the body of a stent and covered by a porous polymeric membrane or layer through which the CNP variant can diffuse. The polymeric matrix, coating, membrane or layer can comprise at least one biodegradable (e.g., hydrophilic) polymer, as is known in the art. In a further embodiment, the CNP variant can be contained in

micropores in the body of a stent. The CNP variant can be delivered from a stent by burst release, pulse release, controlled release or sustained release, or a combination thereof. For example, the stent can locally deliver the CNP variant to the diseased site in a burst release followed by a sustained release. Sustained release can be over a period up to about 2 weeks, 1 month, 2 months, 3 months, 6 months or 1 year.

It will be apparent to one skilled in the art that the CNP variants or compositions thereof can also be administered by other modes. Determination of the most effective mode of administration of the CNP variants or compositions thereof is within the skill of the skilled artisan.

The CNP variants can be administered as pharmaceutical formulations suitable for, e.g., oral (including buccal and sub-lingual), rectal, nasal, topical, pulmonary, vaginal or parenteral (including intramuscular, intraarterial, intrathecal, subcutaneous and intravenous) administration, or in a form suitable for administration by inhalation or insufflation. Depending on the intended mode of administration, the pharmaceutical formulations can be in the form of solid, semi-solid or liquid dosage forms, such as tablets, suppositories, pills, capsules, powders, liquids, suspensions, emulsions, creams, ointments, lotions, and the like. The formulations can be provided in unit dosage form suitable for single administration of a precise dosage. The formulations comprise an effective amount of a CNP variant, and one or more pharmaceutically acceptable excipients, carriers and/or diluents, and optionally one or more other biologically active agents.

Combination Therapy

In one embodiment, a CNP variant can be used in combination with one or more other active agents useful for treating, ameliorating or preventing CNP-responsive conditions or disorders such as, e.g., bone-related disorders (e.g., skeletal dysplasias) and vascular smooth muscle disorders. The other active agent(s) can enhance the effects of the CNP variant and/or exert other pharmacological effects in addition to those of the CNP variant. Non-limiting examples of active agents that can be used in combination with the CNP variants described herein are other natriuretic peptides (e.g., BNP) and inhibitors (e.g., antagonists) of peptidases and proteases (e.g., NEP and furin), NPR-C and tyrosine kinases (e.g., FGFR-3). By preventing NEP cleavage of the CNP variant, an NEP inhibitor can prolong the half-life of the variant. Examples of NEP inhibitors include, without limitation, thiorphan and candoxatril. Co-use of an NPR-C inhibitor can also prolong the half-life of the CNP variant via inhibition of the variant's clearance by NPR-C. A non-limiting example of an NPR-C inhibitor is the fragment FGIPMDRIGRNPR (SEQ ID NO: 82), which would be released at the target site (e.g., bone growth plate) upon proteolytic cleavage of the FGIPMDRIGRNPR-CNP22 chimera (Analog CZ) (SEQ ID NO: 82) or similar chimeras comprising variants of CNP22 (e.g., those containing amino acid substitution(s), addition(s), and/or deletion(s) relative to CNP22). Co-use of a tyrosine kinase inhibitor can accentuate the effects of a CNP therapy by inhibiting the tyrosine kinase receptor FGFR-3, a negative regnlator of chondrocyte and bone growth. Non-limiting examples of tyrosine kinase inhibitors include those disclosed in U.S. Pat. Nos. 6,329,375 and 6,344,459.

To achieve the appropriate therapeutic outcome in the combination therapies, one would generally administer to the subject the CNP composition and other therapeutic(s) in a combined amount effective to produce the desired therapeutic outcome (e.g., restored bone growth). This process may involve administering the CNP composition and other therapeutic agent(s) at the same time. Simultaneous administration can be achieved by administering a single composition or pharmacological protein formulation that includes both the CNP variant and other therapeutic agent(s). Alternatively, the other therapeutic agent(s) can be taken separately at about the same time as a pharmacological formulation (e.g., tablet, injection or drink) of the CNP variant. The CNP variant can also be formulated into a foodstuff such as brownies, pancakes, or cake, suitable for ingestion.

In other alternatives, administration of the CNP variant can precede or follow administration of the other therapeutic agent(s) by intervals ranging from minutes to hours. In embodiments where the other therapeutic agent(s) and the CNP composition are administered separately, one would generally ensure that the CNP variant and the other therapeutic agent(s) are administered within an appropriate time of one another so that both the CNP variant and the other therapeutic agent(s) can exert, synergistically or additively, a beneficial effect on the patient. For example, one can administer the CNP composition within about 0.5-6 hours (before or after) of the other therapeutic agent(s). In one embodiment, the CNP composition is administered within about 1 hour (before or after) of the other therapeutic agent(s).

Identifying and Monitoring Patient Populations

Protocols can be established to identify subjects suitable for CNP therapy and to determine whether a given patient is responsive to CNP therapy. For example, for treatment of bone-related disorders, indicators of growth can be measured, such as long bone growth measurements in utero and neonatal and measurements of bone growth biomarkers such as CNP, cGMP, Collagen II, osteocalcin, and Proliferating Cell Nuclear Antigen (PCNA).

One CNP signaling marker is cGMP (guanosine 3',5' cyclic monophosphate). The level of this intracellular signaling molecule increases after CNP binds to and activates its cognate receptor NPR-B. Elevated levels of cGMP can be measured from cell culture extracts (in vitro) after CNP exposure, conditioned media from bone ex-plant studies (ex vivo) after CNP exposure, and in the plasma (in vivo) within minutes of CNP administration subcutaneously, intravenously, or via other routes of administration known in the art.

Cartilage and bone-specific analytes (or cartilage- and bone-associated markers) can also be measured to assess CNP efficacy. For example, fragments of cleaved collagen type II are a cartilage-specific marker for cartilage turnover. Type II collagen is the major organic constituent of cartilage and fragments of type II collagen (cleaved collagen) are released into circulation, and subsequently secreted into the urine, following cartilage turnover. Cartilage turnover preceeds new bone formation.

A bone-specific biomarker for bone formation which can be measured is N-terminal propeptides of type I procollagen (PINP). The synthesis of type I collagen is an important step in bone formation, as type I collagen is the major organic component in bone matrix. During collagen synthesis, propeptides are released from the procollagen molecule and can be detected in serum. In addition, fragments of collagen type I can be measured as a marker for bone resorption.

Other potential biomarkers for cartilage and bone formation and growth include aggrecan chondroitin sulfate (cartilage-specific marker for cartilage turnover), propeptides of type TT collagen (cartilage-specific marker for cartilage formation), alkaline phosphatase (bone-specific) and osteocalcin (bone-specific marker for bone formation). Cartilage- and bone-associated biomarkers can be measured, e.g., in serum from efficacy/pharmacodynamic in vivo studies and from the conditioned media of ex vivo studies, using commercially available kits.

In one embodiment, the level of at least one bone- or cartilage-associated biomarker is assayed or measured in a subject that has been administered a CNP variant in order to monitor the effects of the CNP variant on bone and cartilage formation and growth in vivo. For example, an increase in the level of at least one bone- or cartilage-associated biomarker may indicate that administration of a CNP variant has a positive effect on bone growth and is a useful treatment for skeletal dysplasias and other bone- or cartilage-related diseases or disorders associated with decreased CNP activity. Exemplary bone- or cartilage-associated biomarkers include, but are not limited to, CNP (e.g, endogenous levels of CNP), cGMP, propeptides of collagen type II and fragments thereof, collagen type II and fragments thereof, osteocalcin, proliferating cell nuclear antigen (PCNA), propeptides of type I procollagen (PINP) and fragments thereof, collagen type I and fragments thereof, aggrecan chondroitin sulfate, and alkaline phosphatase.

In an embodiment, biomarkers are measured by obtaining a biological sample from a subject who will be administered, is being administered or has been administered a CNP variant. Biomarkers can be measured using techniques known in the art, including, but not limited to, Western Blot, enzyme linked immunosorbant assay (ELISA), and enzymatic activity assay. The biological sample can be blood, serum, urine, or other biological fluids.

Additional aspects and details of the disclosure will be apparent from the following examples, which are intended to be illustrative rather than limiting.

F. Examples

Example 1

Synthesis of CNP Variants

CNP variants were prepared using the methods described herein. Substitutions with natural or unnatural amino acids or peptidomimetics were made, as indicated in Tables 1-3 (shown in Example 3), at the respective amino acid residues in the wild-type sequence of CNP22. In certain variants, additional amino acids were added to the N-terminal and/or C-terminal ends of the whole or a portion of the wild-type CNP22 sequence (see Table 3).

Also prepared were CNP variants in which a PEG (or PEO) moiety was conjugated to the N-terminus of CNP22 or variants thereof (see Table 4, shown in Example 3). PEGylation reagents can be obtained from the commercial sources shown in Table 5.

TABLE 5

Vendor	Product Name	Name	MW (Da)	PEGylation Reagent
NOF	Sunbright ME-200CS	mPEG20K	20,000	CH3 (CH2CH2O)450-(CH2)5COO—NHS
NOF	Sunbright ME 050CS	mPEG5K	5,000	CH3(CH2CH2O)110-(CH2)5COO—NHS
Pierce	(Methyl-PEG12)3-PEG4-NHS Ester	(mPEG12)3-PEG4	2,400	[CH3(CH2CH2O)12]3-(CH2CH2O)4-NHCO(CH2)3-COO—NHS
NOF	Sunbright ME-020HS	mPEG2K	2,000	CH3(CH2CH2O)45-(CH2)5COO—NHS
NOF	Sunbright ME-020CS	mPEG2K	2,000	CH3(CH2CH2O)45-(CH2)5COO—NHS
NOF	Sunbright ME-010HS	mPEG1K	1,000	CH3(CH2CH2O)23-CO(CH2)2COO—NHS
Pierce	Methyl PEG24-NHS Ester	MS(PEG)24	1,200	CH3(CH2CH2O)24-(CH2)2COO—NHS
Pierce	EZ-Link NHS-PEG12-Biotin	PEO12-Biotin	940	Biotin-(CH2CH2O)12-(CH2)2COO—NHS
Pierce	Methyl PEG12-NHS Ester	MS(PEG)12	690	CH3(CH2CH2O)12-(CH2)2COO—NHS
Pierce	EZ-Link NHS-PEG4-Biotin	PEO4-biotin	590	Biotin-(CH2CH2O)4-(CH2)2COO—NHS
Pierce	Mono(lactosylamido) mono(succinimidyl)suberate	LSS	590	
Pierce	EZ-link NHS-LC-LC-Biotin	LC-LC-Biotin	570	
Pierce	EZ-link NHS-LC-Biotin	LC-Biotin (LC = long chain)	450	
Pierce	EZ-link NHS-Biotin	Biotin	340	

The PEG (also called PEO) polymers purchased from Pierce Biotechnology (Rockford, Ill.) are monodispersed—i.e., they contain a single discrete polymer of a particular molecular weight. By contrast, the PEG polymers purchased from NOF (Nippon Oil and Fat) are polydispersed—i.e., they contain a mixture of polymers having a distribution of molecular weights.

To PEGylate CNP22 or variants thereof, reaction and purification conditions are optimized for each PEG-CNP conjugate. According to a general PEGylation procedure, reaction mixtures contain about 1 mM CNP22 or a variant thereof, and about 1 to 5 mM NHS-activated PEG in potassium phosphate buffer, pH between about 5.0 and 6.5. To mono-PEGylate selectively at the peptide N-terminus and minimize PEGylation at an internal site (e.g., Lys4 of CNP22), the PEGylation reaction can be conducted under

more acidic conditions (e.g., at a pH between about 5.5 and 6.5) to protonate selectively and hence deactivate the more basic primary amino group on the lysine side chain. After about 1 to 3 hours of incubation at room temperature, the PEGylation reaction is quenched by addition of aqueous glycine buffer. Reaction products are then separated by reverse-phase HPLC, optimized for each PEG-CNP conjugate. Fractionation samples are speedvacced to dryness, and reconstituted/formulated in 1 mM HCl. Identification and purity of each PEG-CNP product are determined by liquid chromatography-mass spectrometry (LC/MS).

Example 2A

Recombinant Production of CNP Variants

CNP variants can be produced using recombinant technology. In certain embodiments, the CNP variants are produced as fusion proteins comprising a cleavable peptide, carrier protein or tag. Exemplary methods for recombinantly producing CNP fusion proteins are disclosed below.

Materials and Methods

Cloning of CNP Fusion Proteins into Expression Vectors

CNP DNA fragments were amplified using polymerase chain reaction (PCR) and the amplified PCR fragments were digested with Nde I and BamHI and cloned into pET21a vector (Novagen, Gibbstown, N.J.). CNP fusion protein DNA was synthesized by DNA2.0 and cloned into different expression vectors (Table 6).

TABLE 6

Construct	Vector	Product	Chemical cleavage	Final product	Expression *E. coli* strain
pJexpress-TAF-CNP	pJexpress401	TAF-CNP inclusion bodies	Formic acid (Asp-Pro)	Pro-CNP	BL21; BL21(DE3)
pJexpress-KSI-CNP(M/N)	pJexpress404	KSI-CNP(M/N) inclusion bodies	CNBr (Met-X)	CNP(M/N)	BL21
pET-31b-KSI-CNP	pET-31b	KSI-CNP inclusion bodies	Formic acid (Asp-Pro)	Pro-CNP	BL21(DE3)
pET-32a-Trx-CNP	pET-32a	Trx-CNP fusion protein (soluble)	Formic acid (Asp-Pro)	Pro-CNP	BL21(DE3)
pMAL-CNP	pMAL-c2X	MBP-CNP fusion protein (soluble)	Formic acid (Asp-Pro)	Pro-CNP	BL21(DE3)

CNP: GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC [Gly-CNP37 (SEQ ID NO: 75);
TAF: human transcription factor TAF12;
KSI: ketosteroid isomerase;
MBP: maltose-binding protein;
Trx: thioredoxin

Expression of CNP Fusion Proteins in E. coli

CNP fusion protein expression plasmids were transformed into E. coli BL21 or BL21(DE3). Transformed cells were plated on LB plates containing 100 ug/ml carbeniciline or 50 ug/ml kanamycin and incubated overnight at 37° C. One single colony was picked and cultured in 4 ml LB medium containing 100 ug/ml of carbeniciline or 50 ug/ml kanamycin at 37° C. with shaking. When an OD_{600} of bacterial culture reached 0.6, 1 mM isopropyl β-D-1-thio-galactopyranoside (IPTG) was added to the cell media and the media was incubated at 37° C. for 3 hours with shaking. For cell harvest, bacterial cells were centrifuged at 4000 rpm for 10 minutes and the cell pellets were stored at −80° C. Cell pellets were lysed with B-PER II Bacterial Extraction Reagent (PIERCE, 0.4 ml per 4 ml of bacterial culture) and Benzonase Nuclease (Novagen, 0.025 U/ml) at room temperature for 10 minutes. Bacterial crude extract was reserved and centrifuged to obtain supernatant. Supernatant and crude extract were assayed for CNP fusion protein expression and solubility by SDS-PAGE and Western Blot.

Detection of CNP Fusion Protein Expression with SDS-PAGE and Western Blot

Ten uL of cell lysates or soluble supernatants was run on sodium dodecyl sulfate-polyacrylimide gel electrophoresis (SDS-PAGE) (Invitrogen, Carlsbad, Calif., NuPAGE 4-12% Bis-tris Gel, MES SDS buffer). The gel was stained using 20 ml Imperial Protein Stain (Thermo Fisher, Rockford, Ill.) at room temperature for 1 hour and de-stained with water. For Western blot, the protein was transferred to membrane with Gel blot (Invitrogen). The membrane was blocked in TBS buffer with 5% milk at room temperature for 1 hour. Rabbit anti-CNP22 antibody (1:2500 dilution) (Bachem, Torrance, Calif.) was added to the membrane, which was then incubated at room temperature with shaking for 2 hours, and then the membrane was washed 3 times with TBS buffer.

Alkaline phosphate (AP) conjugated anti-rabbit IgG (1:5000 dilution) was added to the membrane, which was then incubated at room temperature with shaking for 1 hour, and then the membrane was washed 3 times with TBS buffer. Ten ml WESTERN BLUE®Stabilized Substrate (Promega, Madison, Wis.) was added to the membrane, which was then incubated at room temperature with shaking for 1 to 5 min, and then the membrane was washed with TBS buffer to remove excess stain.

Expression of TAF-CNP Fusion Protein in E. coli BL21

Cells (E. coli strain BL21) expressing TAF-CNP fusion protein were obtained from glycerol stock stored at −80° C. and were grown in 4 ml LB medium containing 50 ug/ml of kanamycin at 37° C. overnight with shaking (250 rpm). Four ml of overnight grown cell culture was transferred to 200 ml LB medium containing 50 ug/ml of kanamycin and was grown at 37° C. with shaking (250 rpm). When the OD_{600} reached 0.6, IPTG was then added to a final concentration of 1 mM to induce protein expression at 37° C. with shaking (250 rpm) for 3 hours. Cells were then spun down at 3000 rpm for 10 minutes and the resulting cell pellet was frozen at −80° C.

Purification of TAF-CNP Inclusion Bodies and Formic Acid Cleavage

The cell pellet (from 200 ml culture) was resuspended in 25 ml of B-PER II buffer (PIERCE), the pellet was sonicated for 10 minutes (50%, 1 second, pause 2 seconds) on ice, centrifuged at 12000 rpm for 20 minutes at 4° C., and then the pellet was resuspended in 25 ml 20× diluted B-PER II buffer. This was repeated until the supernatant became clear

(3-5 times). One mL of resuspended TAF-CNP inclusion bodies was transferred to a 1.5 ml tube and centrifuged at 14000 rpm for 15 min. The supernatant was discarded and the pellet was dissolved with 10 ul of 88% formic acid, and then 490 ul of Millipore filtered water was added immediately. The pellet was mixed well by vortex and incubated at 55° C. for 20 to 24 hours (70° C./6 hours are alternative conditions). The products of the formic acid cleavage were assayed by SDS-PAGE and LC/MS (C4RP).

LC/MS Sample Preparation

Inclusion bodies were isolated from about 8 mL of culture (about 1.5 OD) and the pellet was solubilized in 10 uL nt formate. Resolubilized pellet was immediately diluted to 2% or 10% final formate concentration (0.5 mL) and incubated at 55° C. for 21 hours (pH 2) (cloudiness was more evident in the 2% formate sample the next day). Both samples were centrifuged at 15000 rpm for 2 minutes. Twenty uL of supernatant was injected into an LC/MS (C4 RP) apparatus.

Results

CNP Fusion Proteins were Expressed in E. coli

All CNP fusion proteins were expressed in E. coli induced with 1 mM IPTG at 37° C. for 3 hours (FIG. **1**). The constructs pJexpress-TAF-CNP, pJexpress-KSI-CNP(M/N) and pET-31b-KSI-CNP were expressed as inclusion bodies, while the constructs pET-32a-Trx-CNP and pMAL-CNP were expressed as soluble fusion proteins. Western blot using anti-CNP22 antibody confirmed the expression of the CNP fusion proteins (FIG. **1**).

CNP was Produced from TAF-CNP Inclusion Bodies by Formic Acid Cleavage

TAF-CNP inclusion bodies were partially purified and treated with 2% formic acid at 55° C. for 20 to 24 hours (70° C./6 hours are alternative conditions). The majority of TAF-CNP was cleaved and one extra band having similar size as Gly-CNP37 peptide appeared on SDS-PAGE (FIG. **2**). The cleaved sample was further analyzed by LC/MS (C4 RP). The LC/MS results showed that CNP was released in soluble form from TAF-CNP inclusion bodies after formic acid cleavage. LC/MS analysis indicated that formic acid cleavage of the CNP fusion proteins resulted in formation of cyclized Pro-Gly-CNP37 (MW=4102). Calculation of protein amounts based on analysis suggested that about 60 ug of formic acid-generated CNP was produced from 8 mL of very low OD culture. From a small scale (e.g., about 8 mL) of low OD (e.g., 1.2 OD) cell culture, approximately 8 ug/ml CNP was produced, while fermentation of a larger scale (e.g., about 8 L) of higher OD (e.g., 380D) cell culture can produce approximately 1 mg/ml CNP.

Conclusion

Five expression constructs were generated to express CNP fusion proteins. Expression of all five constructs produced soluble (Trx and MBP) or insoluble (TAF and KSI) CNP fusion proteins. Approximately 1 mg/ml soluble CNP can be produced from TAF-CNP inclusion bodies by a simple formic acid cleavage procedure.

Example 2B

Production of Additional CNP Variants in E. coli

Recombinant production of CNP variants was carried out as described in Example 2A. In this example, additional CNP constructs were generated with a QuikChange II XL site-directed mutagenesis kit (Stratagene) or were synthesized by DNA2.0. Additional CNP constructs and expression vectors are listed in Table 7.

TABLE 7

Construct	Vector	Product	Chemical cleavage	Final product	Expression *E. coli* strain
pJexpress-TAF(C/A)-Pro-CNP38	pJexpress401	TAF(C/A)-Pro-CNP38 inclusion bodies	Formic acid (Asp-Pro)	Pro-CNP38	BL21(DE3)
pJexpress-TAF(4D/4E)-Pro-CNP38	pJexpress401	TAF(4D/4E)-Pro-CNP38 inclusion bodies	Formic acid (Asp-Pro)	Pro-CNP38	BL21(DE3)
pJexpress-TAF (C/A&4D/4E)-Pro-CNP38	pJexpress401	TAF(C/A&4D/4E)-Pro-CNP38 inclusion bodies	Formic acid (Asp-Pro)	Pro-CNP38	BL21(DE3)
pJexpress-TAF (C/A&10D/10E)-Pro-CNP38	pJexpress401	TAF(C/A&10D/10E)-Pro-CNP38 inclusion bodies	Formic acid (Asp-Pro)	Pro-CNP38	BL21(DE3)
pJexpress-TAF-NL-(C/A & 6D/6E)-Pro-CNP38	pJexpress401	TAF-NL-(C/A & 6D/6E)-Pro-CNP38 inclusion bodies	Formic acid (Asp-Pro)	Pro-CNP38	BL21(DE3)
pJexpress-BMP-Pro-CNP38	pJexpress401	BMP-Pro-CNP38 inclusion bodies	Formic acid (Asp-Pro)	Pro-CNP38	BL21; BL21(DE3)
pJexpress-TAF-Pro-CNP37	pJexpress401	TAF-Pro-CNP37 inclusion bodies	Formic acid (Asp-Pro)	Pro-CNP37	BL21; BL21(DE3)
pJexpress-BMP-Pro-CNP37	pJexpress401	BMP-Pro-CNP37 inclusion bodies	Formic acid (Asp-Pro)	Pro-CNP37	BL21; BL21(DE3)
pJexpress-TAF Pro-HSA-CNP	pJexpress401	TAF-Pro-HSA-CNP inclusion bodies	Formic acid (Asp-Pro)	Pro-HSA-CNP	BL21
pJexpress-TAF	pJexpress401	TAF inclusion bodies	N/A	N/A	BL21(DE3)

TABLE 7-continued

Construct	Vector	Product	Chemical cleavage	Final product	Expression *E. coli* strain
pJexpress-BMP	pJexpress401	BMP inclusion bodies	N/A	N/A	BL21(DE3)
pJexpress-TAF(C/A)	pJexpress401	TAF(C/A) inclusion bodies	N/A	N/A	BL21(DE3)
pJexpress-TAF(4D/4E)	pJexpress401	TAF(4D/4E) inclusion bodies	N/A	N/A	BL21(DE3)
pJexpress-TAF (C/A&4D/4E)	pJexpress401	TAF(C/A&4D/4E) inclusion bodies	N/A	N/A	BL21(DE3)
pJexpress-TAF (C/A&10D/10E)	pJexpress401	TAF(C/A&10D/10E) inclusion bodies	N/A	N/A	BL21(DE3)
pJexpress-TAF-NL-(C/A & 6D/6E)	pJexpress401	TAF-NL-(C/A&6D/6E) inclusion bodies	N/A	N/A	BL21(DE3)
pJexpress-TAF-Pro-CNP53	pJexpress401	TAF-Pro-CNP53 inclusion bodies	Formic acid (Asp-Pro)	Pro-CNP53	BL21(DE3)
pJexpress-TAF-CNP34	pJexpress401	TAF-CNP34 inclusion bodies	Formic acid (Asp-Pro)	CNP34	BL21(DE3)

CNP38: GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC [G1y-CNP37 (SEQ ID NO: 75)];
Pro-CNP38: PGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC [Pro-G1y-CNP37](SEQ ID NO: 145);
CNP37: QEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (SEQ ID NO: 60);
HSA-CNP: GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC [HSA-CNP27 (SEQ ID NO: 144)];
Pro-CNP53: PDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIG-SMSGLGC (SEQ ID NO: 185);
CNP34: PNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (SEQ ID NO: 163);
TAF-Pro-CNP38: MVLTKKKLQDLVREVCPNEQLDEDVEEMLLQIADDFIESVVTAA-CQLARIIRKSSTLEVKDVQLILERQWNM-WIMGSSIIIIIIIIISSGLVPRGSIIT-GDDDDKHMDPGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (SEQ ID NO: 196);
TAF: human transcription factor TAF12 histone fold domain (HFD) and linker from pET-15b vector;
TAF-NL: TAF12 HFD without a linker;
TAF12 HFD: VLTKKKLQDLVREVCPNEQLDEDVEEMLLQIADDFIESVVTAACQLA-RHRKSSTLEVKDVQLHLERQWNMWI (SEQ ID NO: 197);
pET-15b linker: MGSSHHHHHHSSGLVPRGSHTGDDDDKHMD (SEQ ID NO: 195);
TAF(C/A): cysteine In TAF is changed to alanine;
TAF(D/E): aspartic acid in TAF is changed to glutamic acid (number indicates which amino acid residue is changed);
TAF(C/A & D/E) : cysteine and aspartic acid in TAF are changed to alanine and glutamic acid, respectively;
BMP: bone morphogenetic protein 2 with seven C/A (cysteine to alanine) mutations;
KSI: ketosteroid isomerase;
MBP: maltose-binding protein;
TRX: thioredoxin

Results

All CNP fusion proteins were expressed in E. coli induced with 1 mM IPTG at 37° C. for 3 hours. Constructs pJexpress-BMP-Pro-CNP38, pJexpress-TAF-Pro-CNP37, pJexpress-BMP-Pro-CNP37, pJexpress-Pro-HSA-CNP, pJexpress-TAF and pJexpress-BMP were expressed as inclusion bodies. A Western Blot with anti-CNP antibody was used to confirm the expression (FIG. **3**).

Pro-Gly-CNP37 ("Pro-CNP38") was Produced from TAF-Pro-CNP38 Inclusion Bodies by Formic Acid Cleavage

Formic acid is used as a denaturant for inclusion body proteins and can specifically cleave the peptide bond between Asp and Pro under optimized conditions. TAF-Pro-CNP inclusion bodies were partially purified as described above and treated with 50% formic acid at 25° C., 37° C., 42° C. and 55° C. for 24 hours. Most of the TAF-Pro-CNP38 was cleaved and one extra band with a similar size as the Gly-CNP37 ("CNP38") peptide showed on SDS-PAGE from the 37° C., 42° C. and 55° C. cleavages (FIG. **4**A). The cleavage reactions at 37° C. and 55° C. were neutralized with 10 M NaOII and centrifuged at 14,000 rpm for 15 minutes. The un-cleaved TAF-Pro-CNP38, TAF and other inclusion bodies precipitated in the pellets. The supernatants contained soluble Pro-CNP38 and were further analyzed by LC/MS. The LC/MS result showed the supernatants contained a mixture of non-specific cleaved peptides generated by excess acidic hydrolysis.

When TAF-Pro-CNP inclusion bodies were treated with 2% and 10% formic acid at 55° C. for 20 hours, the majority of TAF-Pro-CNP38 was cleaved and one extra band having a similar size as Gly-CNP37 was observed on SDS-PAGE (FIG. **4**B). The cleaved sample was further analyzed by LC/MS. The LC/MS analysis showed that correct Pro-CNP38 was released in soluble form from TAF-Pro-CNP38 inclusion bodies after formic acid cleavage. The yields of Pro-CNP38 from 2% and 10% formic acid cleavages were similar (FIG. **4**C).

Formic Acid Cleavage and Neutralization for Pro-Gly-CNP37 ("Pro-CNP38") Production and Purification

Formic acid can dissolve and cleave TAF-Pro-CNP38, BMP-Pro-CNP38 and other inclusion bodies. The pH neutralization results in precipitation of insoluble contaminating proteins/peptides (un-cleaved TAF-Pro-CNP38 and BMP-Pro-CNP38, TAF, BMP and others). The soluble Pro-CNP38 stays in the supernatant after centrifugation. TAF-Pro-CNP38 and BMP-Pro-CNP38 were cleaved in 2% formic acid at 55° C. or 70° C. for 24 hours. The cleavage reactions were neutralized with 1:1 ratio of 0.5 M Tris buffer and

centrifuged at 14,000 rpm for 15 minutes. Results showed that the supernatants contained almost pure peptides and there was no observed recovery loss of Pro-CNP38 upon neutralization. This is a simple and efficient step for Pro-CNP38 purification.

Analysis of Varying Conditions for Formic Acid Cleavage of TAF-Pro-CNP38 Inclusion Bodies for Pro-Gly-CNP37 ("Pro-CNP38") Production

Formic acid can specifically cleave the peptide bond between Asp and Pro under optimized conditions. Non-specific cleavage of peptide bonds between Asp and any other amino acids or even non-specific cleavage of any peptide bond may occur if the formic acid cleavage conditions are not optimized. TAF-Pro-CNP38 inclusion bodies were cleaved with 2% formic acid at 42° C., 55° C. or 70° C. for 6, 24 or 48 hours. FIG. **5**A shows that TAF-Pro-CNP38 was cleaved completely at 70° C. for 24 hours or at 55° C. for 48 hours. The 70° C. cleavage could be completed within 17 hours (FIG. **5**B). Although the 70° C./24 h cleavage gave the highest yield, the non-specific cleavage products (for example, the peptide with molecular weight of 3142 generated from Pro-CNP38 by cleaving between peptide bond Asp and Arg) increased dramatically (FIG. **5**C).

The yield and purity of Pro-CNP38 production were improved when TAF-Pro-CNP38 inclusion bodies were purified or treated with B-PER II buffer before formic acid cleavage. Because B-PER II buffer contains detergent octyl-thio-glucoside and is relatively expensive, other commonly used detergents or buffers were tested for large-scale Pro-CNP38 production. TAF-Pro-CNP38 inclusion bodies were re-suspended in different detergents (Octylsucrose; Triton x-100; Tween-20; NP-40; CA-630) or buffers (B-PER II; B-PER II 1/20 dilution; B-PER; B-PER phosphate buffer; 25 mM tris. 150 mM NaCl, pH 7.9; 25 mM tris, pH 7.5; filtered water; PBS) and incubated at room temperature (RT) for 24 hours. All detergents were 1% in 25 mM tris buffer, pH 7.5. After incubation in detergent or buffer, TAF-Pro-CNP38 inclusion bodies were cleaved by 2% formic acid at 55° C. for 22 hours. Results showed that BPER II continued to exhibit good yield, and positive results were also obtained with CA630 and Triton X-100.

Pro-Gly-CNP37 ("Pro-CNP38") Proteolytic Cleavage Products

One unidentified protease, possibly a membrane associated protease, cleaved the Pro-CNP38 peptide (produced from BL21 strain, MW 4102) into two peptides during Pro-CNP38 purification, resulting in peptides PGQEHPNAR (MW 1004) (SEQ ID NO: 198) and KYKGANKKGLSKGCFGLKLDRIGSMSGLGC (MW 3115) (SEQ ID NO: 199). Not to be bound by theory, one possible reason why detergents may improve the yield and purity of Pro-CNP38 is that detergents can remove most, but possibly not all, of the unidentified protease. High temperature, basic pH and EDTA were tested to identify if these agents could inhibit the protease cleavage. TAF-Pro-CNP38 inclusion bodies were incubated at RT or 120° C. for 2 hrs and centrifuged at 14000 rpm for 15 min. The pellets were re-suspended in 2% formic acid and incubated at 55° C. or 70° C. for 18 hours. A 1:1 ratio of 0.5 M Tris was added to neutralize the cleavage. The neutralized samples were centrifuged at 14000 rpm for 5 min and the supernatants were left at RT for 6 hrs or 22 hrs with or without 10 mM EDTA, pH 10. The proteolytic cleavage was assayed by LC/MS (Table 8).

TABLE 8

LC/MS result of Pro-CNP38 proteolytic cleavage

Sample		A210 Peak Area	Protein Conc. (mg/mL)		Percent MW4102	Percent MW3115
1	H 55 C.	942	0.04	*	91.8	8.2
2	H 70 0	2878	0.11	*	79.2	20.7
3	H 70 6H	2675	0.10	*	80.6	19.4
4	H 70 24	2741	0.11	*	79.2	20.7
5	H 70 EDTA	2385	0.09	*	80.8	19.2
6	H 70 pH 10	1917	0.07	*	81.2	18.8
7	55 C.	1291	0.05		61.1	38.9
8	70 C. 0	4533	0.18		96.8	3.2
9	70 C. 6H	4120	0.16		97.5	2.5
10	70 C. 24	4108	0.16		96.5	3.5
11	70 C. EDTA	4336	0.17		96.9	3.1
12	70 C. pH 10	3425	0.13		97.5	2.4

All samples (8-12) cleaved at 70° C. showed limited proteolytic cleavage (less than 4% cleavage of Pro-CNP38). Almost 40% of Pro-CNP38 was cleaved by protease when cleavage was carried out at 55° C. (sample 7). Basic pH and EDTA did not influence the non-specific proteolytic cleavage. High temperature (120° C. for 2 hrs) non-specifically cleaved Pro-CNP38.

It should be noted that the BL21(DE3) strain from Stratagene does not have the unidentified protease.

Production of Pro-Gly-CNP37 ("Pro-CNP38") and Other CNP Variants from Different Constructs:

Pro-CNP38 can be produced in large scale in E. coli by means of overexpression of a TAF-Pro-CNP38 fusion protein as inclusion bodies followed by formic acid cleavage of the fusion protein. Following the methods described herein, other TAF-CNP fusion proteins (TAF-CNP34 and TAF-Pro-CNP53) were expressed as inclusion bodies and then cleaved with formic acid to generate the CNP variants CNP34 and Pro-CNP53. FIG. **6** depicts the expression of TAF-CNP34, FIG. **7** the expression of TAF-Pro-CNP53, FIG. **8** the products of formic acid cleavage of TAF-CNP34 and TAF-Pro-CNP53, FIG. **9** the peak for CNP-34 in an LC/MS chromatogram, and FIG. **10** the peak for Pro-CNP53 in an LC/MS chromatogram.

Use of formic acid may result in unspecific cleavage(s) at peptide bond(s) other than the targeted Asp-Pro bond. To improve the purity and overall titer of the desired formic acid cleavage product, different residues of aspartic acid in TAF12 or fragments thereof were changed to glutamic acid. Moreover, one or more cysteine residues in TAF12 or fragments thereof were changed to alanine to prevent unspecific disulfide bond formation. All TAF-Pro-CNP38 fusion proteins having such mutations in TAF12 were expressed as inclusion bodies and cleaved with formic acid to produce Pro-CNP38. FIG. **7** shows the expression of TAF-NL-(C/A & 6D/6E)-Pro-CNP38 and TAF(C/A & 10D/10E)-Pro-CNP38, FIG. **11** the expression of TAF(C/A & 4D/4E)-Pro-CNP38 and TAF(4D/4E)-Pro-CNP38, FIG. **12** the products of formic acid cleavage of TAF(4D/4E)-Pro-CNP38 and TAF(C/A & 4D/4E)-Pro-CNP38, and FIG. **13** the products of formic acid cleavage of TAF-NL-(C/A & 6D/6E)-Pro-CNP38 and TAF(C/A & 10D/10E)-Pro-CNP38. Table 9 summarizes the purity (prior to purification) and titer of Pro-CNP38 obtained from the various TAF-Pro-CNP38 constructs.

TABLE 9

Construct	Purity	Titer (ug/mL)
pJexpress-TAF-Pro-CNP38	32%	44
pJexpress-TAF(C/A)-Pro-CNP38	41%	50
pJexpress-TAF(4D/4E)-Pro-CNP38	36%	52
pJexpress-TAF(C/A & 4D/4E)-Pro-CNP38	42%	58
pJexpress-TAF(C/A & 10D/10E)-Pro-CNP38*	32%	26
pJexpress-TAF-NL-(C/A & 6D/6E)-Pro-CNP38	50%	55

*Cells with pJexpress-TAF(C/A&10D/10E)-Pro-CNP38 growed more slowly and the final cell density (OD_{600}) was lower compared to other TAF-Pro-CNP38 constructs.

Large-scale Production of Pro-Gly-CNP37 ("Pro-CNP38") by Fermentation and Formic Acid Cleavage

BL21(DE3) cells comprising the pJexpress-TAF-CNP construct were grown in a 10 liter fermenter at 37° C. for about 16-17 hours until OD_{600} reached 64. The cells were then grown/cultured in the presence of 1 mM IPTG at ahout 35-37° C., to induce expression of the TAF-Pro-CNP38 fusion protein, for about 7-8 hours until OD_{600} reached 160. The fermentation produced a titer of 9 g/L TAF-Pro-CNP38. FIG. **14** displays a Western blot of the TAF-Pro-CNP38 fusion protein produced in the fermentation.

A cell pellet recovered from 750 mL cell culture from the 10 L fermentation was resuspended in phosphate-buffered saline, pH 7.4 (PBS) and lysed by three passes through a pressure homogenizer (10,000 bar). The resulting lysate was centrifuged at 6,500 g for 10 minutes and the supernatant was discarded. The pellet fraction containing insoluble TAF-Pro-CNP38 fusion protein inclusion bodies was resuspended in 500 mL PBS with a rotostator. The suspension was centrifuged at 6,500 g for 10 minutes and the supernatant was discarded. The resulting inclusion body pellet was resuspended in 500 mL water with a roto stator and incubated at 55° C. for 30 minutes. 250 mL 6% formic acid was added to the warmed inclusion body suspension for a final concentration of 2% formic acid and incubated at 55° C. for 20-24 hours. 50 mL of 400 mM Na_2HPO_4 was added after 20-24 hours to begin the neutralization of the formic acid cleavage reaction, and the resulting mixture was titrated with 50% w/v NaOH to pH 6.9-7.4 and left at room temperature for 30 minutes. Upon neutralization, a heavy precipitate formed and was removed by centrifugation at 6,500 g for 10 minutes. The supernatant was retained and contained on average 1.3 g/L culture of 80% pure Pro-CNP38. The majority of the E. coli and TAF-related proteins and peptides remained in the pellet.

The soluble Pro-CNP38 resulting from the formic acid cleavage and neutralized supernatant was 80% pure and contained a mixture of linear and cyclized Pro-CNP38 peptides along with other product-related impurities. The pH neutral supernatant containing Pro-CNP38 was sterile-filtered. Further purification by anion-exchange chromatography using a Fractogel TMAE Hi-CAP column (EMD Biosciences) to remove DNA, endotoxins, and peptide contaminants, which should bind to the column, was performed at pH 7-7.4. The flow-through fraction contained partially cyclized Pro-CNP38. Cupric sulfate was added to the flow-through fraction to a final concentration of 10 uM and incubated at room temperature for 1 hour. The addition of Cu^{++} at neutral pH catalyzed the oxidation of free cysteine sulfhydryl groups on the peptide to form an intramolecular disulfide bond, resulting in 100% cyclized Pro-CNP38 and no detectable linear peptide. The conductivity of the solution was adjusted to <15 mS/cm by the addition of water. Cation-exchange chromatography using an SP-Sepharose column (GE Healthcare) and a sodium phosphate buffer (pH 7) was then performed to remove any remaining DNA and endotoxins in the flow-through and further purify Pro-CNP38 to about 95-96% purity with <0.5% non-product related impurities. FIG. **15** is an SDS-PAGE of eluate fractions from the SP-Sepharose column; the highest concentrations of Pro-CNP38 were found in fractions 22 to 30. Reverse-phase HPLC/MS analysis of non-pooled fraction 24 indicated the presence of 90% Pro-CNP38, 5% Pro-CNP38 with an oxidized methionine residue, 3% Pro-CNP38 cleaved at the Gly-Cys bond to form CNP-17, and 1.6% Pro-CNP38 cleaved at the Asp-Arg bond in the cyclic domain. The yield of pure Pro-CNP38 peptide upon final purification was 0.9 g/L cell culture (36% total recovery).

Pro-CNP38 collected from five separate purifications was pooled for formulation. The pooled product contained 93.5% Pro-CNP38, 3.3% Pro-CNP38 with an oxidized methionine residue, 1.3% deamidated Pro-CNP38, and 1% Pro-CNP38 cleaved at the Gly-Cys bond to form CNP-17. Samples were diluted with 50 mM sodium phosphate (pH 7) to a conductivity of 10 mS/cm and loaded onto a CM-Sepharose column (GE-Healthcare) for concentration and buffer exchange. The weak cation-exchange property of the CM-Sepharose resin allows peptides to dissociate from the column with weak acid solutions rather than typical salt gradients. Acid concentrations required for dissociation of Pro-CNP38 from the CM-Sepharose column depended upon column loading. At 50 mg Pro-CNP38 per mL of resin, 10 mM HCl was sufficient for Pro-CNP38 elution. At 9 mg Pro-CNP38 per mL of resin, 50 mM HCl was required for elution. When the loading was 9 mg Pro-CNP38 per mL of resin, Pro-CNP38 eluted in less than one column volume, which significantly concentrated the peptide. The eluate fraction contained 20.3 mg/mL of 95% pure Pro-CNP38, along with 3% Pro-CNP38 with an oxidized methionine residue, 1% deamidated Pro-CNP38, and <1% Pro-CNP38 cleaved at the Gly-Cys bond to form CNP-17. The concentrated solution of Pro-CNP38 in weak acid is suitable for dilution into appropriate buffers for either liquid or lyophilized formulations.

Example 3

Cleavage of CNP Variants by Neutral Endopeptidase In Vitro

To determine the effects of amino acid substitutions, amino acid extensions, backbone modifications, side chain modifications and PEGylation on the susceptibility of CNP variants to neutral endopeptidase (NEP) cleavage, peptide cleavage assays were carried out using an in vitro assay that monitored disappearance of the non-cleaved CNP variant.

Recombinant human NEP (1 ug/mL final concentration) was added to 100 uM CNP variant diluted in 0.1 M Tris, pH 7. The reaction mixture was incubated at 37° C. for various periods of time, and the reaction was quenched with EDTA (10 mM final) followed by heat denaturation. The reaction mixture was reduced and then the reaction products were analyzed using HPLC and mass spectroscopy. The half-life of the CNP variant was calculated based on the disappearance of intact CNP variant over time. The results for digested CNP variants were compared to a parallel wtCNP22 digestion and normalized to the results for 100 uM CNP22 digested by 1 mg/mL NEP ($t_{1/2}$=80 min).

Table 1 lists the half-lives, based on the in vitro NEP cleavage assay, of various CNP variants having backbone or side chain modifications. Removal of three of the six NEP cleavage sites in Analog L nevertheless resulted in a substantially shorter half-life. Of tested CNP variants, the

greatest resistance to NEP cleavage was exhibited by Analog N, which contains the D-enantiomer of all 22 amino acids of CNP22, and by Analog M, which has an N-methylated amide bond at both Leu9 and Leu11. However, both Analogs N and M failed to stimulate production of cGMP (see below).

Comparing the half-lives of Analogs A, B, E, F. G and H to one another, half-lives were determined to be about 1.5- to about 2.5-fold longer for Analogs E and G compared to those for Analogs A, B, F and H. All of these six analogs showed resistance or improved resistance to cleavage at the Cys6-Phe7 bond relative to wtCNP22 (data not shown). The rank order of analog resistance to NEP at 1 ug/ml, based on half-life, is Analog G (3-Cl-Phe)≥Analog E (D-Phe)>Analog H ("beta-2 Phe"), Analog B (N-Me-Phe), and Analog F (t-Bu-Gly)=wtCNP22>Analog A (Cys-CH_2—NH). Analogs E and G have about 1.5 times longer half-life in comparison to wtCNP22. Besides resistance to cleavage of the Cys6-Phe7 bond, Analogs B, E, F, G and H also exhibited resistance to cleavage of the Gly8-Leu9 bond in the presence of 1 ug/mL NEP (data not shown). These results indicate that CNP variants having backbone or side chain modifications between Cys6 and Gly8 can be resistant to NEP cleavage of the Cys6-Phe7 bond and/or Gly8-Leu9 bond, but do not necessarily have improved overall resistance to NEP or a longer half-life than CNP22. The results seem to be contrary to reports in the literature that NEP first cleaves at the Cys6-Phe7 bond of CNP22 and then elsewhere.

TABLE 1

Analog	Backbone and Side Chain Modifications Natriuretic Peptide	cGMP Response rel. to 1 uM CNP22[1] 10 nM	1 uM	NEP Cleavage ($t_{1/2}$, min)
	CNP22 (SEQ ID NO: 1)	46 ± 10	100 ± 13	80[2]
N	D-CNP22 (all D-amino acids) (SEQ ID NO: 115)	2	1	>>160
A	CNP22, C6—CH2—NH (reduced carbonyl) (SEQ ID NO: 56)	6	66	55
B	CNP22, N-methyl-F7 (methylated amide) (SEQ ID NO: 57)	2	38	80
BD	CNP22, N-methyl-L9 (SEQ ID NO: 116)	2	8	ND
BN	CNP22, N-methyl-L11 (SEQ ID NO: 117)	10	51	ND
BE	CNP22, N-methyl-L20 (SEQ ID NO: 118)	2	5	ND
M	CNP22, N-methyl-L9, N-methyl-L11 (SEQ ID NO: 94)	1	11	>>160
K	CNP22, N-methyl-L9, N-methyl-L20 (SEQ ID NO: 92)	1	1	80
L	CNP22, N-methyl-L9, N-methyl-L11, N-methyl-L20 (SEQ ID NO: 93)	18	10	30
J	CNP22, C6—CH2—NH, N-methyl-L9, N-methyl-L20 (SEQ ID NO: 91)	ND	ND	50
E	CNP22, D-F7 (D-Phe) (SEQ ID NO: 136)	2	6	130
H	CNP22, Beta-2-F7 (3-amino-2-phenylpropionyl) (SEQ ID NO: 57)	2	2	80
G	CNP22, 3-chloro-F7 (SEQ ID NO: 137)	17	93	135
F	CNP22, t-butyl-G8 (SEQ ID NO: 58)	2	18	80
V	CNP22, K4G, 3,4-dichloro-F7 (SEQ ID NO: 119)	ND	ND	68
X	CNP22, K4G, 3-methyl-F7 (SEQ ID NO: 120)	ND	ND	68
	ANP	10	23	ND

[1]Stimulation of cGMP production in NIH3T3 cells by natriuretic peptide relative to cGMP production in the presence of 1 uM CNP22

[2]CNP22 NEP resistance $t_{1/2}$ averaged 80 min. Due to variations in NEP catalytic activity between experiments, all CNP22 $t_{1/2}$ digestions were normalized to 80 min and the difference coefficient was used to calculate analog $t_{1/2}$ in each experiment to obtain an adjusted $t_{1/2}$.

ND = Not Determined

Table 2 lists the half-lives, based on the in vitro NEP cleavage assay, of various CNP variants having substitutions with natural and/or unnatural amino acids. Of tested variants, the greatest resistance to NEP cleavage was shown by Analog BK, which has K4R and G15S substitutions, and Analog BJ, which has K4R and G15N substitutions.

TABLE 2

Analog	Specificity Mutations Natriuretic Peptide	cGMP Response relative to 1 uM CNP22[1] 10 nM	1 uM	NEP Cleavage ($t_{1/2}$, min)
	CNP22 (SEQ ID NO: 1)	46 ± 10	100 ± 13	80
AH	CNP22, K4R (SEQ ID NO: 35)	59	121	80
BP	CNP22, K4R, G5S (SEQ ID NO: 121)	45	ND	ND
BO	CNP22, K4R, G5R (SEQ ID NO: 122)	18	80	ND
P	CNP22, K4G (SEQ ID NO: 123)	ND	ND	68
Z	CNP22, K4R, F7Y (SEQ ID NO: 95)	2	18	ND
AB	CNP22, K4R, G8S (SEQ ID NO: 97)	26 ± 26	86 ± 17	ND
AA	CNP22, K4R, G8V (SEQ ID NO: 96)	3	25	ND
AC	CNP22, K4R, G8T (SEQ ID NO: 98)	11 ± 2	66 ± 16	80
AD	CNP22, K4R, L9T (SEQ ID NO: 99)	4	68	ND
BH	CNP22, K4R, K10R (SEQ ID NO: 112)	12	80	ND
BF	CNP22, K4R, K10Cit (SEQ ID NO: 110)	6	33	ND
BG	CNP22, K4R, K10Q (SEQ ID NO: 111)	9	45	ND
BY	CNP22, K4R, K10S (SEQ ID NO: 124)	16	53	ND
BK	CNP22, K4R, G15S (SEQ ID NO: 114)	13 ± 1	71 ± 11	1.60
BJ	CNP22, K4R, G15N (SEQ ID NO: 113)	4	41	150
AE	CNP22, K4R, G15R (SEQ ID NO: 100)	0.3	0.3	ND
AF	CNP22, K4R, G15Cit (SEQ ID NO: 101)	1.4	2	ND
BZ	CNP22, K4R, S16Q (SEQ ID NO: 125)	42	116	ND
BX	CNP22, K4R, M17N (SEQ ID NO: 126)	40 ± 2	103 ± 17	ND
AG	CNP22, K4R, M17V (SEQ ID NO: 102)	10	65	ND
BQ	CNP22, K4R, G19S (SEQ ID NO: 127)	21	63	ND
BR	CNP22, K4R, G19R (SEQ ID NO: 128)	22 ± 6	84 ± 10	ND

TABLE 2-continued

Analog	Specificity Mutations Natriuretic Peptide	cGMP Response relative to 1 uM CNP22[1] 10 nM	1 uM	NEP Cleavage ($t_{1/2}$, min)
AJ	CNP22, K4R, L20V (SEQ ID NO: 103)	0.2	8	ND
AK	CNP22, K4R, L20t-butyl-Ala (SEQ ID NO: 104)	1	21	ND
AT	CNP22, GlE, K4E (SEQ ID NO: 105)	11	54	60
BS	CNP22, K4R, L20R (SEQ ID NO: 129)	11	8	ND
BT	CNP22, K4R, G21S (SEQ ID NO: 130)	7	39	ND
BU	CNP22, K4R, G21T (SEQ ID NO: 131)	6	21	ND

TABLE 2-continued

Analog	Specificity Mutations Natriuretic Peptide	cGMP Response relative to 1 uM CNP22[1] 10 nM	1 uM	NEP Cleavage ($t_{1/2}$, min)
BW	CNP22, K4R, G21R (SEQ ID NO: 132)	20	21	ND
	ANP	10	23	ND

[1]Stimulation of cGMP production in NIH3T3 cells by natriuretic peptide relative to cGMP production in the presence of 1 uM CNP22
[2]CNP22 NEP resistance $t_{1/2}$ averaged 80 min. Due to variations in NEP catalytic activity between experiments, all CNP22 $t_{1/2}$ digestions were normalized to 80 min and the difference coefficient was used to calculate analog $t_{1/2}$ in each experiment to obtain an adjusted $t_{1/2}$.
ND = Not Determined

Table 3 lists the half-lives, based on the in vitro NEP cleavage assay, of CNP variants having N-terminal and/or C-terminal modifications, including amino acid extensions. Of the analogs tested, Analogs AZ, CC, CF, BL, CS, CK and CL, Pro-Gly-CNP27 and HSA-CNP27 were most resistant to NEP degradation.

TABLE 3

	N- and C-Terminal Modifications Natriuretic Peptide	cGMP Response rel. to 1 uM CNP22[1] 10 nM	1 uM	NEP Cleavage ($t_{1/2}$, min)
	CNP22	46 ± 10	100 ± 13	80
BC	Pentanoic acid (N-term.)-CNP22, G1E (SEQ ID NO: 109)	ND	ND	ND
BB	Heptanoic acid (N-term.)-CNP22, G1E (SEQ ID NO: 108)	32 ± 4	84 ± 19	45-65
AV	Pentanoic acid (N-term.)-CNP22, G1E, K4E (SEQ ID NO: 106)	ND	ND	120
AW	Heptanoic acid (N-term.)-CNP22, G1E, K4E (SEQ ID NO: 107)	ND	ND	<20
AX	CNP17 (delta N-term) (SEQ ID NO: 2)	18	69	ND
	R-CNP22 (SEQ ID NO: 40)	ND	ND	ND
AZ	R-CNP22, K4R (SEQ ID NO: 41)	54 ± 11	106 ± 15	≥160
	ER-CNP22 (SEQ ID NO: 38)	ND	ND	ND
BA	ER-CNP22, K4R (SEQ ID NO: 39)	38 ± 10	113 ± 10	90
	GANRR-CNP22 (SEQ ID NO: 65)	ND	ND	ND
AY	GANRR-CNP22, K4R (SEQ ID NO: 36)	59 ± 9	105 ± 20	65
	GANQQ-CNP22 (SEQ ID NO: 64)	ND	ND	ND
CH	GANQQ-CNP22, K4R (SEQ ID NO: 69)	44 ± 8	95 ± 11	ND
	GANPR-CNP22 (SEQ ID NO: 66)	ND	ND	ND
CI	GANPR-CNP22, K4R (SEQ ID NO: 37)	50 ± 1	105 ± 12	ND
	GANSS-CNP22 (SEQ ID NO: 67)	ND	ND	ND
CG	GANSS-CNP22, K4R (SEQ ID NO: 70)	27 ± 1	88 ± 1	95
CA	AAWARLLQEHPNA-CNP22 (SEQ ID NO: 61)	24	76	ND
CB	AAWARLLQEHPNAR-CNP22 (SEQ ID NO: 62)	36	84	ND
CC	DLRVDTKSRAAWAR-CNP22 (SEQ ID NO: 63)	34	101	>160
CF	GQPREPQVYTLPPS-CNP22 (IgG1(Fc) fragment) (SEQ ID NO: 79)	23 ± 9	72 ± 19	>160
	PNARKYKGANKK-CNP22 (CNP34)	ND	ND	ND
BL	QEHPNARKYKGANKK-CNP22 (CNP37) (SEQ ID NO: 60)	43 ± 15	97 ± 27	>>160
	PQEHPNARKYKGANKK-CNP22 (Pro-CNP37)	ND	ND	ND
CE	GERAFKAWAVARLSQ-CNP22 (IISA fragment) (SEQ ID NO: 81)	15	87	ND
CY	GQHKDDNPNLPRGANPR-CNP22 (HSA fragment) (SEQ ID NO: 80)	ND	ND	ND
CQ	GHHSHEQHPHGANQQ-CNP22 (HRGP fragment) (SEQ ID NO: 76)	16	95	ND
CX	GHHSHEQHPHGANPR-CNP22 (HRGP fragment) (SEQ ID NO: 78)	ND	ND	ND
CS	GQEHPNARKYKGANPK-CNP22 (modified CNP37) (SEQ ID NO: 129)	19	61	>>160
CT	GQEHPNARKYKGANQK-CNP22 (modified CNP37) (SEQ ID NO: 130)	60	121	ND
CU	GQEHPNARKYKGANQQ-CNP22 (modified CNP37) (SEQ ID NO: 131)	9	57	ND
DB	GQEHPNARKYKGANKK-CNP22 (Gly-CNP37) (SEQ ID NO: 75)	50 ± 14	98 ± 17	>>160
	PGQEHPNARKYKGANKK-CNP22 (Pro-Gly-CNP37)	49 ± 6	103 ± 17	>>160
CW	GQEHPNARKYKGANKP-CNP22 (modified CNP37) (SEQ ID NO: 74)	ND	ND	ND

TABLE 3-continued

	N- and C-Terminal Modifications	cGMP Response rel. to 1 uM CNP22[1]		NEP Cleavage
	Natriuretic Peptide	10 nM	1 uM	($t_{1/2}$, min)
CR	GA1-IHPHEHDTHGANQQ-CNP22 (HRGP fragment) (SEQ ID NO: 128)	14 ± 5	77 ± 12	ND
CZ	FGIPMDRIGRNPR-CNP22 (osteocrin "NPR-C inhibitor") (SEQ ID NO: 82)	ND	ND	ND
DA	GKRTGQYKLGSKTGPGPK-CNP22 (FGF2 "heparin-binding domain" fragment) (SEQ ID NO: 83)	ND	ND	ND
CK	GQPREPQVYTGANQQ-CNP22, K4R (IgG1(Fc) fragment) (SEQ ID NO: 84)	2	32	≥160
CL	GVPQVSTSTGANQQ-CNP22, K4R (HSA fragment) (SEQ ID NO: 85)	3	35	>160
	GHKSEVAHRFKGANKK-CNP22 (HSA-CNP27) (SEQ ID NO: 144)	51 ± 9	109 ± 15	>>160
	PGHKSEVAHRFKGANKK-CNP22 (Pro-HSA-CNP27)	32	107	ND
CN	GQTHSSGTQSGANQQ-CNP22, K4R (fibrinogen) (SEQ ID NO: 87)	12	115	ND
CM	GQPSSSSQSTGANQQ-CNP22, K4R (fibronectin) (SEQ ID NO: 86)	ND	ND	ND
CO	GSTGQWHSESGANQQ-CNP22, K4R (fibrinogen) (SEQ ID NO: 88)	2	33	ND
CP	GSSSSSSSSSGANQQ-CNP22, K4R (zinc finger) (SEQ ID NO: 89)	ND	ND	ND
CD	SPKMVQGSG-CNP17-KVLRRH ("BNP tails") (SEQ ID NO: 68)	25	102	ND
CJ	RSSCFGGRIDRIGAC ("C-ANP4-23", ANP-derived) (SEQ ID NO: 133)	ND	ND	ND
	CNP22, K4R, K10R, N.-term.--N-term. dimer/ disuccinimidyl glutarate (SEQ ID NO: 134)	19	44	ND
	CNP22, K4R, K10R, N-term.--N.-term. dimer/Bis-PEO5 (SEQ ID NO: 135)	19	41	ND
BM	CNP53 (SEQ ID NO: 4)	61	101	>>160
	ANP	10	23	ND

[1]Stimulation of cGMP production in N1H3T3 cells by natriuretic peptide relative to cGMP production in the presence of 1 uM CNP22
[2]CNP22 NEP resistance $t_{1/2}$ averaged 80 min. Due to variations in NEP catalytic activity between experiments, all CNP22 $t_{1/2}$ digestions were normalized to 80 min and the difference coefficient was used to calculate analog $t_{1/2}$ in each experiment to obtain an adjusted $t_{1/2}$.
ND = Not Determined

Table 4 lists the half-lives, based on the in vitro NEP cleavage assay, of CNP variants conjugated to PEG (or PEO) polymers at the N-terminus. All the PEGylated CNP variants tested and shown in Table 4 displayed resistance or enhanced resistance to NEP cleavage except for PEO12-GANPR-CNP22(K4R), which had the same half-life as wtCNP22. N-terminal PEGylation of CNP22 having a K4G substitution does not seem to confer substantial improvement in NEP resistance. For example, PEG2K-CNP22 (K4G) was only slightly more resistant to NEP cleavage than CNP22 (data not shown), whereas PEG2K-CNP22 had a much longer half-life in vitro than CNP22.

TABLE 4

N-Terminal PEGylation		cGMP Response rel. to 1 uM CNP22[1]		NEP Cleavage
Natriuretic Peptide	PEG	10 nM	1 uM	($t_{1/2}$, min)
CNP22		46 ± 10	100 ± 13	80
CNP22	PEG20K	0	15	>>160
CNP22	PEG5K	8 ± 1	20 ± 7	>>160
CNP22	PEG2K	6 ± 2	32 ± 4	>>160
CNP22	PEO4-$(PEO12)_3$ (branched)	17 ± 1	52 ± 6	>>160
CNP22	PEO24 (1.2 kDa)	8 ± 5	46 ± 10	>>160
CNP22	PEG1K	15 ± 3	68 ± 17	>160
CNP22	PEO12 (0.6 kDa)	12 ± 7	57 ± 18	160
CNP22	(PEO12)-Biotin	19	81	140

TABLE 4-continued

N-Terminal PEGylation		cGMP Response rel. to 1 uM CNP22[1]		NEP Cleavage
Natriuretic Peptide	PEG	10 nM	1 uM	($t_{1/2}$, min)
CNP22, K4G	(PEO12)-Biotin	10	27	100
CNP22, K4R	PEO24	15	56	ND
CNP22, K4R	PEO12	13	44	ND
CNP-17	PEG2K	5	50	>160
R-CNP22, K4R (SEQ ID NO: 41)	PEO24	15 ± 2	75 ± 12	ND
R-CNP22, K4R (SEQ ID NO: 41)	PEO12	23 ± 2	93 ± 19	≥60
ER-CNP22, K4R (SEQ ID NO: 39)	PEO24	6 ± 2	60 ± 10	ND
ER-CNP22, K4R (SEQ ID NO: 39)	PEO12	20 ± 1	92 ± 25	ND
GANRR-CNP22, K4R (SEQ ID NO: 36)	PEG2K	15 ± 2	45 ± 18	ND
GANRR-CNP22, K4R (SEQ ID NO: 36)	PEO24	28 ± 9	82 ± 18	>>160
GANRR-CNP22, K4R (SEQ ID NO: 36)	PEG1K	15 ± 0.4	56 ± 23	>160
GANRR-CNP22, K4R (SEQ ID NO: 36)	PEO12	40 ± 2	99 ± 13	>160
GANQQ-CNP22, K4R (SEQ ID NO: 69)	PEO24	16 ± 13	73 ± 30	ND
GANQQ-CNP22, K4R (SEQ ID NO: 69)	PEO12	30	78	ND
GANPR-CNP22, K4R (SEQ ID NO: 37)	PEO24	ND	ND	ND
GANPR-CNP22, K4R (SEQ ID NO: 37)	PEO12	ND	ND	80

TABLE 4-continued

N-Terminal PEGylation		cGMP Response rel. to 1 uM CNP22[1]		NEP Cleavage
Natriuretic Peptide	PEG	10 nM	1 uM	($t_{1/2}$, min)
GANSS-CNP22, K4R (SEQ ID NO: 70)	PEO24	8 ± 5	46 ± 21	ND
GANSS-CNP22, K4R (SEQ ID NO: 70)	PEO12	8 ± 0.3	52 ± 13	ND

[1]Stimulation of cGMP production in NIH3T3 cells by natriuretic peptide relative to cGMP production in the presence of 1 uM CNP22
[2]CNP22 NEP resistance $t_{1/2}$ averaged 80 min. Due to variations in NEP catalytic activity between experiments, all CNP22 $t_{1/2}$ digestions were normalized to 80 min and the difference coefficient was used to calculate analog $t_{1/2}$ in each experiment to obtain an adjusted $t_{1/2}$.
ND = Not Determined

FIG. **16** shows the NEP resistance profile of five N-terminal PEGylated conjugates of CNP22. The CNP22 peptides conjugated to PEG (or PEO) polymers of increasing mass exhibited increasing resistance to NEP degradation. In particular, PEO24-CNP22, PEG2K-CNP22 and PEG5K-CNP22 were resistant to NEP degradation over the assay period of 160 minutes.

FIG. **17** displays the NEP resistance profile of CNP variants CNP37 (Analog BL), CNP53 and GANRR-CNP22 (K4R) (SEQ ID NO: 36) having an N-terminal amino acid extension. As can be clearly seen, both CNP37 and CNP53 were resistant to NEP degradation in this in vitro assay, whereas GANRR-CNP22(K4R) (SEQ ID NO: 36) had the same lability to NEP hydrolysis as CNP22.

FIG. **18** depicts the NEP resistance profile of CNP17 and GANRR-CNP22(K4R) (SEQ ID NO: 36) conjugated to a PEG (or PEO) moiety at the N-terminus. PEGylation of GANRR-CNP22(K4R) (SEQ ID NO: 36) greatly improved the NEP resistance of this CNP variant, with PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) being completely resistant to NEP cleavage over the assay period of 160 minutes. Increasing the mass of the PEO moiety from about 0.6 kDa (PEO12) to about 1.2 kDa (PEO24) improved the NEP resistance of PEGylated GANRR-CNP22(K4R) (SEQ ID NO: 36). PEGylation of GANRR-CNP22(K4R) (SEQ ID NO: 36) to a monodispersed PEO24 moiety rather than a polydispersed PEG1K moiety also improved NEP resistance. Finally, although both PEO24-GANRR-CNP22 (K4R) (SEQ ID NO: 36) and PEG2K-CNP17 have a similar total mass (keeping in mind that PEG2K is polydispersed), the former displayed substantially better NEP resistance.

NEP resistance assays were also performed on wtCNP22 and CNP variants G-CNP37, GHKSEVAHRFK-wtCNP27 ("CNP27-HSA", SEQ ID NO: 144) and PEO12-GANRR-CNP22(K4R) ("CNP27-PEO12") (SEQ ID NO: 36). FIG. **19** shows that G-CNP37 and CNP27-HSA were completely resistant to NEP cleavage, and CNP27-PEO12 exhibited much greater stability to NEP degradation compared to wtCNP22.

Example 4

CNP Variant Stimulation of cGMP Production in NIH3T3 Cells

To determine the functional activity of CNP variants, the production of cGMP was measured in NIH3T3 cells exposed to the CNP variants. Murine NIH3T3 cells express endogenously the CNP signaling receptor, NPR-B, which shares 98% protein sequence identity with human NPR-B. NIH3T3 cells were cultured in high glucose Dulbecco's Modified Eagle Medium supplemented with 10% fetal calf serum and antibiotics at 37° C. with 5% CO). Twenty four to 48 hours prior to signaling, cells were passaged to 12-well plates with a density of 2-5x10^5 cells per well at the time of the assay. CNP variants were resuspended in 1 mM HCl to a stock concentration of 1 mg/mL (455 uM for wtCNP22) and subsequently diluted to a 30 uM working stock solution with phosphate-buffered saline (PBS). Ten-fold serial dilutions were prepared in phosphate-buffered saline. Culture medium was removed from the cells and replaced with 0.4 mL PBS/Dulbecco's modified Eagle medium (50/50, v/v) containing 0.75 mM isobutylmethylxanine. Plates were incubated at 37° C., 5% CO_2 for 15 minutes before addition of 0.2 mL CNP variant in PBS and continued incubation at 37° C. for 15 minutes. Reactions were stopped by the addition of 0.2 mL lysis buffer supplied with the CatchPoint cGMP assay kit (Molecular Devices), and cGMP production was determined with the CatchPoint cGMP Assay (Molecular Devices). All stimulation experiments were performed in duplicate.

Tables 1-4 summarize the ability of CNP variants having backbone or side chain modifications, amino acid substitutions, N-terminal amino acid extensions, and/or N-terminal PEGylation, respectively, to stimulate cGMP production in NIH3T3 cells. In all four tables, the values for cGMP production in NIH3T3 cells exposed to 10 nM or 1 uM CNP variant are normalized to cell number and cGMP production in the presence of 1 uM wtCNP22.

Regarding the results in Table 1, only Analog G having 3-Cl-Phe at position 7 displayed substantially the same NPR-B stimulation activity at 1 uM as wtCNP22. With respect to Table 2, various CNP variants with amino acid substitutions, including Analogs AH, BO, AB, BH, BZ, BX and BR, showed substantially similar NPR-B stimulation activity as wtCNP22.

Considering the results in Table 3, many CNP variants having N-terminal and/or C-terminal modifications, including amino acid extensions, exhibited comparable NPR-B stimulation activity as wtCNP22. The functional CNP variants include Analog BB, which is CNP22(G1E) attached to heptanoic acid at the N-terminus, and Analog CD, which is the cyclic domain of CNP22 ("CNP17" retaining the Cys6 to Cys22 sequence) conjugated to the N-terminal and C-terminal "tails" of BNP. FIG. **20** illustrates that GANRR-CNP22(K4R) (SEQ ID NO: 36), CNP37 (Analog BL) and CNP53 all had similar NPR-B stimulation activity as wtCNP22 in the in vitro assay.

Of note from Table 3 is that among the CNP variants assayed for both CNP functionality and NEP resistance, Analog AZ (R-CNP22(K4R)), Analog CC, Analog CF, Analog BL (CNP37), Analog DB (Gly-CNP37) and GHKSEVAHRFK-CNP27 (HSA-CNP27) (SEQ ID NO: 144) all had substantially similar NPR-B stimulation activity as CNP22 while being substantially more resistant to NEP cleavage than CNP22.

With regard to the results in Table 4, nine N-terminal PEGylated CNP variants at 1 uM stimulated cGMP production to at least about 70% of the level achieved by wtCNP22. Several noteworthy aspects appear in Table 4. First, N-terminal PEGylation of GANRR-CNP22(K4R) (SEQ ID NO: 36) with a monodispersed PEO polymer (PEO12 is about 0.6 kDa, PEO24 about 1.2 kDa) resulted in better NPR-B functionality than that with a polydispersed PEG polymer (PEG1K has a polymer number average molecular weight (M_n) of around 1 kDa, PEG2K around 2 kDa) (see also FIG. **21**). Second, N-terminal PEGylation of wtCNP22 with a polydispersed PEG polymer of increasing M_n (PEG1K, PEG2K, PEG5K and PEG20K) or with a monodispersed PEO polymer of greater mass (PEO12 and PEO24) correspondingly decreased the NPR-B activation ability of the CNP variants (see also FIG. **22**). Third, PEO24-GANRR-

CNP22(K4R) (SEQ ID NO: 36), having the N-terminal GANRR (SEQ ID NO: 8) extension, stimulated greater cGMP production than PEO24-CNP22 and PEO24-CNP22 (K4R). Also of note is that among the N-terminal PEGylated CNP variants assayed for both CNP functionality and NEP resistance, PEO12-R-CNP22(K4R), PEO12-GANRR-CNP22(K4R) (SEQ ID NO: 36) and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) all had substantially similar NPR-B stimulation activity as CNP22 while being much more resistant to NEP degradation than CNP22.

Among the CNP variants listed in Tables 1-4 and assayed for both CNP functionality and NEP resistance, Analogs G, BK, AZ, CC, CF, BL and DB, Pro-Gly-CNP37, HSA-CNP27 (GHKSEVAHRFK-CNP27) (SEQ ID NO: 144), PEG1K-CNP22, (PEO12)-biotin-CNP22, PEO12-R-CNP22 (K4R), PEO12-GANRR-CNP22(K4R) (SEQ ID NO: 36), and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) all had substantially similar NPR-B stimulation activity as wtCNP22 while being substantially more resistant to NEP cleavage than wtCNP22.

cGMP production assays were also carried out on wtCNP22 and CNP variants G-CNP37, GHKSEVAHRFK-wtCNP27 ("CNP27-HSA", SEQ ID NO: 144), wtCNP29 and PEO12-GANRR-CNP22(K4R) ("CNP27-PEO12") (SEQ ID NO: 36). FIG. **23** shows that CNP22 and all the CNP variants assayed induced production of similar cGMP levels at either low or high dose of CNP.

Example 5

Binding Specificity for NPR-A, NPR-B and NPR-C

Signaling Competition Assay

To determine the binding specificity of CNP variants for the clearance receptor NPR-C, a signaling competition assay is carried out. Expression plasmids for human NPR-B or NPR-C (each purchased from OriGene) are transiently transfected and receptors are expressed in HEK293T cells. Forty hours after transfection, NPR-B, NPR-C and native HEK293T cells are harvested, counted and plated at a ratio of 1:1 (NPR-B cells:competing cells (either NPR-C or native HEK293T cells)) in 12-well or 96-well plates. Twenty hours after plating, cells are processed for the NPR-B/cGMP stimulation assay described in Example 4. If present, the natriuretic clearance receptor, NPR-C, is expected to bind and internalize CNP, thereby reducing the overall CNP concentration available for signaling through NPR-B, resulting in decreased cGMP production and a shift in the dose-response curve to the right. A rightward shift in the dose-response curve has been verified for wtCNP22. CNP variants having reduced affinity for NPR-C are not expected to induce a shift, or are expected to induce a smaller shift, in the dose-response curve to the right. This signaling competition assay is similar to that previously described by Cunningham (U.S. Pat. No. 5,846,932; B. Cunningham, EMBO J. 13(11): 2508-2515 (1994); H. Jin et al., J. Clin. Invest. 98(4): 969-976 (1996)).

The cGMP stimulation activity of wtCNP-22, Pro-Gly-wtCNP37 and ANP through NPR-B and NPR-A, and their selectivity for NPR-B vs. NPR-C and for NPR-A vs. NPR-C, were evaluated in signaling competition assays. NPR-A, NPR-B and NPR-C individually were transiently tranfected into HEK293T cells. Thirty hours after transfection, the cells were plated into 96-well plates: (A) 20,000 NPR-B cells+20,000 mock transfected cells; (B) 20,000 NPR-B cells+20,000 NPR-C cells; (C) 20,000 NPR-A cells+20,000 mock transfected cells; and (D) 20,000 NPR-A cells+20,000 NPR-C cells. Twenty hours after plating, the culture media was removed and replaced with serum-free media:PBS (1:1)+0.75 uM IBMX for 15 minutes. For cGMP signaling through NPR-B, dose series for ANP, CNP-22 and Pro-Gly-CNP37 were added and incubated at 37° C. for 12 minutes before the assay was stopped by cell lysis. For cGMP signaling through NPR-A, dose series for CNP-22 and Pro-Gly-CNP37 were incubated at 37° C. for 12 minutes, while dose series for ANP were incubated at 37° C. for 6 minutes (because NPR-A appeared to be a "faster" guanylyl cyclase than NPR-B, the incubation time for ANP was shortened in order not to max out (use up all cellular GTP) too soon when signaling with ANP). FIGS. **24**A and B show that CNP-22 and Pro-Gly-CNP37 ("Pro-CNP38") stimulated cGMP production through NPR-B with similar dose-response curves, and to a much greater extent than through NPR-A, and exhibited a similar profile for NPR-B vs. NPR-C selectivity in the signaling competition assays.

Determination of Binding Affinities (K_i) for NPR-A, NPR-B and NPR-C

The binding affinities (K_i) of CNP variants for NPR-A, NPR-B and NPR-C are determined in a heterologous competition binding assay (U.S. Pat. No. 5,846,932; B. Cunningham, EMBO J. 13(11): 2508-2515 (1994); H. Jin et al., J. Clin. Invest. 98(4): 969-976 (1996)). Membranes from HEK293 cells, or another suitably transfectable cell line (e.g., HeLa cells), expressing human NPR-A, NPR-B or NPR-C are prepared for radio-labeled ligand binding assays. Membrane preparations are diluted in an appropriate buffer and varying concentrations of wtCNP22 or CNP variant (competitor) are added with I^{125}-labeled wtCNP22 (Bachem). Samples are incubated at room temperature to allow for ligand/receptor equilibration and bound peptide is separated from free peptide by filtration through PVDF filter membranes. Filters are washed before the addition of scintillant and counting by a scintillation counter. Binding is measured in duplicate for each concentration of competitor peptide. CNP variant affinity (K_i, equilibrium dissociation constant) and B_{max}, (receptor number) are calculated by non-linear regression analysis and/or the Cheng-Prusoff equation.

CNP variants exhibiting reduced affinity to NPR-C are expected to have reduced susceptibility to clearance by NPR-C and thus a longer plasma or serum half-life. Increased half-life of the CNP variants in circulation would increase the availability of the variants for therapeutic activity.

Example 6

Effect of CNP Variants on the Growth of Rat Chondrosarcoma (RCS) Cells and cGMP Production in RCS Cells

To assess the ability of CNP variants to stimulate bone growth, skeletal dysplasia is simulated in cell culture by treating rat chondrosarcoma (RCS) cells with fibroblast growth factor 2 (FGF-2), which activates fibroblast growth factor receptor 3 (FGFR-3) and indnces growth arrest (Krejci et al., J. Cell Sci., 118(21):5089-5100 (2005)).

Optimal CNP treatment parameters are determined by varying CNP concentration (0.05, 0.1, 0.2 and 0.5 uM), and treatment duration and interval (continuous; 2.5 min, 10 min, 30 min, 1 hr, 2 hr, 4 hr and 8 hr once a day; 2.5 min, 10 min, 30 min, 1 hr, 2 hr and 4 hr twice a day). After 72 hours, cells are counted using an automated cell counter, and the amount of extracellular matrix is estimated using alcian blue staining.

RCS cells are then treated with a CNP variant using the optimal conditions determined from the growth experiments with wtCNP22. The concentration of cGMP is measured by competitive ELISA for nntreated RCS cells, RCS cells treated with CNP, and RCS cells treated with the CNP

variant. Cell growth and matrix synthesis resulting from treatment with the CNP variant are also monitored and compared to those resulting from CNP treatment.

To assess the effect of CNP variants in a human cell culture system, primary re-differentiated human chondrocytes in alignate beads are treated with wtCNP22 and CNP variants, and cGMP concentration is determined by competitive ELISA as a measure of effective CNP signaling.

The methods described herein can be employed to assess the ability of CNP variants to stimulate cGMP production in and growth of rat chondrosarcoma cells in vitro.

Example 7

Dose Response Study in Rat Chondrosarcoma Cells

The tyrosine kinase receptor fibroblast growth factor receptor 3 (FGFR-3), a negative regulator of chondrocyte growth, is contitutively on in achondroplasia subjects. Stimulation of the FGFR-3 receptor with FGF-2 causes growth arrest by prolonged activation of Erk MAPK, and causes decreased matrix synthesis and loss of matrix, as well as a change in cell shape. Continuous exposure of rat chondrosarcoma (RCS) cells to fibroblast growth factor 2 (FGF-2) simulates achondroplasia in cell culture by activating FGFR-3 and inducing growth arrest (Krejci et al., J. Cell Sci., 118(21): 5089-5100 (2005)). To determine the dose of CNP variant and frequency of dosing that stimulate sufficient growth of bone cells, a dose response study was performed using the RCS cell assay as described in Example 6.

RCS cells were seeded at 10×10^3 cells per well in 24-well plates, grown for 24 hr, treated for 72 hr, and then counted. RCS cells were continuously exposed to FGF-2 (5 ng/mL) to simulate a constitutively active FGFR-3, which induced cell growth arrest (see bar #5 in FIG. **25**). Wild-type CNP22 (0.2 uM) was cultured continuously (72 hr), 1 hr daily or 2 hr daily. All stimulants were changed daily Continuous exposure of RCS cells to 0.2 uM CNP22 in the presence of 5.0 ng/mL FGF-2 partially reversed FGF2-induced growth arrest, leading to the growth of approximately 200×10^3 cells per well (bar #6 in FIG. **25**), compared to approximately 100×10^3 cells per well in the absence of CNP22 (bar #5 in FIG. **25**).

Both 1 hr exposure to CNP22 (0.2 uM) once a day and 2 hr exposure to CNP22 (0.2 uM) once a day achieved about 84% of the effect of continuous CNP22 (0.2 uM) exposure on chondrocyte growth (bars #7 and 8 in FIG. **25**). These results demonstrate that continuous exposure of growth-arrested chondrocytes to CNP22 is not required for reversal of cell growth arrest. Additionally, dose response studies demonstrate that lower doses of CNP22 are capable of reversing growth arrest (FIG. **26**A).

Furthermore, histological and cell morphological analysis of the extracellular matrix showed that CNP22 treatment antagonized FGF2-mediated loss of chondrosarcoma extracellular matrix and increased matrix synthesis. Exposure to FGF-2 decreased matrix synthesis and increased degradation, while addition of CNP22 to the FGF-2 cell culture increased matrix synthesis and partially inhibited FGF-2, as assessed by ^{35}S-sulfate and ^{3}H-Pro incorporation into, or decrease from, matrix (FIGS. **27**A-D). Analysis of aggrecan and fibronectin production (mRNA and protein) in RCS cells cultured with FGF-2 and CNP22 shows that FGF-2 decreased aggrecan level and increased fibronectin level, which was inhibited by addition of CNP22 (FIGS. **28**A-C). FGF-2 induces and activates matrix-processing molecules predominantly via Erk, and addition of CNP22 shows some effect on this activation.

Additional high-throughput assays for measuring growth arrest, such as crystal violet staining, are useful for measuring the effects of CNP22 and variants thereof on RCS cells.

Similar dose response studies can be conducted with the CNP variants described herein to determine their effective dose for reversing FGF2-induced growth arrest of RCS cells.

Example 8A

Ex Vivo Stimulation of Growth of Tibia and Femur from Mice with Mild Achondroplasia

Mouse tibial organ culture model has been used to demonstrate the efficacy of wild-type CNP22 in stimulating longitudinal bone growth. Treatment of wild-type tibiae with CNP22 at 10^{-8}, 10^{-7} or 10^{-6} M for 6 days increased longitudinal growth by 31%, 40% and 42%, respectively. Histological evaluation also showed expansion of the hypertrophic zone, e.g., an increase in the number and size of hypertrophic chondrocytes in the growth plate (Agoston et al., BMC Dev. Biol. 7:18 (2007)). Similar findings were observed in tibiae isolated from $FGFR3^{Ach}$ mice (Yasoda et al., Nat. Med. 10: 80-86 (2004)).

To determine the efficacy of CNP variants in stimulating longitudinal bone growth, CNP variants were tested in a mouse organ culture model of endochondral bone growth in wild-type mice and transgenic mice having a G380R mutation in the human FGFR-3 gene ($FGFR3^{wt/Ach}$ heterozygote) which represent a mouse model of mild achondroplasia. In brief, the pharmacological activity of wild-type CNP22 and CNP variants was compared in an organ culture model of embryonic or neonatal mouse tibiae, isolated from wild-type and $FGFR3^{wt/Ach}$ littermates. Overall hone growth and histological changes within the growth plate were assessed. Conditioned culture medium is also assessed for biomarkers of intracellular signaling (cGMP), cartilage metabolism (type II collagen, other collagens, aggrecan chondroitin sulfate), bone metabolism (bone alkaline phosphatase, osteocalcin, type I collagen [C-telopeptide, N-telopeptide]), and inflammation (interleukin-1, interleukin 6, interleukin-11).

Effective CNP variants are identified by their ability, e.g., to stimulate production of cGMP, and bone growth as measured by increased longitudinal bone length and expansion of the cells in the hypertrophic zone of the growth plate.

Measurement of Bone Growth

The efficacy of wtCNP22, CNP37 and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) in stimulating longitudinal femoral growth was evaluated in the mouse organ culture model. For these experiments femora were isolated from 2-3 day old wild-type mice and cultured in alphaMEM supplemented with 0.2% BSA, 0.5 mM L-glutamine, 40 units penicillin/mL and 40 ug streptomycin/mL, for 8 days in the presence of vehicle, CNP22 or CNP variants. The treatment commenced at day 0 and was repeated every two days thereafter, as the medium was changed. Bones were measured prior to treatment and every two days thereafter, using a dissection microscope fitted with a 1 cm eye-piece reticule. Conditioned medium was used for biomarker analysis. At day 8 bones were fixed in 4% paraformaldehyde for 24 hr, decalcified in 5% formic acid for 24 hrs, dehydrated and embedded in paraffin. Bones were sectioned at 5 um (microns), which were then deparaffinized, rehydrated, and stained with Alcian Blue for 30 min (pH 2.5; MasterTech). Alcian Blue stains cartilage blue. Stained sections were visualized and photographed by brightfield microscopy. The thickness of the hypertrophic region of the growth plate cartilage was determined by image analysis.

FIG. **29** illustrates the effect of wtCNP22, CNP37 and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) on longitudinal growth of 3-day old wild-type mouse femurs treated with the CNP peptides every two days. The results were normalized to measurements prior to treatment (day 0). The studies were performed in triplicate (vehicle) or quadruplicate (CNP peptides). As shown in FIG. **29**, CNP37 and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36), as well as CNP22, were effective in stimulating longitudinal femoral growth, with the N-terminal PEGylated CNP variant being the most effective.

The growth of wild-type and FGFR3^{ach} mouse femur and tibia in response to CNP22, CNP37 and PEO24-GANRR-CNP22(K4R) ("CNP27-PEO24") (SEQ ID NO: 36) was also assessed. Culture of either wild-type or achondroplastic (FGFR3^{ach}) mouse tibia showed that CNP37 and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) both increased the longitudinal growth of the tibia compared to vehicle or CNP22 (FIGS. **30** and **31**). CNP22, CNP37 and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) also stimulated the growth of wild-type mouse femur (FIG. **32**). Moreover, each of CNP22, PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) and CNP37 increased the longitudinal growth of FGFR3^{ach} mouse femur compared to vehicle (FIG. **33**).

Furthermore, ex vivo distribution of CNP37 in the growth plate of FGFR3^{ach} mouse tibia was evaluated. Bone samples were prepared as described above. Paraffin sections were cut and heat fixed for 1 hour at 60° C. Antigen retrieval with 1% hyaluronidase at 37° C. (30 minutes) was followed by a 1 hour serum block (10% Normal Goat Serum). CNP22 antibody (1:500 dilution; Peninsula Laboratories Inc., San Carlos, Calif.) was applied overnight at 4° C. For immunodetection, Vectastain ELITE ABC kit (Vector, Burlingame, Calif.) was used according to the manufacturer's recommendations. Specific bound peroxidase was visualized by incubation with DAB substrate kit (Vector) and the reaction was developed for 3 minutes. Slides were then dehydrated and mounted and photographed using a brightfield microscope. Staining for CNP in the growth plate of FGFR3^{ach} mouse tibia showed that CNP immunoreactivity was increased in the regions of articular and hypertrophic chondrocytes (FIG. **34**), indicating that CNP37 was delivered to the chondrocytes.

In addition to the distribution of CNP variants in the bone growth plate, the ex vivo effects of CNP37, CNP22 and vehicle on cells in the FGFR3^{ach} and wild-type growth plate, e.g., hypertrophic cell size and cellularity of proliferating zone, were also evaluated. Bone samples were prepared, including Alcian Blue staining, as described above. Images of the entire proximal growth plate were taken at 4× magnitude. The growth plate is divided into three zones, starting from the epiphyseal side of cartilage: the resting zone (individual small chondrocytes), proliferating zone (columns of stacked chondrocytes parallel to the long axis of the bone), and hypertrophic zone (large chondrocytes and thin septa between the chondrocytes). In these regions, measurements were made by ImageJ software, including the number of proliferating chondrocytes per column and the density of hypertrophic chondrocytes. A test square (4×4 mm^2) at five different regions of the hypertrophic zone was used to determine the density of hypertrophic chondrocytes. The cell size of hypertrophic chondrocytes was calculated by 1 over determined cell density. Cellularity of proliferating columns was increased by CNP37 and CNP22 in both wild-type and FGFR3^{ach} mice (FIGS. **35**B and C). Chondrocyte hypertrophy in FGFR3^{ach} mice was also increased as a result of culture with CNP22 or CNP37 (FIGS. **36**B and C).

Ex vivo studies of cultures of mouse bones indicated that CNP37 was delivered to the growth plate and was able to increase chondrocyte cellularity and hypertrophy, which are associated with growth plate expansion and longitudinal bone growth. To assess the biodistribution of CNP37 in the bone growth plate in vivo and the in vivo effects of CNP37 on the growth plate (including total growth plate thickness, hypertrophic zone thickness, and cellularity of proliferating zone), bone samples were obtained from FGFR3^{ach} mice treated with vehicle or CNP37 as described above. For biodistribution and in vivo effects studies, tibias were fixed and stored in 70% ethanol. For immunohistochemistry, the samples were decalcified in 5% formic acid for 2 days, dehydrated and embedded in paraffin. Bones were sectioned at 5 um (microns), which were then deparaffinized, rehydrated, and used for CNP immunohistochemistry as described above. For cellular image analysis, bones were sectioned at 5 (microns), and then deparaffinized, rehydrated, and stained with Alcian Blue for 30 minutes (pH 2.5; MasterTech) and Hematoxylin & Eosin for 30 seconds. Stained sections were visualized and photographed by brightfield microscopy. Thicknesses of the growth plate and the proliferating and hypertrophic zones were measured using ImageJ software.

In vivo biodistribution studies demonstrated that, similar to the ex vivo studies, CNP immunoreactivity was increased in the regions of articular and hypertrophic chondrocytes in the tibia growth plate of FGFR3^{ach} mice treated with CNP37, indicating that CNP37 was delivered in vivo to the growth plate of FGFR3^{ach} mouse tibia (FIG. **37**). Furthermore, CNP37 treatment significantly increased the total growth plate thickness, proliferating zone thickness and hypertrophic zone thickness of FGFR3^{ach} mouse tibia in vivo (FIGS. **38**A-C).

These results demonstrate that CNP variants of the disclosure penetrate into the growth plate of wild-type and achondroplastic animals, increase the number and size of chondrocytes, increase the thickness of the proliferating zone and the hypertrophic zone of the growth plate, and increase longitudinal bone growth in treated wild-type and achondroplasic animals. Therefore, the CNP variants are useful for stimulating bone growth in achondroplastic subjects.

Measurement of Biomarkers

In addition to measurement of bone growth in response to CNP variants, assay of the levels of biomarkers for cartilage and bone formation and growth induced in response to CNP variants is useful for evaluating the effect of CNP variants on bone growth.

Femurs and tibias were isolated from wild-type and FGFR3^{ach} mice as described above. Bones were cultured with CNP22 or a variant thereof for eight days with the replacement of media every two days. On the eighth day, media was collected and analyzed for the biomarkers cGMP (cyclic guanosine 3', 5' cyclic monophosphate) and fragments of cleaved collagen type II, a cartilage-specific marker for cartilage turnover. Both markers were measured using commercially available enzyme-linked immunosorbent assays (ELISA) for cGMP (Cayman Chemical Co., Ann Arbor, Mich.) and cleaved collagen type II (Cartilaps) (Immunodiagnostic Systems, Fountain Hills, Ariz.), following the manufacturer's protocol.

Levels of cGMP and collagen type II fragments were measured from cell culture extracts after exposure to CNP22, CNP37 or PEO24-GANRR-CNP22(K4R) ("CNP27-PEO24") (SEQ ID NO: 36). FIGS. **39-42** show a large increase ($p<0.01$) in the levels of cGMP in the media after exposure of ex-planted wild-type and FGFR3^{ach} mouse femurs and tibias to CNP22, CNP37 or PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36). In addition, exposure of wild-type and FGFR3^{ach} mouse femurs to CNP22, CNP37 or PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36)

increased the levels of cleaved collagen type II, with the treated $FGFR3^{ach}$ mouse femurs showing significantly increased ($p<0.05$) levels of collagen type II fragments (FIG. **43**). Elevated levels of collagen type II fragments indicate a turnover of the cartilage matrix, and cartilage turnover typically precedes new bone formation in growing bones.

Example 8B

Ex Vivo Stimulation of Growth of Femur from Mice with Severe Achondroplasia

The effect of a CNP variant on the growth of bones from mice with severe achondroplasia was evaluated ex vivo. Used in the study were transgenic mice expressing a human FGFR-3gene having a Y367C mutation ($FGFR3^{Y367C}$) [S. Pannier et al., Biochim. Biophys. Acta, 1792(2): 140-147 (2009)], which represent a mouse model of severe achondroplasia. Femurs were isolated at embryonic day 16.5 and cultured for 6 days in the presence of 1 uM Pro-Gly-CNP37. Bone lengths were measured at Day 1 and Day 7. The bones were then paraffin-embedded, sectioned and stained with hematoxylin and eosin to assess histological changes and cellular morphology. Treatment of bone explants isolated from $FGFR3^{Y367C}$ mice with Pro-Gly-CNP37 ("ProCNP38") resulted in increase in bone growth and expansion in the growth plate (FIG. **44**). Femurs from $FGFR3^{Y367C}$ mice treated with vehicle for 6 days showed an 18% deficiency in growth length-wise compared to wild-type femurs treated with vehicle. Treatment of femurs from $FGFR3^{Y367C}$ mice with 1 uM Pro-Gly-CNP37 for 6 days reduced the growth deficiency to just 11%, i.e., reduced the growth defect by around 40%.

Example 9

Serum/Plasma Stability of CNP Variants In Vitro

In preparation for pharmacokinetics (PK) studies, the stability of CNP variants in serum and/or plasma is evaluated.

Briefly, the analyte is isolated by the removal of serum or plasma proteins by either a 2% trichloroacetic acid precipitation or a 1:3 serum:acetonitrile precipitation. The precipitation mixture is vortexed at 14,000 rpm for five minutes, and a portion of the supernatant is removed and diluted with water prior to transfer to a silanized autosampler vial for analysis. Serum extracts are then analyzed by reverse-phase high performance liquid chromatography (RP-HPLC) with electrospray ionization mass spectrometry (ESI-MS). A single mass (m/z), shown to be specific for the CNP variant, is monitored for quantitation purposes.

Initially, analytical stability and recovery is determined. Analytical (RP-HPLC and ESI-MS) parameters are optimized through the analysis of matrix standards (serum extracts fortified with analyte post-precipitation). After optimization, analytical recovery is determined by spiking serum samples at known concentrations and comparison of the analyte response to that of matrix standards prepared at similar concentrations. Analyte stability in serum extracts is also determined to assure no significant losses occur after serum precipitation and prior to actual analysis. To test the effect of freezing on serum stability, a two-cycle freeze/thaw study is also performed. In this study a serum sample is spiked with CNP variant and analyzed prior to freezing overnight at −20° C. The sample is then thawed at room temperature and re-analyzed. The process is repeated for a second freeze/thaw cycle.

Serum stability of CNP variant is determined by spiking of serum/plasma samples with CNP variant at a concentration of 10 ug/mL. The sample is placed in a 37° C. water bath for a period of three hours. At 30 minute intervals duplicate aliquots of serum are removed and analyzed. If rapid losses of analyte are evident (>50% in 30 minutes), the study may be repeated with 10 minute timepoints.

In an exemplary method for determining the stability of CNP variants in murine plasma, a mixture of CNP variant (10 uL of a stock solution of about 2.5-5.0 mg/mL), heparinized murine plasma (50 uL, Bioreclamation, CD-1 Lith Hep 62231), and 5 M NaCl (10 uL) is incubated at 37° C. and 5% CO_2 for 0-5 hr, and then quenched with 10× protease inhibitor cocktail (15 uL, Sigma P2714). For extraction, 150 uL of MeOH/0.1% FA is added to 85 uL of the reaction mixture, and the resulting mixture is vortexed for 1 min and then centrifuged at 15° C. for 15 min. 75 uL of the supernatant is added to 300 uL of aqueous 0.1% FA. A small portion of the resulting mixture is subjected to analysis by LC/MS.

Example 10

Pharmacokinetics and cGMP Production in Rats and Mice

Studies were conducted in normal rats to evaluate the pharmacokinetics (PK) profile of CNP22 and certain CNP variants and the time courses of plasma cGMP concentration after single intravenous (i.v.) or subcutaneous (s.c.) administration of the CNP peptides. Plasma CNP immunoreactivity was determined by using a competitive radioimmunoassay (RIA) with an anti-CNP rabbit polyclonal antibody. Plasma cGMP concentration was determined by RIA using a commercially available kit (YAMASA cyclic GMP Assay kit, YAMASA Corporation).

Normal male rats, 7-8 weeks of age, were used. Recombinant wild-type CNP22, CNP37 and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) were evaluated. A dosage of 20 nmol/kg of each CNP peptide as a solution in 5% mannitol was intravenously injected once into the tail, or a dosage of 50 nmol/kg of each CNP peptide as a solution in 0.03 mol/L acetic acid buffer solution, pH 4.0, containing 1% (w/v) benzyl alcohol and 10% (w/v) sucrose, was subcutaneously injected once into the back.

Plasma CNP immunoreactivity was determined by the competitive RIA using anti-CNP rabbit polyclonal antibody. Standard and QC samples were prepared. Fifty uL of the standard, QC and assay samples were added, respectively, to test tubes containing 50 uL of RIA buffer. Diluted anti-CNP rabbit polyclonal antibody (100 uL) was added to the tubes. All tubes were kept at 4° C. overnight. ^{125}I-[Tyr^0]-CNP22 solution (100 uL) and rabbit IgG solution (100 uL) were added and left at approximately 4° C. overnight. One milliliter of anti-rabbit IgG goat serum containing 10% polyethylene glycol was added, vortexed and left at approximately 4° C. for at least 1 hour, and then the insoluble fraction was precipitated by centrifugation. After aspiration of the supernatant, the amount of radiation (gamma line) in the sediment was measured by a gamma-counter. Each sample was measured in duplicate, and the mean was adopted as the value determined.

Plasma cGMP concentrations in the sample at 5, 30, 60 and 90 minutes after i.v. dosing, or at 5, 30, 60, 120 and 180 minutes after s.c. dosing, were determined by the competitive RIA using anti-cGMP monoclonal antibody. Standard samples were prepared. 100 uL of the assay samples (standard solutions for the calibration curve or the diluted plasma samples for cGMP determination) were transferred to test tubes. Then 100 uL of anti-cGMP monoclonal antibody solution and 100 uL of ^{125}I-labeled succinyl cGMP tyrosine

methyl ester solution were added to the tubes, respectively. All tubes were kept at 4° C. overnight. After the addition of 500 uL dextran charcoal solution, the tubes were vortexed and then placed on ice for 10 minutes. The reaction mixture was centrifuged and 500 uL of the supernatant was transferred from each sample to a new test tube. The amount of radiation (gamma line) in the supernatant was measured by a gamma-counter. Each sample was measured in duplicate, and the mean was adopted as the value determined.

Plasma CNP immunoreactivity was employed for pharmacokinetics (PK) analysis. PK analysis was performed using WINNONLIN® Professional (Pharsight Corporation). The PK profiles of CNP22, CNP37 and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) after i.v. administration were calculated using PK parameters such as concentration at 0 hour (C_0: extrapolation, pmol/mL), total body clearance (CL_{tot}: ml/min/kg), distribution volume at steady state (V_{dss}: mL/kg), area under the plasma concentration-time curve (AUC: pmol·min/mL), mean residence time (MRT: min), and half-life ($T_{1/2}$: min). The PK profiles of the CNP peptides after s.c. administration were calculated using PK parameters such as maximum plasma concentration (C_{max}: pmol/mL), time to reach C_{max} (T_{max}: min), area under the plasma concentration-time curve (AUC: pmol·min/mL), mean residence time (MRT: min), and half-life ($T_{1/2}$: min).

In plasma spike recovery experiments, the RIA detected CNP22, CNP37 and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) similarly (data not shown).

Procedures similar to those described above are employed to study in mice the PK profiles of CNP22 and variants thereof and their ability to stimulate cGMP production.

The PK profiles of CNP22, CNP37 and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) after i.v. administration in three rats are illustrated in FIG. **45**. As shown by FIG. **45**, CNP37 and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) had a much longer half-life and a much greater bioavailability than CNP22. The half-life, $T_{1/2}$ (min), was 1.42 (±0.45) for CNP22, 22.3 (±1.5) for PEO24-GANRR-CNP22 (K4R) (SEQ ID NO: 36), and 49.5 (±28.0) for CNP37. The area under the curve, AUC (pmol·min/mL), was 320 (±54) for CNP22, 1559 (±568) for CNP37, and 2084 (±424) for PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36).

The PK profiles of the three CNP peptides after s.c. administration in three rats are depicted in FIG. **46**. Compared to CNP22, PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) had a much longer half-life (78.1 min (±16.4) vs. 10.0 (±5.0)) and a much greater bioavailability (60% (±6%) vs. 19% (±9%)).

The time courses of plasma cGMP concentrations after i.v. administration of the three CNP peptides in three rats are displayed in FIG. **47**. FIG. **47** clearly demonstrates that i.v. administration of CNP37 and PEO24-GANRR-CNP22 (K4R) (SEQ ID NO: 36) resulted in much higher plasma levels of cGMP at 30, 60 and 90 minutes than i.v. administration of CNP22.

The time profiles of plasma cGMP concentrations after s.c. administration of the three CNP peptides in three rats are shown in FIG. **48**. Subcutaneous administration of PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) and CNP37 also resulted in substantially higher plasma concentrations of cGMP than s.c. administration of CNP22, with the difference relative to CNP22 increasing over time for PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36), but decreasing over time for CNP37.

The rat studies indicate that compared to wtCNP22, the CNP variants CNP37 and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) had a substantially longer half-life in vivo, had a substantially greater bioavailability in vivo, and stimulated substantially higher levels of cGMP production in vivo for an extended period of time. The resistance of CNP37 and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) to NEP degradation correlates to a longer plasma half-life in vivo, which in turn correlates to prolonged NPR-B/cGMP signaling in vivo. These results show that compared to CNP22, the CNP variants of the disclosure, administered by i.v. or s.c. injection (e.g., once daily), can be more effective in treating CNP-responsive conditions or disorders, such as bone-related disorders and vascular smooth muscle disorders.

Example 11

Pharmacokinetics Study in Mice

To determine CNP variants having increased NEP resistance, for efficacy study in $FGFR3^{ach}$ mice (see Example 13), a pharmacokinetics (PK) study is carried out that compares the pharmacokinetics properties of CNP variants to wild-type CNP22. The $FGFR3^{ach}$ mouse is a mutant mouse model of mild achondroplasia, containing a single transgene on a background of FVB mice.

Wild-type CNP22 or variant thereof is administered as a single intravenous (i.v.) dose in 6-week old wild-type FVB mice. Exemplary PK studies were conducted using wtCNP22. Six-week old FVB/N mice were intravenously administered wtCNP22 in a single dose at 100 nmol/kg. Mean plasma levels of CNP22 were calculated, and the estimated half-life of CNP22 was determined to be from 0.76 min to 1.03 min.

CNP variants displaying greater resistance to NEP degradation are expected to exhibit increased serum concentrations over time and a longer half-life in vivo.

Example 12

Efficacy of CNP Variants in Wild-Type Mice

The in vivo effects of CNP variants on bone growth were assessed in wild-type mice. Three week old FVB wild-type male mice received daily subcutaneous (s.c.) injection of either vehicle, G-CNP37 (200 nmol/kg) or PEO12-GANRR-CNP22(K4R) ("CNP27-PEO12") (SEQ ID NO: 36) (200 nmol/kg) for 5 weeks. Body weight was measured at least once weekly. Tail length was measured at least once weekly using digital caliper readings, and body length (naso-anal length), bone length (tibia, femur, ulna and humerus), skull length (anterior to posterior cranial segment) and lumbar vertebrae 5 (LV5) length were measured after 5 weeks of treatment using a caliper. X-rays were taken at baseline and after 5 weeks of treatment.

Five weeks of treatment of wild-type mice with G-CNP37 resulted in significant body weight gain, with increased body weight being observed beginning at Day 9 ($p<0.05$) (FIG. **49**). Treatment with G-CNP37 also resulted in significantly increased tail length, beginning at the second week after treatment ($p<0.01$) (FIG. **50**).

Table 10 shows the percentage change in tail length, body (naso-anal) length, skull (anterior to posterior cranial segment) length, bone (femur, tibia, humerus and ulna) lengths, and lumbar vertebrae 5 (LV5) length in wild-type mice injected s.c. once daily with 200 nmol/kg of either G-CNP37 or PEO12-GANRR-CNP22(K4R) ("CNP27-PEO12") (SEQ ID NO: 36) for 5 weeks, relative to a value of 100% for wild-type mice treated with vehicle only.

TABLE 10

	Tail	Naso-Anal	Anterior to Posterior (Skull)	Femur	Tibia	Humerus	Ulna	LV5
G-CNP37	119%**	114%**	104%*	109%**	105%**	104%**	101%	108%*
CNP27-PEO12	101%	104%**	101%	102%*	100%	102%*	96%*	103%

**p < 0.01, *p < 0.05

Treatment with G-CNP37 resulted in significantly increased tail length, body (naso-anal) length, skull length, proximal bone (femur and humerus) length, distal bone (tibia) length, and vertebral (lumbar vertebrae 5) length compared to treatment with vehicle.

Lower doses of Pro-Gly-CNP37 dosed daily s.c. at 5 nmol/kg, 20 nmol/kg or 70 nmol/kg for five weeks resulted in a dose-dependent increase in tail length, body (naso-anal) length, and bone lengths compared to vehicle. Table 11 shows the percentage change in tail length, body (naso-anal) length, and bone (femur, tibia, humerus and ulna) lengths in wild-type mice injected s.c. once daily with 5 nmol/kg, 20 nmol/kg or 70 nmol/kg Pro-Gly-CNP37 for 5 weeks, relative to a value of 100% for wild-type mice treated with vehicle only.

TABLE 11

	Tail	Naso-Anal	Femur	Tibia	Humerus	Ulna
5 nmol/kg	107.6%**	102.7%**	103.3%**	101.7%**	101.1%	100.9%*
20 nmol/kg	107.8%**	107.6%**	107.1%**	103.4%**	102.3%**	102.5%**
70 nmol/kg	113.6%**	112.5%**	109.3%**	105.9%**	103.6%**	104.1%**

**p < 0.01,
*p < 0.05

In another study, various dosing regimens of Pro-Gly-CNP37 were administered for nine weeks followed by one week of recovery. Wild-type FVB mice were dosed s.c. with:

(1) vehicle daily for nine weeks, followed by one week of recovery;
(2) 20 nmol/kg Pro-Gly-CNP37 daily for one week, followed by three doses per week for eight weeks and one week of recovery;
(3) 20 nmol/kg Pro-Gly-CNP37 on alternating weeks; or
(4) 5 nmol/kg Pro-Gly-CNP37 daily for nine weeks followed by one week of recovery.

Increased growth in tail length, body length, and bone lengths was observed in all treatment groups (Groups 2, 3 and 4) at the end of the study. Table 12 shows the percentage change in tail length, body (naso-anal) length, and bone (femur, tibia, humerus and ulna) lengths in wild-type mice administered Pro -Gly-CNP37 ("Pro-CNP38") under the dose regimens described above, relative to a value of 100% for wt mice treated with vehicle only.

TABLE 12

	Total Pro-CNP38 received (nmol/kg)	Tail	Naso-Anal	Femur	Tibia	Humerus	Ulna
Group 2	620	104.8%**	106.0%**	105.3%**	100.8%	102.5%	101.7%
Group 3	700	106.7%**	106.5%**	104.1%**	102.5%**	102.6%*	102.8%*
Group 4	350	104.9%**	105.1%**	107.3%**	102.7%**	102.3%**	103.6%**

**p < 0.01, *p < 0.05

While all dose regimens of Pro-Gly-CNP37 increased axial and appendicular growth parameters measured, daily dosing of Pro-Gly-CNP37 promoted appendicular growth (femur, tibia, humerus, and ulna) at a lower total dose level (Group 4) compared to regimens with less frequent dosing (Groups 2 and 3).

Example 13

Efficacy in Mouse Model of Mild Achondroplasia

The efficacy of CNP variants in enhancing growth and correcting achondroplasia was tested in a mouse model of mild achondroplasia, using a strain of transgenic mice expressing a human FGFR-3 gene having a G380R mutation ($FGFR3^{ach}$) (Wang et al., Proc. Natl. Acad. Sci. USA, 96(8): 4455-4460 (1999); Naski et al., Development USA, 125: 4977-4988 (1998); U.S. Pat. Nos. 6,265,632 and 6,136,040).

At 3 weeks of age, $FGFR3^{ach}$ mice and their wild-type littermates were anesthetized to have lateral whole-body X-ray images taken by Faxitron, and randomized by body weight into the following treatment groups (n=8/group): (1) wild-type/vehicle, (2) $FGFR3^{ach}$/vehicle, (3) $FGFR3^{ach}$/CNP37, and (4) $FGFR3^{ach}$/PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36). The mice received once daily subcutaneous (s.c.) administration of designated test article (vehicle or 200 nmol/kg CNP variant) for 5 weeks. Satellite groups (n=3) were used to confirm exposure of each test article following a single subcutaneous administration on Day 1. Wild-type male FVB mice that received daily s.c. injection of vehicle for 5 weeks were used as a control for normal growth.

X-ray measurements at baseline and at the end of the study were performed to determine change in the head length, area of the skull, and the external auditory meatus (EAM, the ear canal running from the outer ear to the middle ear). Body weight and tail length were measured at least once weekly, using a digital caliper to measure tail length, and body (naso-anal) length was measured after 5 weeks of treatment. Bone (tibia, femur, ulna and humerus) lengths were measured using a digital caliper at necropsy.

On Day 37, all mice were sacrificed by terminal anesthesia and whole-animal photographs and X-ray images by Faxitron were taken. Left and right tibia, femur, humerus, and ulna were collected and measured using a digital caliper. The left portions of each bone were processed for histology, and the right portions were snap-frozen for archival. Samples obtained from the bones were used to evaluate the effects of CNP variants on endochondral bone growth.

Treatment of FGFR3^{ach} mice with CNP37 by once daily s.c. injection for 5 weeks resulted in significantly increased body length (FIG. **51**), tail length (FIG. **52**), distal bone (ulna and tibia) lengths (FIGS. **53**A and B), and proximal bone (humerus and femur) lengths (FIGS. **54**A and B). Moreover, treatment with CNP37 increased the head length (FIG. **56**), area of the external auditory meatus (FIG. **57**), and spine length (FIG. **58**) via extension of the vertebral bodies. Additionally, treatment with CNP37 corrected rhizomelia (disproportion of the length of the proximal limbs) of FGFR3^{ach} mice, i.e., restored proportional growth of proximal bones, as assessed by the femur:tibia ratio (FIG. **55**). Table 13 summarizes the percentage change in tail length, body (naso-anal) length, and bone (femur, tibia, humerus and ulna) lengths in FGFR3^{ach} mice injected s.c. once daily with 200 nmol/kg of either CNP37 or PEO24-GANRR-CNP22(K4R) ("CNP27-PEO24") (SEQ ID NO: 36) for 5 weeks, relative to a value of 100% for FGFR3^{ach} mice treated with vehicle only.

TABLE 13

	Tail	Naso-Anal	Femur	Tibia	Humerus	Ulna
CNP37	115%**	109%**	112%**	107%**	105%*	106%**
CNP27-PEO24	100%	99%	100%	102%	100%	101%

**$p < 0.01$, *$p < 0.05$

The results of these studies show that CNP37 can stimulate spinal and long bone growth, help to correct rhizomelia by preferentially increasing the length of the femur over the tibia, and help to restore craniofacial proportions in FGFR3^{ach} mice. These results indicate that CNP37 and potentially other CNP variants may be effective in correcting the symptoms of achondroplasia and treating subjects having defects in bone growth or in need of increased bone growth.

Example 14

Measurement of Biomarkers and Evaluation of Immunogenicity in Wild-Type and Achondroplastic Mice

Measurement of Biomarkers After CNP Administration

The levels of bone growth biomarkers were measured in wild-type and achondroplastic (FGFR3^{ach}) mice.

Transgenic FVB FGFR3^{ach} mice (a mouse model of mild achondroplasia) were treated daily for 5 weeks by subcutaneous injection with either vehicle (30 mM acetic acid/acetate buffer, 1% benzyl alcohol, 10% sucrose, pH 4.0), CNP22, CNP37 or PEO24-GANRR-CNP22(K4R) ("CNP27-PEO24") (SEQ ID NO: 36) at 200 nmol/kg for each CNP compound, as described above. Plasma and serum were collected during the study. Harvested plasma was stored with 10× protease inhibitor at −80° C. until analysis. cGMP levels were measured 15 minutes post-injection on Day 36 from K2-ETDA plasma collection tubes. Fragments of cleaved collagen type II (cartilage-associated biomarkers), osteocalcin (bone-associated biomarker), and IgG (relating to immunogenicity) were measured from terminal bleed serum at the end of the study (Day 37). cGMP was measured using a commercially available ELISA kit (Cayman Chemical Co., Cat. No. 581021.1), cleaved collagen type II (Cartilaps) was measured using a commercially available kit from Immuno-diagnostic Systems (Cat. No. 3CAL4000), and osteocalcin was measured using a commercially available kit from Biomedical Technologies Inc. (Stoughton, Mass.).

FIG. **59** shows an increase in cGMP plasma levels in FGFR3^{ach} mice treated with CNP37 or PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) compared to vehicle. FIGS. **60** and **61** show that administration of CNP37 resulted in the greatest elevation of serum levels of cleaved collagen type II and osteocalcin. These results indicate that administration of the CNP peptides, in particular CNP37, to FGFR3^{ach} mice led to increased levels of bone growth markers, suggesting increased bone formation and growth in CNP peptide-treated FGFR3^{ach} mice.

For evaluation of biomarkers in wild-type mice, wild-type FVB mice were treated daily for 5 weeks by subcutaneous injection with either vehicle (30 mM acetic acid/acetate buffer, 1% benzyl alcohol, 10% sucrose, pH 4.0), 200 nmol/kg G-CNP37, 200 nmol/kg PEO12-GANRR-CNP22 (K4R) ("CNP27-PEO12") (SEQ ID NO: 36), or 20 nmol/kg or 70 nmol/kg Pro-Gly-CNP37. Plasma and serum were collected during the study. Harvested plasma was stored with 10× protease inhibitor at −80° C. until analysis. cGMP levels were measured 15 minutes post-injection on Day 36 from K2-ETDA plasma collection tubes. The levels of cleaved collagen type II, bone-specific alkaline phosphatase, and IgG (relating to immunogenicity) were measured from terminal bleed serum at the end of the study (Day 37). cGMP and cleaved collagen type II (Cartilaps) were measured as described above. Bone-specific alkaline phosphatase was measured using a commercially available kit (Cusabio, Cat. No. CSB-E11914m).

Administration of G-CNP37 significantly increased ($p<0.05$) the level of cGMP (FIG. **62**) and particularly the level of fragments of cleaved collagen type II (FIG. **63**) in wild-type mice compared to vehicle. The significantly higher level of collagen type II fragments resulting from administration of G-CNP37 indicates turnover of the cartilage matrix, suggesting that G-CNP37 stimulated new bone formation in growing bones in wild-type mice.

Both doses of Pro-Gly-CNP37 ("Pro-CNP38"), 20 and 70 nmol/kg, significantly increased ($p<0.05$) plasma cGMP level 15 minutes after administration compared to wild-type mice treated with vehicle (FIG. **64**). Administration of the higher dose (70 nmol/kg) of Pro-Gly-CNP37 also significantly increased ($p<0.05$) the level of cleaved collagen type II compared to vehicle-treated mice (FIG. **65**), suggesting that the higher dose of Pro-Gly-CNP37 stimulated cartilage matrix turnover prior to new bone formation in wild-type mice. Furthermore, administration of the higher dose (70 nmol/kg) of Pro-Gly-CNP37 increased ($p<0.05$) the level of

bone-specific alkaline phosphatase compared to vehicle-treated mice (FIG. **66**), suggesting that the higher dose of Pro-Gly-CNP37 increased bone remodeling in wild-type mice.

Evaluation of Immunogenicity of CNP Variants

Because the CNP variants are peptide derivatives, it is possible that administration of the peptides may lead to an immunogenic response in vivo. To assess whether an immune response occurred after successive administrations of CNP variant, measurement of serum antibody levels was performed.

An IgG assay was performed to assess whether an IgG immune response was triggered by 5 week exposure of achondroplastic FGFR3^{ach} mice to CNP22, CNP37 or PEO24-GANRR-CNP22(K4R) ("CNP27-PEO24") (SEQ ID NO: 36). IgG is the most predominant immunoglobulin in mouse and human serum and is produced as part of the secondary immune response to an antigen. The IgG response to administration of the CNP peptides was determined as follows. 96-well plates were coated with 100 ng/mL CNP22, CNP37 or PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) in BupH PBS buffer (Pierce/Thermo, Cat. No. 28372, Rockford, Ill.). After overnight incubation, plates were blocked with Casein-PBS blocking buffer (Pierce/Thermo, Cat. No. 37528) for two hours at room temperature with shaking at 300 rpm. After washing with wash buffer (1×PBS with 0.05% Tween), diluted serum samples from a terminal bleed (diluted 1:25) were added to the plate. Positive and negative controls were also loaded onto the plate. Positive control was 1:25 diluted serum (pooled from 6 individual FVB mice) with anti-CNP22 antibody added (Bachem rabbit anti-CNP22 IgG, Cat. No. T-4222) at 1:1000 dilution. Negative control was the diluted pooled serum. After two hours of incubation, the plates were washed and secondary antibody diluted in blocking buffer was added to the wells. For the mouse serum samples, anti-mouse IgG Fcγ (peroxidase-conjugated affini-pure goat anti-mouse IgG, Fcγ fragment, Cat. No. 115-035-071, Jackson Immunoresearch, West Grove, Pa.) was added at a 1:10,000 dilution. For the positive and negative controls, anti-rabbit IgG-HRP (Santa Cruz Biotechnology, Cat. No. SC-2004, Santa Cruz, Calif.) was added to blocking buffer. After two hours of incubation at room temperature with shaking at 300 rpm, plates were washed with wash buffer. 100 uL of TMB (One-step TMB, Pierce/Thermo, Cat. No. 34022) was added to all wells. Plates were incubated at room temperature for 15 minutes with shaking at 300 rpm. Colorimetric reactions were stopped by the addition of 100 uL of 2 N H_2SO_4. Plates were read at 450 nm (Spectramax, Molecular Devices, Sunnyvale, Calif.) and data was analyzed using SoftMax Pro software (Molecular Devices).

Serum samples from FGFR3^{ach} mice treated with CNP22 or PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) indicated no positive IgG immune response in any of the mice. Only one out of nine CNP37-treated FGFR3^{ach} mice showed a slightly positive IgG response.

The immunogenic response of wild-type mice administered G-CNP37, PEO12-GANRR-CNP22(K4R) (SEQ ID NO: 36) or Pro-Gly-CNP37, as measured by an increase in serum IgG level, was also assessed by examining terminal bleed serum samples as described above. None of the wild-type mice treated with PEO12-GANRR-CNP22(K4R) (SEQ ID NO: 36) or Pro-Gly-CNP37 (20 or 70 nmol/kg) showed a positive IgG immune response, and only one out of six wild-type mice administered G-CNP37 showed a slightly positive IgG response.

Measurement of Biomarkers Under Different CNP Dosing Regimens

Wild-type FVB mice were treated daily for 9 weeks by subcutaneous (s.c.) injection of vehicle (30 mM acetic acid/acetate buffer, 1% benzyl alcohol, 10% sucrose, pH 4.0) or Pro-Gly-CNP37 ("Pro-CNP38") under different dosing regimens. Group 1 included vehicle-treated mice injected s.c. daily for 9 weeks followed by 1 week of no treatment. Group 2 included mice treated with Pro-Gly-CNP37 (20 nmol/kg) once daily for 1 week, followed by three doses per week for 8 weeks, followed by 1 week of no treatment. Group 3 contained mice treated with Pro-Gly-CNP37 (20 nmol/kg) once daily in alternate weeks (weeks 1, 3, 5, 7 and 9), with 1 week of no treatment following each week of treatment. Group 4 contained mice treated with Pro-Gly-CNP37 (5 nmol/kg) once daily for 9 weeks followed by 1 week of no treatment. Finally, Group 5 included mice treated with Pro-Gly-CNP37 (5 nmol/kg) once daily for 5 weeks without any week of no treatment. Terminal bleed serum was collected from the mice, and cleaved collagen type II, total alkaline phosphatase and total antibody levels were measured therefrom. Cleaved collagen type II levels were measured as described above. Total alkaline phosphatase levels, predominantly from the liver and bones, were measured with the assistance of a veterinary diagnostic laboratory testing facility (Antech).

A total antibody assay was developed to evaluate potential immune response. The platform used for the total antibody assay was an electrochemiluminescent assay (ECLA). The ECLA platform utilizes a biotin-labeled drug (here, biotin-Pro-Gly-CNP37) and a ruthenium-labeled drug (here, Ru—Pro-Gly-CNP37). The biotin-labeled drug binds to a streptavidin-coated plate that contains electrodes, and the ruthenium-labeled drug functions as the detection component of the assay, as ruthenium can be electrochemically stimulated. A drug-specific antibody (here, a CNP-specific antibody) binds to the biotin-labeled drug and the ruthenium-labeled drug and "bridges" the two labeled drugs. Among the advantages of the ECLA platform, any isotype of antibody (IgG, IgM, etc.) can be detected, and the ECLA assay is species-independent.

The total antibody assay was performed as follows. Pro-Gly-CNP37 was labeled with biotin in a 4:1 challenge ratio, and Pro-Gly-CNP37 was also labeled separately with ruthenium in a 10:1 challenge ratio. Both separate labeling reactions were quenched by the addition of glycine, and the samples from both reactions were buffer-exchanged to PBS. Low and high QCs were prepared by using commercially available anti-CNP22 antibody (Bachem rabbit anti-CNP22 IgG, Cat. No. T-4222) added at low and high concentrations to 5% diluted FVB mouse serum. Mouse serum samples were diluted to 5% using assay diluent (5% BSA in PBS, MSD Cat. No. R93AA-1). A working solution was prepared by adding biotin-labeled Pro-Gly-CNP37 to assay diluent, then adding ruthenium-labeled Pro-Gly-CNP37 to assay diluent, and then combining the two solutions together. Twenty-five uL of low and high QCs were loaded onto the plate as controls. Then 25 uL of sample was added to a non-binding 96-well plate, followed by the addition of 50 uL of working solution to all wells. Samples and QCs were incubated with the working solution at room temperature for 2 hours with shaking at 350 rpm. In the meantime, an MSD streptavidin plate (MSD Cat. No. L13SA-1) was blocked with blocking buffer (MSD Cat. No. R93AA-1) at room temperature for 2 hours with shaking at 350 rpm. At the end of the two hour incubation, the MSD streptavidin plate was washed, and then 50 uL of sample or QC was transferred to

the MSD plate. The plate was then incubated at room temperature for 1 hour with shaking at 350 rpm. At the end of the 1 hour incubation, the MSD plate was washed and 150 uL of 2× read buffer (MSD Cat. No. R92TC-2) was added to the plate. The plate was read using an MSD PR400 machine.

All five groups of mice exhibited substantially comparable levels of cleaved collagen type II (FIG. **67**). Treatment of the mice in Group 5 with 5 nmol/kg Pro-Gly-CNP37 once daily for 5 weeks significantly increased ($p<0.001$) total alkaline phosphatase level (FIG. **68**), which is indicative of bone remodeling. Serum samples from each of the four groups of mice treated with Pro-Gly-CNP37 did not show a positive antibody response in the total antibody assay.

Not to be bound by any theory, there are possible explanations as to why the four groups of CNP-treated mice did not exhibit statistically significant difference in cleaved collagen type II level compared to vehicle-treated mice, and why only the CNP-treated mice in Group 5 exhibited a statistically significant increase in total alkaline phosphatase level compared to vehicle-treated mice. It is possible that because the vehicle-treated mice in the study were also growing, cleaved collagen type II and alkaline phosphatase, which are biomarkers of growth, were also produced in the vehicle-treated mice. In addition, there was a one week period of no treatment for the mice in each of Groups 1 to 4, which possibly diluted out any changes in the cleaved collagen type II and total alkaline phosphatase levels between the CNP-treated mice in Groups 1-3 and the vehicle-treated mice. There was no period of no treatment for the CNP-treated mice in Group 5, and those mice exhibited significantly ($p<0.001$) greater total alkaline phosphatase level compared to vehicle-treated mice.

Example 15

Dose Responses of CNP Variants in Mice

The effects of varying doses of CNP variants were assessed in wild type FVB mice.

For the dose study, in two separate studies (S1 and S2), groups of 10 mice were administered Pro-Gly-wtCNP37 ("Pro-CNP38") at 20 and 70 nmol/kg subcutaneously once daily for 36 injections. Tail length and body weight were measured over the course of treatment. Animals were sacrificed at the end of the experiment and bone length assessed.

Tail length of vehicle treated animals was approximately 8 cm at day 36, whereas animals treated with 20 nmol/kg Pro-CNP38 exhibited tail length of approximately 8.75 cm and 70 nmol/kg Pro-CNP38 increased tail length to approximately 9.5 cm. Administration of Pro-CNP38 in either dose induces a significant ($p<0.05$) relative increase in total body length compared to control treated animals, demonstrating approximately a 130% increase in growth velocity at 20 nmol/kg and approximately a 160% increase in growth velocity at 70 nmol/kg Pro-CNP38 (FIG. **69**).

Treatment with Pro-CNP 38 also significantly increased bone length in most of the long bones assessed as well as total naso-anal (body length) of the animal. Table 14 is representative of the relative % increase in bone length in treated animals in comparison to vehicle treated animals.

TABLE 14

Pro-CNP38	Tail	Naso-Anal	LV4-6	Femur	Tibia	Humerus	Ulna
20 nmol/kg	8%*	7%*	10%*	7%*	4%*	3%*	3%
70 nmol/kg	16%*	13%*	13%*	10%*	7%*	4%*	5%*

Relative % increase,
*$p < 0.05$ ANNOVA (Dunnett's) v vehicle

Bone mineral density (BMD) and bone mineral content (BMC) were also evaluated after administration of Pro-CNP38 at different doses. Results (FIG. **70**) showed that administration of Pro-CNP38 at 70 nmol/kg significantly decreased bone mineral density (FIG. **70**A) and increased bone mineral content (FIG. **70**B), suggesting there is a delay in bone mineralization in treated animals, but the mineralization process itself is not adversely affected by treatment with CNP.

There were no significant changes in organ weight between Pro-CNP38 or vehicle treated animals.

Bioanalytical Studies of Dose Response in Mice

Bioanalytical studies were carried out to measure markers of CNP activity after in vivo administration of varying doses of Pro-CNP38. Plasma cGMP levels, serum levels of collagen type II and serum levels of alkaline phosphatase from in vivo samples were analyzed. Wild type mice were administered 20 and 70 nmol/kg Gly-wtCNP37 ("CNP38"), 20 and 70 nmol/kg Pro-Gly-wtCNP37 ("Pro-CNP38") and 70 and 200 nmol/kg of GHKSEVAHRFK-wtCNP27 ("HSA-CNP27") (SEQ ID NO: 144) subcutancously once daily for 36 days. Plasma was collected 15 minutes after the last injection on day 36 and mice were sacrified 24 hr later. At sacrifice, terminal bleed serum was collected and used for biomarker analysis as described previously.

FIG. **71** shows that 20 nmol/kg CNP38 and 70 nmol/kg HSA-CNP27 signficantly increased plasma cGMP ($p<0.01$), raising plasma cGMP levels to approximately 300 pmol and 400 pmol, respectively. Adminsitration of 70 nmol/kg CNP38 increased cGMP to approximately 500 pmol ($p<0.01$), while administration of 70 nmol/kg Pro-CNP38 increased cGMP to approximately 575 pmol ($p<0.001$). Administration of 200 nmol/kg HSA-CNP27 increased cGMP to approximately 675 pmol ($p<0.001$).

CNP variants also significantly increased serum levels of cleaved collagen type II (FIG. **72**). CNP38 at 20 nmol/kg increased collagen to approximately 9 pg/ml ($p<0.05$), CNP38 at 70 nmol/kg increased collagen to approximately 8 pg/ml ($p<0.05$), Pro-CNP38 at 20 nmol/kg incrcased collagen to approximately 12 pg/ml ($p<0.05$), Pro-CNP38 at 70 nmol/kg increased collagen to approximately 16 pg/ml ($p<0.05$), HSA-CNP27 at 70 nmol/kg increased collagen to approximately 10 pg/ml, and HSA-CNP27 at 200 nmol/kg increased collagen to approximately 10 pg/ml ($p<0.05$).

Serum alkaline phosphatase (AP) levels also increased after administration of CNP variants (FIG. **73**). CNP38 at 20 nmol/kg increased AP to approximately 130 IU/L, CNP38 at 70 nmol/kg increased AP to approximately 160 IU/L ($p<0.001$), Pro-CNP38 at 20 nmol/kg increased AP to approximately 155 IU/L ($p<0.001$), Pro-CNP38 at 70 nmol/kg increased AP to approximately 180 IU/L ($p<0.001$), IISA-CNP27 at 70 nmol/kg increased AP to approximately 120 IU/L, and HSA-CNP27 at 200 nmol/kg increased AP to approximately 140 IU/L ($p<0.01$). Table 15 illustrates the percent of total AP that is bone specific.

TABLE 15

	Total AP IU/L	Bone-specific AP IU/L	% of Total Alk Phos
Vehicle	109.8	24.7	22.5
CNP38 (20 nmol/kg)	135.9	47.9	35.2
CNP38 (70 nmol/kg)	166.3	63.5	38.2
Pro-CNP38 (20 nmol/kg)	159.9	65.9	41.2
Pro-CNP38 (70 nmol/kg)	183.6	78.4	42.7
HSA-CNP27 (20 nmol/kg)	121.4	51.7	42.6
HSA-CNP27 (70 nmol/kg)	136.1	76.5	56.2

Analysis of anti-CNP antibodies showed that only HSA-CNP27 elicited an IgG antibody response in mice.

The results above illustrate that administration of CNP variants increases the concentration of collagen type II and alkaline phosphatase in serum, indicating that CNP increases factors relevant for increased bone growth, and suggest that administration of CNP variants at doses as low as 20 nmol/kg are effective in increasing bone growth in vivo.

cGMP Response After Different Dose Regimens

Bioanalytical analysis was also assessed at different times after administration of Pro-Gly-wtCNP37 ("Pro-CNP38") to wild type CD-1 mice, 8-10 weeks old, (n=3 per treatment group). Pro-CNP38 was given in a single subcutaneous dose of 200 nmol/kg and levels of cGMP measured at 15 minutes, 3 hours, 1 day, 2 days and 3 days post injection in the plasma, epiphysis, cortical bone (marrow removed), lung and brain. Blood was collected on K2EDTA. Tibial and femoral epiphysis and cortical bone, ear pinna, brains, kidneys and lungs were harvested, placed in boiling water for 5 minutes, then frozen to −70° C. Both plasma and tissue were assayed for cGMP (Cayman Chemical cyclic GMP ELISA kit).

Results showed that cGMP levels increased at 15 minutes post injection in plasma (approximately 1300 pmol/ml) and epiphysis (approximately 2.5 pmol/ml/mg). By 3 hours, plasma levels had decreased approximately to control levels, while levels in the epiphysis were approximately 3 fold lower than cGMP levels at 15 minutes post injection, but higher than control levels. Levels of cortical bone increased to approximately 0.5 pmol/ml/mg at 15 minutes and remained at this level at 3 hours. Levels of cGMP at all timepoints were back to control levels by 1 day after injection. Little to no cGMP was detected in lung or brain at any timepoint.

cGMP levels were also measured in mice administered multiple injections of Pro-CNP38. Groups of mice (n=3) were given Pro-CNP38 as follows: 20 nmol/kg single dose, subcutaneously; 200 nmol/kg single dose, subcutaneously; 20 nmol/kg subcutaneously on days 0 and 1; 200 nmol/kg subcutaneously on days 0 and 1; 20 nmol/kg subcutaneously on days 0 and 3; 200 nmol/kg subcutaneously on days 0 and 3. Mice were sacrificed 15 minutes after the final dose of Pro-CNP38 and plasma levels of cGMP analyzed. There does not appear to be a modulation of the plasma cGMP signal due to the different dose regimens. cGMP responses in the cartilage are also investigated.

Evaluation of Potential Desensitization of NPR-B Receptor

Histological analysis shows that CNP, when administered daily at 200 nmol/kg, accumulates in the growth plate of animals, based on increased CNP immunoreactivity. It is possible that this accumulation, or daily stimulation of the CNP receptor, in the growth plate could desensitize the CNP receptor.

To determine if multiple dosing desensitizes the NPR-B receptor in vitro, normal human articular chondrocytes were cultured with Pro-Gly-wtCNP37 ("Pro-CNP38") for varying times and cGMP secretion measured.

Primary normal human chondrocytes isolated from articular cartilage were cultured as recommended by the supplier (Lonza). At 60-80% confluence the chondrocytes were treated with 1 uM Pro-CNP38 twice, with increasing amounts of time between treatments (first treatment at time 0, then at 15 min, 30 min, 60 min, 2 hrs, 3 hrs, 4 hrs, 6 hrs after the initial treatment). In the following experiment the treatments were either applied twice, with increasing amounts of time between treatment (first treatment at time 0, then at 6, 16, 24, 48 hrs after initial treatment) or only once, in parallel to the second treatment (only at 6, 16, 24 and 48 hrs-naïve responses). Treatments were either applied for 15 min only (acute treatment), or for the duration of the experiment (chronic treatment when CNP was left in the medium). Cell lysates and conditioned medium were collected and analyzed for total cGMP secretion (Molecular Devices ELISA).

In a short term experiment, cells were stimulated with Gly-wtCNP37 ("CNP38") 1 uM two times for 15 minutes (acute treatment), or two times with CNP38 throughout culture (chronic treatment). The periods between treatments were 15 min, 30 min, 60 min, 2 hrs, 3 hrs, 4 hrs, and 6 hrs. Peak cGMP stimulation was obtained by treating cells once only, at the last time point of the experiment, (6 hours; >0.1 pmol/well in the acute experiment and 0.2 pmol/well in the chronic experiment). In the acute experiment, when the cells are treated twice, the response decreases to 0.1 pmol/well if the cells are treated at time 0 and then again at 15 minutes. In the acute experiment, when the cells are treated twice, the response decreases to approximately 0.5 pmol/well if the cells are treated at time 0 and again at 30 min, 60 min, 2, 3, and 4 hours. In the chronic experiment, when the cells are treated twice, the response decreases to approximately 0.16 pmol/well if the cells are treated at time 0 and at 15 minutes. In the chronic experiment, when the cells are treated twice, the response decreases to approximately 0.6 pmol/well if the cells are treated at time 0 and again at 30 min, 60 min, or 2 hours. In the chronic experiment, when the cells are treated twice, the response decreases to <0.5 pmol/well if the cells are treated at time 0 and again at 2, 4 or 6 hours.

Long term studies were also carried out. For acute treatment, 1 uM CNP38 was added to cell culture as described above. For chronic treatment, 1 uM CNP38 was added to cell culture throughout the experiment duration as described above. Results indicate that the NPR-B receptor can desensitize after repeated, daily CNP administration in vitro, and 48 hours between doses is sufficient to recover to 60% of the maximial NPR-B response to CNP38, if the CNP is removed after the first dose, and to <40% if the CNP is incubated throughout the experiment (FIG. **74**).

To evaluate whether treatment with a CNP variant desensitizes the NPR-B receptor in vivo, experiments were conducted with wild-type mice. CD-1 male mice 8-10 weeks of age were injected subcutaneously with vehicle control or Pro-Gly-wtCNP37 ("Pro-CNP38") at 200 nmol/kg on the appropriate days. The mice were either injected with Pro-CNP38 daily for up to 8 days, or were injected with Pro-CNP38 on the first day, the first and second days, or the first and third days of the study. Fifteen minutes following the final injection, the mice (n=3 per treatment group) were deeply anesthetized and ex-sanguinated via thoracotomy and aortic cannulation. Circulating blood was flushed from the body with PBS via an aortic cannula. The kidneys and right tibias, right femurs and left femurs were collected, boiled in water for 5 minutes and/or snap frozen in liquid

nitrogen and stored on dry ice or in a −70° C. freezer until cGMP assay. For estimation of cGMP production in cartilage, distal femurs and/or proximal tibias were dissected, weighed and pulverized using Covaris Cryoprep CP02. Powdered samples were homogenized using a Covaris E210 sonicator in 5% perchloric acid, and neutralized in 60% KOH. Samples were then centrifuged at 10,000 rpm at 4° C. for 5 minutes, and the supernatant was used for the cGMP assay (Cyclic GMP Enzyme Immunoassay Kit, Cayman, Mich.). Additionally, left tibias were collected, fixed in 10% normal buffered formalin, and saved for further immunohistochemical analysis.

FIG. **75**A shows that repeated daily treatment of wild-type mice with 200 nmol/kg of Pro-CNP38 for 1, 4, 6, 7 and 8 days did not result in desensitization of the cGMP response. To the contrary, potentiation of the cGMP response was observed after 4 days of daily treatment. Further daily treatment resulted in a plateau of the cGMP reponse, up to 8 days. The results indicate that daily treatment of wild-type mice with 200 nmol/kg of Pro-CNP38 for up to 8 days do not desensitize the cGMP response.

The kinetics of the cGMP response in distal femoral cartilage after treatment of the wild-type mice with Pro-CNP38 was also investigated. Treatment of the mice once a day for two days potentiated the cGMP response to Pro-CNP38, compared with the cGMP response to a single treatment (FIG. **75**B). When the mice were treated on the first and third days, but not on the first and second days, the cGMP response after the second treatment (on the third day) was substantially similar to the cGMP response observed after a single treatment on day one (FIG. **75**B). The results suggest that dosing on consecutive days is beneficial for potentiating the cGMP response to Pro-CNP38 in this mouse study.

Activation of NPR-B in Different Tissues

To evaluate potential activation of NPR-B in different tissues by a CNP variant, wild-type male CD-1 mice were injected with 200 nmol/kg of Gly-CNP37, and the cGMP response was measured in certain tissues at different time points. Two mice were used for each treatment group. Each mouse received one subcutaneous injection of Gly-CNP37 or vehicle control. Fifteen, 30 or 60 minutes or 3 hours following the injection, the mice were deeply anesthetized and ex-sanguinated via thoracotomy and aortic cannulation. Circulating blood was removed by a PBS flush with an aortic cannula. Heart, liver, lung, kidney, ear pinna, aorta and brain were collected. All tissues were boiled in water for 5 minutes, finely dissected (including flushing of marrow from femoral cortices with PBS), weighed, cooled in liquid nitrogen, and pulverized in a BioPulverizer. The resulting powdered samples were homogenized with a Polytron in 6% pre-chilled perchloric acid and neutralized with 60% KOH. Samples were then centrifuged at 10,000 rpm and 4° C. for 5 minutes, and the supernatant was used for the assay of cGMP (Cyclic GMP Enzyme Immunoassay Kit, Cayman Chemical Company, Ann Arbor, Mich.). The results were normalized for tissue weight.

Secretion of cGMP in response to treatment with Gly-CNP37 ("CNP" in FIG. **76**) was detectable in distal femurs (cartilage and bone), femoral cortices (bone), ear pinna (cartilage), and kidney (FIGS. **76**A-D). Maximal cGMP responses in those tissues were observed 15 min after treatment. Liver, heart, lung and brain tissues did not exhibit appreciable cGMP secretion in response to Gly-CNP37 relative to vehicle control at the studied time points (FIGS. **76**E-H). The results indicate that treatment with 200 nmol/kg of Gly-CNP37 stimulated cGMP secretion in cartilage, bone and renal tissues.

Example 16

Dose Responses of CNP Variants in Monkeys

The effects of the CNP variant Pro-Gly-CNP37 on bone growth and the levels of bone growth-related biomarkers are evaluated in cynomolgus monkeys. Eight normal juvenile cynomolgus monkeys (about 2.5 years of age at the start of the on-going study) are subcutaneously injected daily with 10 or 36 μg/kg/day of Pro-Gly-CNP37 (n=4 per dose group). Four such monkeys are administered vehicle as control. The total length of treatment is 6 months. Various measures of growth plate expansion and bone growth are made by digital X-ray and magnetic resonance imaging, and by measurement of limb and body lengths externally. Blood and urine samples are collected periodically for clinical pathology and measurement of levels of Pro-Gly-CNP37 and biomarkers. Following termination of the study, gross pathology is performed and tissue samples are evaluated histologically for assessment of efficacy and safety.

Data obtained thus far in the on-going study show that both doses of Pro-Gly-CNP37 have increased growth plate width by digital X-ray (FIG. **77**), increased right and left tibia lengths by digital X-ray (FIGS. **78**A and B), increased leg length by external measurement (FIG. **79**), increased arm length by external measurement (FIG. **80**), increased body length by external measurement (FIG. **81**), and increased the serum level of alkaline phosphatase, a biomarker for bone formation (FIG. **82**). The data demonstrate that Pro-Gly-CNP37 can stimulate bone growth in normal juvenile cynomolgus monkeys at hemodynamically acceptable doses.

Example 17

Effects of CNP Variants on Cardiovascular System in Mice

Natriuretic peptides such as CNP have been reported to affect the cardiovascular system. Wang et al. (Eur J Heart Fail. 9:548-57. 2007) describe that CNP has been shown to have a cardioprotective effect in preventing myocardial ischaemia/reperfusion injury and improving cardiac remodelling after myocardial infarction in rats. Wang demonstrated that mice overexpressing CNP have reduced incidence of cardiac hypertrophy caused by myocardial infarction. Additionally, CNP has been shown to cause endothelium-independent vasodilation (M. Honing et al., Hypertension, 37:1179-1183 (2001)) and therefore may transiently decrease blood pressure in vivo.

To assess the effects of CNP variants on the cardiovascular system, the blood pressure and heart rate in anesthetized wild-type FVB mice is studied following subcutaneous injection of the variants.

After a pilot study to define a broad dose range of cardiovascular activity, a dose-response study is conducted to examine the effects of three different dose levels of each CNP variant. Three male FVB mice aged 8 weeks comprise each treatment group. Doses are administered subcutaneously to anesthetized mice, and systolic, diastolic and mean arterial pressure (MAP), as well as heart rate, are monitored via implanted intraarterial pressure transducers.

Example 18

Formulation of CNP Variants

CNP preformulation studies were carried out to assess the stability of CNP variant Gly-wtCNP37 ("CNP38") at different pH's (pH 3, 4, 5, 6, 7 and 8) and temperatures (5° C., 25° C., and 40° C.) overtime. CNP38 exhibited greater stability at pH 4-6 than at the other pH's in the studies. CNP38 was stable at 5° C. at pH 4-6, with ≥about 95% of CNP38 remaining after 15 weeks. When the temperature was raised to 25° C., at pH 4 about 85% of CNP38 remained after 15 weeks, at pH 5 about 85% remained after 15 weeks, and at pH 6 about 80% remained after 15 weeks. When the temperature was raised to 40° C., at pH 4 about 55-60% of CNP38 remained after 15 weeks, at pH 5 about 65% remained after 15 weeks, and at pH 6 about 40% remained after 15 weeks. FIG. **83** illustrates the observed plot of pseudo-first order degradation rate constant (K_{obs}) vs. pH from pH 3 to 8 and at 5° C., 25° C. and 40° C. The stability data for CNP38 in the preformulation studies suggests CNP formulations having a pH in the range from about 4 to about 6. An acidic pH (e.g., pH≤about 6) can promote the stability of a CNP variant by, e.g., minimizing or avoiding deamidation of asparagine and/or glutamine residue(s), isomerization of aspartic acid residue(s), or degradation of the CNP variant by other pathways.

CNP variants can be formulated in pharmaceutical carriers for administration to subjects affected by, e.g., bone growth conditions. In some embodiments, liquid formulations of CNP variants are formulated according to any combinations of the ingredients and their amounts or concentrations in Table 16.

TABLE 16

Ingredient Class	Ingredient	Concentration Range
Active ingredient	CNP variant	10 mg/mL ± 9.9 mg/mL
Buffering agent	Acetic acid/acetate	10 mM ± 5 mM, or pH 5 ± 1
Buffering agent	Citric acid/citrate	10 mM ± 5 mM, or pH 5 ± 1
Isotonicity-adjusting agent	NaCl	140 mM ± 20 mM
Isotonicity-adjusting agent	Sucrose	10% ± 5%
Preservative	m-Cresol	0.4% ± 0.1% or 0.2%
Preservative/anti-adsorbent	Benzyl alcohol	1.5% ± 0.5%
Stabilizer	Glycerin (glycerol)	5%-100% (neat)[1]
Stabilizer	Methionine	0.01%-0.2%
Stabilizer	Ascorbic acid/ ascorbate salt	0.1%-l%
Stabilizer	Thioglycerol	0.1%-l%
Anti-adsorbent	Polysorbate 20	0.001%-0.5%
	Polysorbate 80	0.001%-0.5%
	Benzyl alcohol	0.5%-1.5%

[1]Glycerin is used to minimize or prevent water-driven hydrolysis, deamidation, isomerization or cleavage of CNP variants. For lyophilized formulations, 4-6% or 6-20% mannitol or sucrose can be substituted for NaCl.

In certain embodiments, lyophilized formulations of CNP variants are prepared from formulations formulated according to any combinations of the ingredients and their amounts or concentrations in Table 17.

TABLE 17

Ingredient Class	Ingredient	Concentration Range
Active ingredient	CNP variant	10 mg/mL ± 9.9 mg/mL
Buffering agent	Acetic acid/acetate	10 mM ± 5 mM, or pH 5 ± 1
Buffering agent	Citric acid/citrate	10 mM ± 5 mM, or pH 5 +± 1
Isotonicity-adjusting agent/bulking agent	Sorbitol	5% ± 3%
Isotonicity-adjusting agent/bulking agent	Mannitol	5% ± 3%
Isotonicity-adjusting agent/bulking agent	Sucrose	10% ± 5%
Preservative	m-Cresol	0.4% ± 0.2%
Preservative/anti-adsorbent	Benzyl alcohol	1.5% ± 0.5%
Stabilizer	Glycerin (glycerol)	5%-100% (neat)[1]
Stabilizer	Methionine	0.01%-0.2%
Stabilizer	Ascorbic acid/ ascorbate salt	0.1%-l%
Stabilizer	Thioglycerol	0.1%-l%
Anti-adsorbent	Polysorbate 20	0.001%-0.5%
	Polysorbate 80	0.001%-0.5%
	Benzyl alcohol	0.5%-1.5%

[1]Glycerin is used to minimize or prevent water-driven hydrolysis, deamidation, isomerization or cleavage of CNP variants.

In certain embodiments, a formulation comprising a CNP variant has a pH of about 3-7, or about 3-6, or about 3.5-6.5, or about 4-6, or about 4-5, or about 4.5-5.5. In some embodiments, for pH 4-5.5 a suitable buffering agent is acetic acid/acetate (e.g., sodium acetate), and for pH 5.5-6 a suitable buffering agent is citric acid/citrate. Citric acid/citrate (e.g., sodium citrate) is also a suitable buffering agent in the range of pH 3-6 or pH 4-6. In certain embodiments, the buffering agent has a concentration in the formulation of about 2-50 mM, or about 2-40 mM, or about 2-30 mM, or about 5-30 mM, or about 2-20 mM, or about 5-20 mM, or about 5-15 mM.

To minimize or avoid deamidation of a CNP variant, the variant can be formulated in pharmaceutically acceptable organic cosolvents, such as glycerin, ethanol, and propylene glycol. Because deamidation occurs by hydrolysis, substitution of an organic cosolvent for water minimizes contact of the CNP variant with water. The concentration of one or more organic solvents in an organic-aqueous solvent system can be, e.g., from about 10% to about 99%, or about 100% if water is not used.

Also to minimize or avoid deamidation of a CNP variant, water can be removed from the formulation by lyophilization. In some embodiments, lyophilized formulations contain any combinations of the following components:

- buffer: sodium acetate and acetic acid, or sodium citrate and citric acid;
- isotonicity/bulking agent: mannitol (e.g., 3-10%, 2-8% or 4-6%);
  sucrose (e.g., 6-20%, 5-15% or 8-12%);
- antioxidants: methionine and/or ascorbic acid with molal ratio of each antioxidant to CNP variant from about 0.1:1 to about 1:1, or from about 0.5:1 to about 5:1, or from about 1:1 to about 15:1, or from about 1:1 to about 10:1, or from about 3:1 to about 10:1.

Deamidation can also be minimized or avoided by storing a CNP composition (e.g., a liquid formulation or a lyophilized formulation) at lower temperature, such as at about 5° C., 0° C., −10° C., −20° C., −30° C., −40° C., −50° C., −60° C., −70° C., −80° C., −90° C., or −100° C.

To minimize or avoid oxidation of oxidizable residues (e.g., methionine) in a CNP variant, the variant can be formulated with one or more antioxidants. Exemplary antioxidants include, but are not limited to, methionine, ascorbic acid, and thioglycerol. Oxidation of, e.g., methionine residues can also be minimized or prevented by purging oxygen from a liquid medium (if a liquid formulation) with nitrogen or argon, and/or by purging oxygen from a container or packaging with nitrogen or argon.

In some embodiments, to minimize or prevent adsorption (e.g., adsorption of a CNP variant to plastic or glass),

Polysorbate 20, Polysorbate 80 or benzyl alcohol, or a combination thereof, is added to a CNP formulation. In certain embodiments, each of the anti-adsorbent(s) is in a concentration from about 0.001% to about 0.5%, or from about 0.01% to about 0.5%, or from about 0.1% to about 1%, or from about 0.5% to about 1%, or from about 0.5% to about 1.5%, or from about 0.5% to about 2%, or from about 1% to about 2%. Exemplary range(s) of anti-adsorbent(s) in the formulation include without limitation from about 0.001% to about 0.5% of Polysorbate 20, from about 0.001% to about 0.5% of Polysorbate 80, and/or from about 0.5% to about 1.5% of benzyl alcohol.

In certain embodiments, a liquid CNP formulation comprises, or a lyophilized CNP formulation is prepared from a formulation that comprises, (1) an acetic acid/acetate (e.g., sodium acetate) buffer having a concentration of about 30 mM±5 or 10 mM buffering agent and a pH of about 4±0.5 or 1, and (2) benzyl alcohol (e.g., as a preservative and/or anti-adsorbent) at a concentration of about 1%±0.5%, and optionally (3) sucrose at a concentration of about 10%±5%.

Example 19

Clinical Evaluation of CNP Variants

The following example provides guidance on the parameters to be used for the clinical evaluation of compositions comprising CNP22 or variants thereof in the therapeutic methods of the present disclosure. As discussed herein, CNP22 or variants thereof will be used in the treatment of disorders responsive to CNP, including disorders of the bone and vascular smooth muscle. Clinical trials will be conducted which will provide an assessment of doses of CNP22 or variants thereof for safety, pharmacokinetics, and initial response of both surrogate and defined clinical endpoints. The trial will be conducted for a minimum, but not necessarily limited to, 24 weeks to collect sufficient safety information on about 100 evaluable patients. The initial dose for the trials will vary from about 0.001 to about 1.0 mg/kg/week, or any of the doses described herein. In the event that the initial dose in this range does not produce a significant direct clinical benefit, the dose should be increased within this range or beyond this range as necessary, and maintained for an additional minimal period of, but not necessarily limited to, 24 weeks to establish safety and to evaluate efficacy further.

Measurements of safety will include adverse events, allergic reactions, complete clinical chemistry panel (including kidney and liver functions), urinalysis, and CBC with differential. In addition, other parameters relevant to clinical benefit will be monitored. The present example also includes the determination of pharmacokinetic parameters of CNP22 or variants thereof, including absorption, distribution, metabolism, excretion, and half-life and bioavailability in the blood. It is anticipated that such analyses will help relate dose to clinical response.

Methods

Patients will undergo a baseline medical history and physical exam, and a standard set of clinical laboratory tests (including CBC, Panel 20, CH50, and UA). The patients will be followed closely with weekly visits to the clinic. The patients will return to the clinic for a complete evaluation one week after completing the treatment period. Should dose escalation be required, the patients will follow the same schedule outlined above. Safety will be monitored throughout the trial.

Diagnosis and Inclusion Criteria

The patients may be male or female, with a documented diagnosis of a potentially CNP-responsive disorder. A specific example of a potentially CNP-responsive, bone-related disorder is achondroplasia, which may be confirmed by genetic testing and other evidence of an FGFR-3 mutation or dysfunction. The ideal age range of achondroplasia patients will be from infant (<1 year of age) to pre-adolescent (<13 years of age). A patient will be excluded from this study if the patient is pregnant or lactating; has received an investigational drug within 30 days prior to study enrollment; or has a medical condition, serious intercurrent illness, or other extenuating circumstance that may significantly decrease study compliance.

Safety

Therapy with CNP22 or variants thereof will be determined to be safe if no significant acute or chronic drug reactions occur during the course of the study. The longer-term administration of the drug will be determined to be safe if no significant abnormalities are observed in the clinical examinations, clinical labs, or other appropriate studies.

It has been shown that compared to wild-type CNP22, certain CNP variants of the disclosure are much more resistant to NEP degradation in vitro, have a much longer plasma half-life and bioavailability in rats, stimulate a much higher level of cGMP production in rats, and/or induce a significantly greater increase in long bone length and body length in achondroplastic mice. Furthermore, it has been shown that short duration dose regimen treatments with CNP22 are nearly as effective as continuous CNP22 treatment in reversing FGF2-induced arrest of chondrocyte growth in vitro. These results, among others described herein, demonstrate the utility of CNP variants of the disclosure in treating CNP-responsive conditions or disorders such as, e.g., bone-related disorders and vascular smooth muscle disorders.

It is understood that every embodiment of the disclosure described herein may optionally be combined with any one or more of the other embodiments described herein. Every patent literature and every non-patent literature cited herein are incorporated herein by reference in their entirety.

Numerous modifications and variations to the disclosure, as set forth in the embodiments and illustrative examples described herein, are expected to occur to those skilled in the art. Consequently only such limitations as appear in the appended claims should be placed on the disclosure.

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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Gly is optionally modified with an amino acid sequence derived from a natriuretic polypeptide, a non-natriuretic polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (22)..(22)
<223> OTHER INFORMATION: Cys is optionally modified with an amino acid sequence derived from a natriuretic polypeptide, a non-natriuretic polypeptide

<400> SEQUENCE: 5

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 6
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Gly is modified with a synthetic or natural polymeric group or combination thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: Xaa is Lys or a conservative amino acid substitution thereof, any natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amine on a side chain, Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Glu or Ser
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is Lys or a conservative amino acid substitution thereof, any natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amine on a side chain, Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Glu or Ser
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (22)..(22)
<223> OTHER INFORMATION: Cys is optionally modified with a synthetic or natural polymeric group or combination thereof

<400> SEQUENCE: 6

Gly Leu Ser Xaa Gly Cys Phe Gly Leu Xaa Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 7
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

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<400> SEQUENCE: 7

Gly Ala Asn Lys Lys
1 5

<210> SEQ ID NO 8
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 8

Gly Ala Asn Arg Arg
1 5

<210> SEQ ID NO 9
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 9

Gly Ala Asn Pro Arg
1 5

<210> SEQ ID NO 10
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 10

Gly Ala Asn Gln Gln
1 5

<210> SEQ ID NO 11
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 11

Gly Ala Asn Ser Ser
1 5

<210> SEQ ID NO 12
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 12

Gly Ala Asn Arg Gln
1 5

<210> SEQ ID NO 13
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 13

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Gly Ala Asn Arg Met
1 5

<210> SEQ ID NO 14
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 14

Gly Ala Asn Arg Thr
1 5

<210> SEQ ID NO 15
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 15

Gly Ala Asn Arg Ser
1 5

<210> SEQ ID NO 16
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 16

Ala Ala Trp Ala Arg Leu Leu Gln Glu His Pro Asn Ala
1 5 10

<210> SEQ ID NO 17
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 17

Ala Ala Trp Ala Arg Leu Leu Gln Glu His Pro Asn Ala Arg
1 5 10

<210> SEQ ID NO 18
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 18

Asp Leu Arg Val Asp Thr Lys Ser Arg Ala Ala Trp Ala Arg
1 5 10

<210> SEQ ID NO 19
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 19

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Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys
1 5 10 15

<210> SEQ ID NO 20
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 20

Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Arg Arg
1 5 10 15

<210> SEQ ID NO 21
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 21

Asp Leu Arg Val Asp Thr Lys Ser Arg Ala Ala Trp Ala Arg Leu Leu
1 5 10 15

Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys
20 25 30

<210> SEQ ID NO 22
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 22

Asp Leu Arg Val Asp Thr Lys Ser Arg Ala Ala Trp Ala Arg Leu Leu
1 5 10 15

Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Arg Arg
20 25 30

<210> SEQ ID NO 23
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 23

Ser Leu Arg Arg Ser Ser
1 5

<210> SEQ ID NO 24
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 24

Asn Ser Phe Arg Tyr
1 5

<210> SEQ ID NO 25
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 25

Ser Pro Lys Met Val Gln Gly Ser Gly
1 5

<210> SEQ ID NO 26
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 26

Met Val Gln Gly Ser Gly
1 5

<210> SEQ ID NO 27
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 27

Lys Val Leu Arg Arg Tyr
1 5

<210> SEQ ID NO 28
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 28

Lys Val Leu Arg Arg His
1 5

<210> SEQ ID NO 29
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 29

Gly Gln His Lys Asp Asp Asn Pro Asn Leu Pro Arg
1 5 10

<210> SEQ ID NO 30
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 30

Gly Val Pro Gln Val Ser Thr Ser Thr
1 5

<210> SEQ ID NO 31
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 31

Gly Glu Arg Ala Phe Lys Ala Trp Ala Val Ala Arg Leu Ser Gln
1 5 10 15

<210> SEQ ID NO 32
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 32

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
1 5 10

<210> SEQ ID NO 33
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Amino acid extention

<400> SEQUENCE: 33

Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys
1 5 10 15

<210> SEQ ID NO 34
<211> LENGTH: 37
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: CNP-22 with amino acid extention
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (19)..(19)
<223> OTHER INFORMATION: Xaa is Lys or a conservative amino acid substitution thereof, any natural or unnatural amino acid, Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Glu or Ser
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (25)..(25)
<223> OTHER INFORMATION: Xaa is Lys or a conservative amino acid substitution thereof, any natural or unnatural amino acid, Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Glu or Ser
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (37)..(37)
<223> OTHER INFORMATION: Cys is optionally modified with a synthetic, natural or combination polymeric group

<400> SEQUENCE: 34

Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly
1 5 10 15

Leu Ser Xaa Gly Cys Phe Gly Leu Xaa Leu Asp Arg Ile Gly Ser Met
20 25 30

Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 35
<211> LENGTH: 22
<212> TYPE: PRT

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: CNP22 (K4R)

<400> SEQUENCE: 35

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 36
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: GANRR-CNP22(K4R) - CNP27(Arg4)

<400> SEQUENCE: 36

Gly Ala Asn Arg Arg Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu
1 5 10 15

Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25

<210> SEQ ID NO 37
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: GANPR-CNP22(K4R) - CNP27(Pro4) - Analog CI

<400> SEQUENCE: 37

Gly Ala Asn Pro Arg Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu
1 5 10 15

Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25

<210> SEQ ID NO 38
<211> LENGTH: 24
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: ER-CNP22

<400> SEQUENCE: 38

Glu Arg Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile
1 5 10 15

Gly Ser Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 39
<211> LENGTH: 24
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

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<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BA - ER-CNP22(K4R)

<400> SEQUENCE: 39

Glu Arg Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile
1 5 10 15

Gly Ser Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 40
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: R-CNP22

<400> SEQUENCE: 40

Arg Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly
1 5 10 15

Ser Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 41
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog AZ - R-CNP22(K4R)

<400> SEQUENCE: 41

Arg Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly
1 5 10 15

Ser Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 42
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: CNP22 (K4R)(K10R)

<400> SEQUENCE: 42

Gly Leu Ser Arg Gly Cys Phe Gly Leu Arg Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 43
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: CNP-22 (Cys6-Phe7 variable bond)

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<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -CH2-NH-
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -C(=O)-N(R)- where R is methyl, ethyl, n-propyl, isopropyl, cyclopropyl, n-butyl, isobutyl, sec-butyl or tert-butyl
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -C(=O)-NH-CH2-
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -CH2-S-
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -CH2-S(O)n where n is 1 or 2
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -CH2-CH2-
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -CH=CH-
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -C(=O)-CH2-
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -CH(CN)-NH-
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -CH(OH)-CH2-
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -O-C(=O)-NH-
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with NHC(=O)NH-

<400> SEQUENCE: 43

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 44
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES

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<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Xaa is 3-amino-2-phenylpropionic acid or D-Phe

<400> SEQUENCE: 44

Gly Leu Ser Lys Gly Cys Xaa Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 45
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: CNP22 - modified Cys6
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(6)
<223> OTHER INFORMATION: Xaa is Cys, homocysteine, penicillamine, 2-mercaptopropionic acid, or 3-mercaptopropionic acid.

<400> SEQUENCE: 45

Gly Leu Ser Lys Gly Xaa Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 46
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Gly is optionally modified with a bone or cartilage targeting compound, a biphosphonate, polyAsp, polyGlu, osteopontin, osteocalcin, or sialoprotein peptides, polymers, PEG, carbohydrates, hydrophobic acids, or amino acids
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: Xaa is Lys or a conservative amino acid substitution thereof, any natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amine on a side chain, Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser, or Glu
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Xaa is Cys with peptide bond isosteres between positions 6 and 7
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Xaa is L-Phe, D-Phe, 3-amino-2-phenylpropionic acid, or N-alkylated derivatives of Phe
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Xaa is Gly, tert-butyl-Gly (tBu-Gly), Thr, Ser, Val or Asn
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: Xaa is Leu, Ser, Thr or peptide bond isosteres thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES

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<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is Lys or a conservative amino acid substitution thereof, any natural or unnatural amino acid, Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (11)..(11)
<223> OTHER INFORMATION: Xaa is Leu or peptide bond isosteres thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (14)..(14)
<223> OTHER INFORMATION: Xaa is Ile, tBu-Gly and peptide bond isosteres thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (17)..(17)
<223> OTHER INFORMATION: Xaa is Met, Val, Asn, beta-Cl-Ala, 2-aminobutyric acid (Abu) or 2-amino-isobutyric acid (Aib)
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Xaa is Leu, norleucine (Nle), homoleucine (Hleu), Val, tert-butyl-Ala (tBu-Ala), Ser, Thr, Arg or peptide bond isosteres thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (22)..(22)
<223> OTHER INFORMATION: Cys is optionally modified with a bone or cartilage targeting compound, a bisphosphonate, polyAsp, polyGlu, osteopontin, osteocalcin or sialoprotein peptides, or amino acid sequences derived from non-CNP polypeptides

<400> SEQUENCE: 46

Gly Leu Ser Xaa Gly Xaa Xaa Xaa Xaa Xaa Xaa Asp Arg Xaa Gly Ser
1 5 10 15

Xaa Ser Gly Xaa Gly Cys
20

<210> SEQ ID NO 47
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Gly is optionally modified with a peptide sequence containing one to five amino acids from a natriuretic or non-natriuretic polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Gly is optionally modified with a peptide sequence containing one to five amino acids from a natriuretic or non-natriuretic polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: Xaa is Lys or a conservative amino acid substitution thereof, any natural or unnatural amino acid or peptidomimetic, Arg, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser, or Glu
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(6)
<223> OTHER INFORMATION: Xaa is Cys or decarboxy Cys
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Peptide bond between positions 6 and 7 may be replaced with -CH2-NH-
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Peptide bond between positions 6 and 7 may be

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      replaced with -C(=O)-N(R)- where R is methyl, ethyl, n-propyl,
      isopropyl, cyclopropyl, n-butyl, isobutyl, sec-butyl or tert-butyl
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Peptide bond between positions 6 and 7 may be
      replaced with -C(=O)-NH-CH2-
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Peptide bond between positions 6 and 7 may be
      replaced with -CH2-S-
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Peptide bond between positions 6 and 7 may be
      replaced with -CH2-S(O)n where n is 1 or 2
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Peptide bond between positions 6 and 7 may be
      replaced with -CH2-CH2-
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Peptide bond between positions 6 and 7 may be
      replaced with -CH=CH-
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Peptide bond between positions 6 and 7 may be
      replaced with -C(=O)-CH2-
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Peptide bond between positions 6 and 7 may be
      replaced with -CH(CN)-NH-
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Peptide bond between positions 6 and 7 may be
      replaced with -CH(OH)-CH2-
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Peptide bond between positions 6 and 7 may be
      replaced with -O-C(=O)-NH-
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Peptide bond between positions 6 and 7 may be
      replaced with -NHC(=O)NH-
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Xaa is L-Phe, D-Phe, 3-amino-2-phenylpropionic
      acid, or N-alkylated derivatives of Phe
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Xaa is Gly, tert-butyl-Gly (tBu-Gly), Val, Ser,
      Thr or Asn
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: Xaa is Leu, Ser, Thr or peptide bond isosteres
      thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is Arg, Gly, 6-hydroxy-norleucine,
      citrulline (Cit), Gln, Ser or Glu
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (11)..(11)
<223> OTHER INFORMATION: Xaa is Leu or peptide isosteres thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (14)..(14)
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-continued

<223> OTHER INFORMATION: Xaa is Ile, tBu-Gly or peptide isosteres thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (17)..(17)
<223> OTHER INFORMATION: Xaa is Met, Val, Asn, beta-Cl-Ala, 2-aminobutyric acid (Abu) or 2-amino-isobutyric acid (Aib)
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Xaa is Leu, norleucine (Nle), homoleucine (Hleu), Val, tert-butyl-Ala (tBu-Ala), Ser, Thr, Arg, or peptide bond isosteres thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (22)..(22)
<223> OTHER INFORMATION: Cys is optionally modified with a synthetic bone targeting compound, a bisphosphonate, polyAsp, polyGlu, osteopontin, osteocalcin or sialoprotein peptides, or charged PEG molecules

<400> SEQUENCE: 47

Gly Leu Ser Xaa Gly Xaa Xaa Xaa Xaa Xaa Xaa Asp Arg Xaa Gly Ser
1 5 10 15

Xaa Ser Gly Xaa Gly Cys
20

<210> SEQ ID NO 48
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Xaa is a synthetic or natural polymeric group or combination thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is absent or may be one or more amino acids from Gly-Leu-Ser-Lys-Gly and/or substitutions at one or more of these positions
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Xaa is Lys, a conservative amino acid substitution, any natural or unnatural amino acid or peptidomimetic that does have a reactive primary amine on a side chain
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (19)..(19)
<223> OTHER INFORMATION: Cys is optionally modified with a synthetic or natural polymeric group or a combination thereof

<400> SEQUENCE: 48

Xaa Xaa Cys Phe Gly Leu Xaa Leu Asp Arg Ile Gly Ser Met Ser Gly
1 5 10 15

Leu Gly Cys

<210> SEQ ID NO 49
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Xaa is absent or may be a peptide fragment or water soluble polymer

-continued

<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is Cys or peptide bond isosteres thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (3)..(3)
<223> OTHER INFORMATION: Xaa is L-Phe, D-Phe, 3-amino-2-phenylpropionic acid or N-alkylated derivatives of Phe
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: Xaa is Gly, tert-butyl-Gly (tBu-Gly), Val, Ser, Thr or Asn
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (5)..(5)
<223> OTHER INFORMATION: Xaa is Leu, Ser, Thr or N-Me-Leu
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(6)
<223> OTHER INFORMATION: Xaa is Lys or a conservative amino acid substitution thereof, any natural or unnatural amino acid, Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Xaa is Leu and peptide bond isosteres thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is Ile, tBu-Gly or peptide bond isosteres thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (13)..(13)
<223> OTHER INFORMATION: Xaa is Met, Val, Asn, beta-Cl-Ala, 2-aminobutyric acid (Abu) or 2-amino-isobutyric acid (Aib)
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (16)..(16)
<223> OTHER INFORMATION: Xaa is Leu, norleucine (Nle), homoleucine (Hleu), Val, tert-butyl-Ala (tBu-Ala), Ser, Thr, Arg or peptide bond isosteres thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (18)..(18)
<223> OTHER INFORMATION: Cys is optionally modified with amino acid sequences useful in bone/cartilage targeting, polyAsp or polyGlu, osteopontin, osteocalcin, sialoprotein or sequences derived from NPPC or non-CNP polypeptides

<400> SEQUENCE: 49

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asp Arg Xaa Gly Ser Xaa Ser Gly Xaa
1 5 10 15

Gly Cys

<210> SEQ ID NO 50
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Gly is optionally modified with bone targeting compound, moieties to reduce renal clearance, charged PEG molecules or hydrophilic polymers
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: Xaa is Lys or a conservative amino acid substitution thereof, any natural or unnatural amino acid, Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu
<220> FEATURE:

-continued

<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(6)
<223> OTHER INFORMATION: Xaa is Cys or peptide isosteres between positions 6 and 7
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Xaa is L-Phe, D-Phe, 3-amino-2-phenylpropionic acid or N-alkylated derivatives of Phe
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Xaa is Gly, tert-butyl-Gly, Thr, Ser, Val or Asn
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: Xaa is Leu, Ser, Thr or peptide bond isosteres thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is Lys or a conservative amino acid substitution thereof, any natural or unnatural amino acid, Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (11)..(11)
<223> OTHER INFORMATION: Xaa is Leu, Asn or peptide bond isosteres thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (14)..(14)
<223> OTHER INFORMATION: Xaa is Ile, tert-butyl-Gly (tBu-Gly), Asn or peptide bond isosteres thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: Xaa is Gly, Arg, Ser or Asn
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (17)..(17)
<223> OTHER INFORMATION: Xaa is Met, Val, Asn, beta-Cl-Ala, 2-aminobutyric acid (Abu) or 2-amino-isobutyric acid (Aib)
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Xaa is Leu, norleucine (Nle), homoleucine (Hleu), Val, tert-butyl-Ala (tBu-Ala), Arg, Thr, Ser or peptide bond isoteres thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (22)..(22)
<223> OTHER INFORMATION: Cys is optionally modified with bone targeting compound, moieties to reduce renal clearance, charged PEG molecules or hydrophilic polymers

<400> SEQUENCE: 50

Gly Leu Ser Xaa Gly Xaa Xaa Xaa Xaa Xaa Xaa Asp Arg Xaa Xaa Ser
1 5 10 15

Xaa Ser Gly Xaa Gly Cys
20

<210> SEQ ID NO 51
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Xaa is Arg or Glu

<400> SEQUENCE: 51

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Xaa Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 52
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (5)..(5)
<223> OTHER INFORMATION: Xaa is Arg, Gln or Ser

<400> SEQUENCE: 52

Gly Leu Ser Lys Xaa Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 53
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: Xaa is Ser, Asn, Arg, or Cit

<400> SEQUENCE: 53

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Xaa Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 54
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (19)..(19)
<223> OTHER INFORMATION: Xaa is Ser, Arg, or Asn

<400> SEQUENCE: 54

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Xaa Leu Gly Cys
20

<210> SEQ ID NO 55
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (21)..(21)
<223> OTHER INFORMATION: Xaa is Ser, Thr, or Arg

<400> SEQUENCE: 55

-continued

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Xaa Cys
20

<210> SEQ ID NO 56
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog A
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (6)..(6)
<223> OTHER INFORMATION: CH2NH

<400> SEQUENCE: 56

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 57
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analogs B
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Xaa replaced with N-Me-Phe

<400> SEQUENCE: 57

Gly Leu Ser Lys Gly Cys Xaa Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 58
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog F
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: tBu

<400> SEQUENCE: 58

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 59
<211> LENGTH: 22

-continued

<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog H
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Xaa is replaced with NHCH2-CH(Phe)CO

<400> SEQUENCE: 59

Gly Leu Ser Lys Gly Cys Xaa Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 60
<211> LENGTH: 37
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BL - CNP37

<400> SEQUENCE: 60

Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly
1 5 10 15

Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met
20 25 30

Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 61
<211> LENGTH: 35
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CA

<400> SEQUENCE: 61

Ala Ala Trp Ala Arg Leu Leu Gln Glu His Pro Asn Ala Gly Leu Ser
1 5 10 15

Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly
20 25 30

Leu Gly Cys
35

<210> SEQ ID NO 62
<211> LENGTH: 36
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CB

<400> SEQUENCE: 62

Ala Ala Trp Ala Arg Leu Leu Gln Glu His Pro Asn Ala Arg Gly Leu
1 5 10 15

-continued

Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser
20 25 30

Gly Leu Gly Cys
35

<210> SEQ ID NO 63
<211> LENGTH: 36
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CC

<400> SEQUENCE: 63

Asp Leu Arg Val Asp Thr Lys Ser Arg Ala Ala Trp Ala Arg Gly Leu
1 5 10 15

Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser
20 25 30

Gly Leu Gly Cys
35

<210> SEQ ID NO 64
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: GANQQ-CNP22

<400> SEQUENCE: 64

Gly Ala Asn Gln Gln Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu
1 5 10 15

Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25

<210> SEQ ID NO 65
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: GANRR-CNP22

<400> SEQUENCE: 65

Gly Ala Asn Arg Arg Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu
1 5 10 15

Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25

<210> SEQ ID NO 66
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: GANPR-CNP22

<400> SEQUENCE: 66

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Gly Ala Asn Pro Arg Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu
1 5 10 15

Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25

<210> SEQ ID NO 67
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: GANSS-CNP22

<400> SEQUENCE: 67

Gly Ala Asn Ser Ser Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu
1 5 10 15

Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25

<210> SEQ ID NO 68
<211> LENGTH: 32
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequene
<220> FEATURE:
<223> OTHER INFORMATION: Sythetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CD
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: N-terminus BNP tail
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (32)..(32)
<223> OTHER INFORMATION: N-terminus BNP tail

<400> SEQUENCE: 68

Ser Pro Lys Met Val Gln Gly Ser Gly Cys Phe Gly Leu Lys Leu Asp
1 5 10 15

Arg Ile Gly Ser Met Ser Gly Leu Gly Cys Lys Val Leu Arg Arg His
20 25 30

<210> SEQ ID NO 69
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: GANQQ-CNP22(K4R) - Analog CH

<400> SEQUENCE: 69

Gly Ala Asn Gln Gln Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu
1 5 10 15

Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25

<210> SEQ ID NO 70
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

-continued

<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: GANSS-CNP22(K4R) - Analog CG

<400> SEQUENCE: 70

Gly Ala Asn Ser Ser Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu
1 5 10 15

Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25

<210> SEQ ID NO 71
<211> LENGTH: 38
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CS

<400> SEQUENCE: 71

Gly Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Pro Lys
1 5 10 15

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
20 25 30

Met Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 72
<211> LENGTH: 38
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CT

<400> SEQUENCE: 72

Gly Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Gln Lys
1 5 10 15

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
20 25 30

Met Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 73
<211> LENGTH: 38
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CU

<400> SEQUENCE: 73

Gly Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Gln Gln
1 5 10 15

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
20 25 30

Met Ser Gly Leu Gly Cys
35

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<210> SEQ ID NO 74
<211> LENGTH: 38
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CW

<400> SEQUENCE: 74

Gly Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Pro
1 5 10 15

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
20 25 30

Met Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 75
<211> LENGTH: 38
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 75

Gly Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys
1 5 10 15

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
20 25 30

Met Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 76
<211> LENGTH: 37
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CQ

<400> SEQUENCE: 76

Gly His His Ser His Glu Gln His Pro His Gly Ala Asn Gln Gln Gly
1 5 10 15

Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met
20 25 30

Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 77
<211> LENGTH: 38
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CR

<400> SEQUENCE: 77

Gly Ala His His Pro His Glu His Asp Thr His Gly Ala Asn Gln Gln
1 5 10 15

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser

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```
            20                  25                  30

Met Ser Gly Leu Gly Cys
        35


<210> SEQ ID NO 78
<211> LENGTH: 37
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequencce
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CX

<400> SEQUENCE: 78

Gly His His Ser His Glu Gln His Pro His Gly Ala Asn Pro Arg Gly
1               5                   10                  15

Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met
            20                  25                  30

Ser Gly Leu Gly Cys
        35


<210> SEQ ID NO 79
<211> LENGTH: 36
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CF

<400> SEQUENCE: 79

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gly Leu
1               5                   10                  15

Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser
            20                  25                  30

Gly Leu Gly Cys
        35


<210> SEQ ID NO 80
<211> LENGTH: 39
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CY

<400> SEQUENCE: 80

Gly Gln His Lys Asp Asp Asn Pro Asn Leu Pro Arg Gly Ala Asn Pro
1               5                   10                  15

Arg Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly
            20                  25                  30

Ser Met Ser Gly Leu Gly Cys
        35


<210> SEQ ID NO 81
<211> LENGTH: 37
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
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<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CE

<400> SEQUENCE: 81

Gly Glu Arg Ala Phe Lys Ala Trp Ala Val Ala Arg Leu Ser Gln Gly
1 5 10 15

Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met
20 25 30

Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 82
<211> LENGTH: 35
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CZ

<400> SEQUENCE: 82

Phe Gly Ile Pro Met Asp Arg Ile Gly Arg Asn Pro Arg Gly Leu Ser
1 5 10 15

Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly
20 25 30

Leu Gly Cys
35

<210> SEQ ID NO 83
<211> LENGTH: 40
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog DA

<400> SEQUENCE: 83

Gly Lys Arg Thr Gly Gln Tyr Lys Leu Gly Ser Lys Thr Gly Pro Gly
1 5 10 15

Pro Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile
20 25 30

Gly Ser Met Ser Gly Leu Gly Cys
35 40

<210> SEQ ID NO 84
<211> LENGTH: 37
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CK

<400> SEQUENCE: 84

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Gly Ala Asn Gln Gln Gly
1 5 10 15

Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met
20 25 30

Ser Gly Leu Gly Cys
35

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<210> SEQ ID NO 85
<211> LENGTH: 36
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CL

<400> SEQUENCE: 85

Gly Val Pro Gln Val Ser Thr Ser Thr Gly Ala Asn Gln Gln Gly Leu
1 5 10 15

Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser
20 25 30

Gly Leu Gly Cys
35

<210> SEQ ID NO 86
<211> LENGTH: 37
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CM

<400> SEQUENCE: 86

Gly Gln Pro Ser Ser Ser Ser Gln Ser Thr Gly Ala Asn Gln Gln Gly
1 5 10 15

Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met
20 25 30

Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 87
<211> LENGTH: 37
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CN

<400> SEQUENCE: 87

Gly Gln Thr His Ser Ser Gly Thr Gln Ser Gly Ala Asn Gln Gln Gly
1 5 10 15

Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met
20 25 30

Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 88
<211> LENGTH: 37
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CO

<400> SEQUENCE: 88

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Gly Ser Thr Gly Gln Trp His Ser Glu Ser Gly Ala Asn Gln Gln Gly
1 5 10 15

Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met
20 25 30

Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 89
<211> LENGTH: 37
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CP

<400> SEQUENCE: 89

Gly Ser Ser Ser Ser Ser Ser Ser Ser Ser Gly Ala Asn Gln Gln Gly
1 5 10 15

Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met
20 25 30

Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 90
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Gly is optionally modified with bone targeting compound, a bisphosphonates, polyAsp, polyGlu, osteopontin, osteocalcin, or sialoprotein peptides, negatively charged PEG molecules or natural polymers
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(6)
<223> OTHER INFORMATION: Xaa is Cys or another natural or unnatural amino acid
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Xaa is Phe or another natural or unnatural amino acid
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Xaa is Gly, a larger natural or unnatural amino acid or peptidomimetic
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (22)..(22)
<223> OTHER INFORMATION: Cys is optionally modified with bone targeting compound, a bisphosphonates, polyAsp, polyGlu, osteopontin, osteocalcin, or sialoprotein peptides, negatively charged PEG molecules or natural polymers

<400> SEQUENCE: 90

Gly Leu Ser Lys Gly Xaa Xaa Xaa Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 91

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<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog J
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(6)
<223> OTHER INFORMATION: Xaa is CH2-NH
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: N-Me
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: N-Me

<400> SEQUENCE: 91

Gly Leu Ser Lys Gly Xaa Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 92
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog K
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: N-Me
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: N-Me

<400> SEQUENCE: 92

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 93
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog L
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: N-Me
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (11)..(11)
<223> OTHER INFORMATION: N-Me
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: N-Me

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<400> SEQUENCE: 93

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 94
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog M
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: N-Me
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (11)..(11)
<223> OTHER INFORMATION: N-Me

<400> SEQUENCE: 94

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 95
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog Z

<400> SEQUENCE: 95

Gly Leu Ser Arg Gly Cys Tyr Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 96
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog AA

<400> SEQUENCE: 96

Gly Leu Ser Arg Gly Cys Phe Val Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 97
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog AB

<400> SEQUENCE: 97

Gly Leu Ser Arg Gly Cys Phe Ser Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 98
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog AC

<400> SEQUENCE: 98

Gly Leu Ser Arg Gly Cys Phe Thr Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 99
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog AD

<400> SEQUENCE: 99

Gly Leu Ser Arg Gly Cys Phe Gly Thr Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 100
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog AE

<400> SEQUENCE: 100

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Arg Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 101
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE

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<223> OTHER INFORMATION: Analog AF
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: Xaa is Cit

<400> SEQUENCE: 101

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Xaa Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 102
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog AG

<400> SEQUENCE: 102

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Val Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 103
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog AJ

<400> SEQUENCE: 103

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Val Gly Cys
20

<210> SEQ ID NO 104
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog AK
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Xaa is tBu-Ala

<400> SEQUENCE: 104

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Xaa Gly Cys
20

<210> SEQ ID NO 105
<211> LENGTH: 22
<212> TYPE: PRT

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog AT

<400> SEQUENCE: 105

Glu Leu Ser Glu Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 106
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog AV
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: N-terminus modified with pentanoic acid

<400> SEQUENCE: 106

Glu Leu Ser Glu Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 107
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog AW
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Heptanoic acid modifying N-terminus

<400> SEQUENCE: 107

Glu Leu Ser Glu Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 108
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BB
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: N-terminus modified with heptanoic acid

<400> SEQUENCE: 108

Glu Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser

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```
1               5                   10                  15

Met Ser Gly Leu Gly Cys
            20


<210> SEQ ID NO 109
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BC
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: N-terminus modified with pentanoic acid

<400> SEQUENCE: 109

Glu Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1               5                   10                  15

Met Ser Gly Leu Gly Cys
            20


<210> SEQ ID NO 110
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BF
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is Cit

<400> SEQUENCE: 110

Gly Leu Ser Arg Gly Cys Phe Gly Leu Xaa Leu Asp Arg Ile Gly Ser
1               5                   10                  15

Met Ser Gly Leu Gly Cys
            20


<210> SEQ ID NO 111
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BG

<400> SEQUENCE: 111

Gly Leu Ser Arg Gly Cys Phe Gly Leu Gln Leu Asp Arg Ile Gly Ser
1               5                   10                  15

Met Ser Gly Leu Gly Cys
            20


<210> SEQ ID NO 112
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
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<223> OTHER INFORMATION: Analog BH

<400> SEQUENCE: 112

Gly Leu Ser Arg Gly Cys Phe Gly Leu Arg Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 113
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BJ

<400> SEQUENCE: 113

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Asn Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 114
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BK

<400> SEQUENCE: 114

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Ser Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 115
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog N
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(22)
<223> OTHER INFORMATION: All D-amino acids

<400> SEQUENCE: 115

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 116
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:

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<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BD
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: N-Me

<400> SEQUENCE: 116

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 117
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BN
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (11)..(11)
<223> OTHER INFORMATION: N-Me

<400> SEQUENCE: 117

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 118
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BE
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: N-Me

<400> SEQUENCE: 118

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 119
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog V
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: 3,4-dichloro

<400> SEQUENCE: 119

Gly Leu Ser Gly Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser

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```
1               5                   10                  15

Met Ser Gly Leu Gly Cys
            20


<210> SEQ ID NO 120
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog X
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: 3-methyl

<400> SEQUENCE: 120

Gly Leu Ser Gly Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1               5                   10                  15

Met Ser Gly Leu Gly Cys
            20


<210> SEQ ID NO 121
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BP

<400> SEQUENCE: 121

Gly Leu Ser Arg Ser Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1               5                   10                  15

Met Ser Gly Leu Gly Cys
            20


<210> SEQ ID NO 122
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BO

<400> SEQUENCE: 122

Gly Leu Ser Arg Arg Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1               5                   10                  15

Met Ser Gly Leu Gly Cys
            20


<210> SEQ ID NO 123
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog P

<400> SEQUENCE: 123
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Gly Leu Ser Gly Gly Cys Phe Gly Leu Arg Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 124
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BY

<400> SEQUENCE: 124

Gly Leu Ser Arg Gly Cys Phe Gly Leu Ser Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 125
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BZ

<400> SEQUENCE: 125

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Gln
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 126
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BX

<400> SEQUENCE: 126

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Asn Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 127
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BQ

<400> SEQUENCE: 127

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

-continued

Met Ser Ser Leu Gly Cys
20

<210> SEQ ID NO 128
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BR

<400> SEQUENCE: 128

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Arg Leu Gly Cys
20

<210> SEQ ID NO 129
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BS

<400> SEQUENCE: 129

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Arg Gly Cys
20

<210> SEQ ID NO 130
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BT

<400> SEQUENCE: 130

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Ser Cys
20

<210> SEQ ID NO 131
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BU

<400> SEQUENCE: 131

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Thr Cys
20

-continued

<210> SEQ ID NO 132
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BW

<400> SEQUENCE: 132

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Arg Cys
20

<210> SEQ ID NO 133
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CJ

<400> SEQUENCE: 133

Arg Ser Ser Cys Phe Gly Gly Arg Ile Asp Arg Ile Gly Ala Cys
1 5 10 15

<210> SEQ ID NO 134
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: N-terminal linked to CNP22 by disuccinimidyl glutarate

<400> SEQUENCE: 134

Gly Leu Ser Arg Gly Cys Phe Gly Leu Arg Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 135
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: N-terminal linked to CNP22 by Bis-PEO5

<400> SEQUENCE: 135

Gly Leu Ser Arg Gly Cys Phe Gly Leu Arg Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 136
<211> LENGTH: 22

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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog E
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Xaa is D-Phe

<400> SEQUENCE: 136

Gly Leu Ser Lys Gly Cys Xaa Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 137
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Sythetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog G
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Xaa is 3-Cl-Phe

<400> SEQUENCE: 137

Gly Leu Ser Lys Gly Cys Xaa Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 138
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Xaa comprises one or more amino acids selected from Gly-Leu-Ser-Lys-Gly or substitutions at one or more of these amino acids and further comprises a hydrophilic or water soluable polymer

<400> SEQUENCE: 138

Xaa Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu
1 5 10 15

Gly Cys

<210> SEQ ID NO 139
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Cys is modified with a natural and/or synthetic polymer
<220> FEATURE:
<221> NAME/KEY: MOD_RES

-continued

<222> LOCATION: (17)..(17)
<223> OTHER INFORMATION: Cys is modified with a natural and/or synthetic polymer

<400> SEQUENCE: 139

Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly
1 5 10 15

Cys

<210> SEQ ID NO 140
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Gly is modified with a natural and/or synthetic polymer
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (22)..(22)
<223> OTHER INFORMATION: Cys is modified with a natural and/or synthetic polymer

<400> SEQUENCE: 140

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 141
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Cys is modified with a natural and/or synthetic polymeric group or combination thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (5)..(5)
<223> OTHER INFORMATION: Xaa is Lys or a conservative amino acid substitution thereof, any natural or unnatural amino acid, Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Glu or Ser
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (17)..(17)
<223> OTHER INFORMATION: Cys is optionally modified with a natural and/or synthetic polymeric group or combination thereof

<400> SEQUENCE: 141

Cys Phe Gly Leu Xaa Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly
1 5 10 15

Cys

<210> SEQ ID NO 142
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Xaa is optionally modified with bone- or

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cartilage-targeting moieties, moieties that reduce renal clearance, hydrophilic polymers, carbohydrates, hydrophobic acids and combinations thereof

<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is absent or may comprise 1 to 5 amino acids from Gly-Leu-Ser-Lys-Gly, optionally substituted with a natural or unnatural amino acid
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Xaa is Lys, any natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amine on a side chain
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (19)..(19)
<223> OTHER INFORMATION: Xaa is optionally modified with bone- or cartilage-targeting moieties, moieties that reduce renal clearance, hydrophilic polymers, carbohydrates, hydrophobic acids and combinations thereof

<400> SEQUENCE: 142

Xaa Xaa Cys Phe Gly Leu Xaa Leu Asp Arg Ile Gly Ser Met Ser Gly
1 5 10 15

Leu Gly Cys

<210> SEQ ID NO 143
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Gly is optionally modified with cartilage or bone targeting compounds, a bisphosphonate, polyAsp, polyGlu, osteopontin peptides, osteocalcin peptides, sialoprotein peptides, charged PEG molecules or hydrophilic polymers
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: Xaa is Lys or a conservative amino acid substitution thereof, any natural or unnatural amino acid, Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(6)
<223> OTHER INFORMATION: Xaa is Cys or peptide isosteres between positions 6 and 7
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Xaa is L-Phe, D-Phe, N-alkylated Phe, or Phe analog
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Xaa is Gly, tert-butyl-Gly, Thr, Ser, Val or Asn
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: Xaa is Leu, Ser, Thr or peptide bond isosteres thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is Lys or a conservative amino acid substitution thereof, any natural or unnatural amino acid, Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (11)..(11)

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<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (14)..(14)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: Xaa is Gly, Arg, Ser or Asn
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (17)..(17)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Xaa is Leu, norleucine (Nle), homoleucine (Hleu), Val, tert-butyl-Ala (tBu-Ala), Arg, Thr, Ser or peptide bond isoteres thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (22)..(22)
<223> OTHER INFORMATION: Cys is optionally modified with cartilage or bone targeting compounds, a bisphosphonate, polyAsp, polyGlu, osteopontin peptides, osteocalcin peptides, sialoprotein peptides, charged PEG molecules or hydrophilic polymers

<400> SEQUENCE: 143

Gly Leu Ser Xaa Gly Xaa Xaa Xaa Xaa Xaa Xaa Asp Arg Xaa Xaa Ser
1 5 10 15

Xaa Ser Gly Xaa Gly Cys
20

<210> SEQ ID NO 144
<211> LENGTH: 38
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 144

Gly His Lys Ser Glu Val Ala His Arg Phe Lys Gly Ala Asn Lys Lys
1 5 10 15

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
20 25 30

Met Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 145
<211> LENGTH: 39
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 145

Pro Gly Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys
1 5 10 15

Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly
20 25 30

Ser Met Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 146
<211> LENGTH: 52
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

-continued

<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 146

Leu Arg Val Asp Thr Lys Ser Arg Ala Ala Trp Ala Arg Leu Leu Gln
1 5 10 15

Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu
20 25 30

Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser
35 40 45

Gly Leu Gly Cys
50

<210> SEQ ID NO 147
<211> LENGTH: 51
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 147

Arg Val Asp Thr Lys Ser Arg Ala Ala Trp Ala Arg Leu Leu Gln Glu
1 5 10 15

His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser
20 25 30

Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly
35 40 45

Leu Gly Cys
50

<210> SEQ ID NO 148
<211> LENGTH: 50
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 148

Val Asp Thr Lys Ser Arg Ala Ala Trp Ala Arg Leu Leu Gln Glu His
1 5 10 15

Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys
20 25 30

Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu
35 40 45

Gly Cys
50

<210> SEQ ID NO 149
<211> LENGTH: 49
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 149

Asp Thr Lys Ser Arg Ala Ala Trp Ala Arg Leu Leu Gln Glu His Pro
1 5 10 15

Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly
20 25 30

Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly
35 40 45

-continued

Cys

<210> SEQ ID NO 150
<211> LENGTH: 48
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 150

Thr Lys Ser Arg Ala Ala Trp Ala Arg Leu Leu Gln Glu His Pro Asn
1 5 10 15

Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys
20 25 30

Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
35 40 45

<210> SEQ ID NO 151
<211> LENGTH: 47
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 151

Lys Ser Arg Ala Ala Trp Ala Arg Leu Leu Gln Glu His Pro Asn Ala
1 5 10 15

Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe
20 25 30

Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
35 40 45

<210> SEQ ID NO 152
<211> LENGTH: 46
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 152

Ser Arg Ala Ala Trp Ala Arg Leu Leu Gln Glu His Pro Asn Ala Arg
1 5 10 15

Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly
20 25 30

Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
35 40 45

<210> SEQ ID NO 153
<211> LENGTH: 45
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 153

Arg Ala Ala Trp Ala Arg Leu Leu Gln Glu His Pro Asn Ala Arg Lys
1 5 10 15

Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu
20 25 30

Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
35 40 45

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<210> SEQ ID NO 154
<211> LENGTH: 44
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 154

Ala Ala Trp Ala Arg Leu Leu Gln Glu His Pro Asn Ala Arg Lys Tyr
1 5 10 15

Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys
20 25 30

Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
35 40

<210> SEQ ID NO 155
<211> LENGTH: 43
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 155

Ala Trp Ala Arg Leu Leu Gln Glu His Pro Asn Ala Arg Lys Tyr Lys
1 5 10 15

Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu
20 25 30

Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
35 40

<210> SEQ ID NO 156
<211> LENGTH: 42
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 156

Trp Ala Arg Leu Leu Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly
1 5 10 15

Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp
20 25 30

Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
35 40

<210> SEQ ID NO 157
<211> LENGTH: 41
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 157

Ala Arg Leu Leu Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala
1 5 10 15

Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg
20 25 30

Ile Gly Ser Met Ser Gly Leu Gly Cys
35 40

<210> SEQ ID NO 158
<211> LENGTH: 40
<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 158

Arg Leu Leu Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn
1 5 10 15

Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile
20 25 30

Gly Ser Met Ser Gly Leu Gly Cys
35 40

<210> SEQ ID NO 159
<211> LENGTH: 39
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 159

Leu Leu Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys
1 5 10 15

Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly
20 25 30

Ser Met Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 160
<211> LENGTH: 38
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 160

Leu Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys
1 5 10 15

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
20 25 30

Met Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 161
<211> LENGTH: 36
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 161

Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu
1 5 10 15

Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser
20 25 30

Gly Leu Gly Cys
35

<210> SEQ ID NO 162
<211> LENGTH: 35
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

-continued

<400> SEQUENCE: 162

His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser
1 5 10 15

Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly
20 25 30

Leu Gly Cys
35

<210> SEQ ID NO 163
<211> LENGTH: 34
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 163

Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys
1 5 10 15

Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu
20 25 30

Gly Cys

<210> SEQ ID NO 164
<211> LENGTH: 33
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 164

Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly
1 5 10 15

Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly
20 25 30

Cys

<210> SEQ ID NO 165
<211> LENGTH: 32
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 165

Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys
1 5 10 15

Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25 30

<210> SEQ ID NO 166
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 166

Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe
1 5 10 15

Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25 30

<210> SEQ ID NO 167
<211> LENGTH: 30
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 167

Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly
1 5 10 15

Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25 30

<210> SEQ ID NO 168
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 168

Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu
1 5 10 15

Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25

<210> SEQ ID NO 169
<211> LENGTH: 28
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 169

Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys
1 5 10 15

Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25

<210> SEQ ID NO 170
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 170

Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu
1 5 10 15

Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25

<210> SEQ ID NO 171
<211> LENGTH: 26
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 171

Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp
1 5 10 15

Arg Ile Gly Ser Met Ser Gly Leu Gly Cys

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20 25

<210> SEQ ID NO 172
<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 172

Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg
1 5 10 15

Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25

<210> SEQ ID NO 173
<211> LENGTH: 24
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 173

Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile
1 5 10 15

Gly Ser Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 174
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 174

Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly
1 5 10 15

Ser Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 175
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 175

Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met
1 5 10 15

Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 176
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 176

Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser
1 5 10 15

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Gly Leu Gly Cys
20

<210> SEQ ID NO 177
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 177

Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly
1 5 10 15

Leu Gly Cys

<210> SEQ ID NO 178
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 178

Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu
1 5 10 15

Gly Cys

<210> SEQ ID NO 179
<211> LENGTH: 54
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 179

Gly Asp Leu Arg Val Asp Thr Lys Ser Arg Ala Ala Trp Ala Arg Leu
1 5 10 15

Leu Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys
20 25 30

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
35 40 45

Met Ser Gly Leu Gly Cys
50

<210> SEQ ID NO 180
<211> LENGTH: 53
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 180

Asp Leu Arg Val Asp Thr Lys Ser Arg Ala Ala Trp Ala Arg Leu Leu
1 5 10 15

Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly
20 25 30

Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Asn
35 40 45

Ser Gly Leu Gly Cys
50

<210> SEQ ID NO 181

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<211> LENGTH: 38
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 181

Gly Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys
1 5 10 15

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
20 25 30

Asn Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 182
<211> LENGTH: 37
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 182

Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly
1 5 10 15

Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Asn
20 25 30

Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 183
<211> LENGTH: 38
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Sythetic Polypeptide

<400> SEQUENCE: 183

Gly His Lys Ser Glu Val Ala His Arg Phe Lys Gly Ala Asn Lys Lys
1 5 10 15

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
20 25 30

Asn Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 184
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 184

Gly Ala Asn Arg Arg Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu
1 5 10 15

Asp Arg Ile Gly Ser Asn Ser Gly Leu Gly Cys
20 25

<210> SEQ ID NO 185
<211> LENGTH: 54
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

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<400> SEQUENCE: 185

Pro Asp Leu Arg Val Asp Thr Lys Ser Arg Ala Ala Trp Ala Arg Leu
1 5 10 15

Leu Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys
20 25 30

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
35 40 45

Met Ser Gly Leu Gly Cys
50

<210> SEQ ID NO 186
<211> LENGTH: 38
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 186

Pro Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys
1 5 10 15

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
20 25 30

Met Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 187
<211> LENGTH: 34
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 187

Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys
1 5 10 15

Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu
20 25 30

Gly Cys

<210> SEQ ID NO 188
<211> LENGTH: 39
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 188

Pro Gly His Lys Ser Glu Val Ala His Arg Phe Lys Gly Ala Asn Lys
1 5 10 15

Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly
20 25 30

Ser Met Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 189
<211> LENGTH: 28
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 189

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Pro Gly Ala Asn Arg Arg Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys
1 5 10 15

Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25

<210> SEQ ID NO 190
<211> LENGTH: 54
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 190

Met Asp Leu Arg Val Asp Thr Lys Ser Arg Ala Ala Trp Ala Arg Leu
1 5 10 15

Leu Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys
20 25 30

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
35 40 45

Met Ser Gly Leu Gly Cys
50

<210> SEQ ID NO 191
<211> LENGTH: 39
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 191

Met Gly Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys
1 5 10 15

Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly
20 25 30

Ser Met Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 192
<211> LENGTH: 38
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 192

Met Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys
1 5 10 15

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
20 25 30

Met Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 193
<211> LENGTH: 39
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 193

Met Gly His Lys Ser Glu Val Ala His Arg Phe Lys Gly Ala Asn Lys
1 5 10 15

```
Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly
            20                  25                  30

Ser Met Ser Gly Leu Gly Cys
        35


<210> SEQ ID NO 194
<211> LENGTH: 28
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 194

Met Gly Ala Asn Arg Arg Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys
1               5                   10                  15

Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
            20                  25


<210> SEQ ID NO 195
<211> LENGTH: 30
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 195

Met Gly Ser Ser His His His His His His Ser Ser Gly Leu Val Pro
1               5                   10                  15

Arg Gly Ser His Thr Gly Asp Asp Asp Asp Lys His Met Asp
            20                  25                  30


<210> SEQ ID NO 196
<211> LENGTH: 142
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 196

Met Val Leu Thr Lys Lys Lys Leu Gln Asp Leu Val Arg Glu Val Cys
1               5                   10                  15

Pro Asn Glu Gln Leu Asp Glu Asp Val Glu Glu Met Leu Leu Gln Ile
            20                  25                  30

Ala Asp Asp Phe Ile Glu Ser Val Val Thr Ala Ala Cys Gln Leu Ala
        35                  40                  45

Arg His Arg Lys Ser Ser Thr Leu Glu Val Lys Asp Val Gln Leu His
    50                  55                  60

Leu Glu Arg Gln Trp Asn Met Trp Ile Met Gly Ser Ser His His His
65                  70                  75                  80

His His His Ser Ser Gly Leu Val Pro Arg Gly Ser His Thr Gly Asp
                85                  90                  95

Asp Asp Asp Lys His Met Asp Pro Gly Gln Glu His Pro Asn Ala Arg
            100                 105                 110

Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly
        115                 120                 125

Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
    130                 135                 140


<210> SEQ ID NO 197
<211> LENGTH: 72
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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 197

Val Leu Thr Lys Lys Lys Leu Gln Asp Leu Val Arg Glu Val Cys Pro
1 5 10 15

Asn Glu Gln Leu Asp Glu Asp Val Glu Glu Met Leu Leu Gln Ile Ala
20 25 30

Asp Asp Phe Ile Glu Ser Val Val Thr Ala Ala Cys Gln Leu Ala Arg
35 40 45

His Arg Lys Ser Ser Thr Leu Glu Val Lys Asp Val Gln Leu His Leu
50 55 60

Glu Arg Gln Trp Asn Met Trp Ile
65 70

<210> SEQ ID NO 198
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 198

Pro Gly Gln Glu His Pro Asn Ala Arg
1 5

<210> SEQ ID NO 199
<211> LENGTH: 30
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 199

Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly
1 5 10 15

Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25 30

<210> SEQ ID NO 200
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 200

Gly Ala Asn Lys Pro
1 5

<210> SEQ ID NO 201
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 201

Gly Ala Asn Pro Lys
1 5

<210> SEQ ID NO 202

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<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 202

Gly Ala Asn Pro Gln
1 5

<210> SEQ ID NO 203
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 203

Gly Ala Asn Gln Lys
1 5

<210> SEQ ID NO 204
<211> LENGTH: 41
<212> TYPE: PRT
<213> ORGANISM: Triakis scyllium

<400> SEQUENCE: 204

Arg Leu Leu Lys Asp Leu Ser Asn Asn Pro Leu Arg Phe Arg Gly Arg
1 5 10 15

Ser Lys Lys Gly Pro Ser Arg Gly Cys Phe Gly Val Lys Leu Asp Arg
20 25 30

Ile Gly Ala Met Ser Gly Leu Gly Cys
35 40

<210> SEQ ID NO 205
<211> LENGTH: 39
<212> TYPE: PRT
<213> ORGANISM: Triakis scyllium

<400> SEQUENCE: 205

Leu Lys Asp Leu Ser Asn Asn Pro Leu Arg Phe Arg Gly Arg Ser Lys
1 5 10 15

Lys Gly Pro Ser Arg Gly Cys Phe Gly Val Lys Leu Asp Arg Ile Gly
20 25 30

Ala Met Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 206
<211> LENGTH: 38
<212> TYPE: PRT
<213> ORGANISM: Triakis scyllium

<400> SEQUENCE: 206

Lys Asp Leu Ser Asn Asn Pro Leu Arg Phe Arg Gly Arg Ser Lys Lys
1 5 10 15

Gly Pro Ser Arg Gly Cys Phe Gly Val Lys Leu Asp Arg Ile Gly Ala
20 25 30

Met Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 207
<211> LENGTH: 22
<212> TYPE: PRT

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<213> ORGANISM: Triakis scyllium

<400> SEQUENCE: 207

Gly Pro Ser Arg Gly Cys Phe Gly Val Lys Leu Asp Arg Ile Gly Ala
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 208
<211> LENGTH: 41
<212> TYPE: PRT
<213> ORGANISM: Lamna ditropis

<400> SEQUENCE: 208

Arg Leu Leu Lys Asp Leu Ser Asn Asn Pro Leu Arg Phe Lys Gly Arg
1 5 10 15

Ser Lys Lys Gly Pro Ser Arg Gly Cys Phe Gly Val Lys Leu Asp Arg
20 25 30

Ile Gly Ala Met Ser Gly Leu Gly Cys
35 40

<210> SEQ ID NO 209
<211> LENGTH: 39
<212> TYPE: PRT
<213> ORGANISM: Lamna ditropis

<400> SEQUENCE: 209

Leu Lys Asp Leu Ser Asn Asn Pro Leu Arg Phe Lys Gly Arg Ser Lys
1 5 10 15

Lys Gly Pro Ser Arg Gly Cys Phe Gly Val Lys Leu Asp Arg Ile Gly
20 25 30

Ala Met Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 210
<211> LENGTH: 38
<212> TYPE: PRT
<213> ORGANISM: Lamna ditropis

<400> SEQUENCE: 210

Lys Asp Leu Ser Asn Asn Pro Leu Arg Phe Lys Gly Arg Ser Lys Lys
1 5 10 15

Gly Pro Ser Arg Gly Cys Phe Gly Val Lys Leu Asp Arg Ile Gly Ala
20 25 30

Met Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 211
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Lamna ditropis

<400> SEQUENCE: 211

Phe Lys Gly Arg Ser Lys Lys Gly Pro Ser Arg Gly Cys Phe Gly Val
1 5 10 15

Lys Leu Asp Arg Ile Gly Ala Met Ser Gly Leu Gly Cys
20 25

<210> SEQ ID NO 212
<211> LENGTH: 22
<212> TYPE: PRT

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<213> ORGANISM: Lamna ditropis

<400> SEQUENCE: 212

Gly Pro Ser Arg Gly Cys Phe Gly Val Lys Leu Asp Arg Ile Gly Ala
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 213
<211> LENGTH: 41
<212> TYPE: PRT
<213> ORGANISM: Squalus acanthias

<400> SEQUENCE: 213

Arg Leu Leu Gln Asp Leu Ser Asn Asn Pro Leu Arg Phe Lys Gly Arg
1 5 10 15

Ser Lys Lys Gly Pro Ser Arg Ser Cys Phe Gly Leu Lys Leu Asp Arg
20 25 30

Ile Gly Ala Met Ser Gly Leu Gly Cys
35 40

<210> SEQ ID NO 214
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Squalus acanthias

<400> SEQUENCE: 214

Gly Pro Ser Arg Ser Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ala
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 215
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Oryzias latipes

<400> SEQUENCE: 215

Gly Trp Asn Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 216
<211> LENGTH: 24
<212> TYPE: PRT
<213> ORGANISM: Oryzias latipes

<400> SEQUENCE: 216

Pro Met Val Ala Gly Gly Gly Cys Phe Gly Met Lys Met Asp Arg Ile
1 5 10 15

Gly Ser Ile Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 217
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Arothron maculatus

<400> SEQUENCE: 217

Gly Arg Ser Ser Met Val Gly Gly Arg Gly Cys Phe Gly Met Lys Ile
1 5 10 15

-continued

Asp Arg Ile Gly Ser Ile Ser Gly Leu Gly Cys
20 25

<210> SEQ ID NO 218
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Oryzias latipes

<400> SEQUENCE: 218

Gly Gly Met Arg Ser Cys Phe Gly Val Arg Leu Glu Arg Ile Gly Ser
1 5 10 15

Phe Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 219
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Arothron maculatus

<400> SEQUENCE: 219

Gly Gly Leu Arg Ser Cys Phe Gly Val Arg Leu Ala Arg Ile Gly Ser
1 5 10 15

Phe Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 220
<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: Oryzias latipes

<400> SEQUENCE: 220

Gly Gly Ser Thr Ser Arg Ser Gly Cys Phe Gly His Lys Met Asp Arg
1 5 10 15

Ile Gly Thr Ile Ser Gly Met Gly Cys
20 25

<210> SEQ ID NO 221
<211> LENGTH: 24
<212> TYPE: PRT
<213> ORGANISM: Arothron maculatus

<400> SEQUENCE: 221

Gly Gly Ser Ser Arg Ser Gly Cys Phe Gly His Lys Met Asp Arg Ile
1 5 10 15

Gly Thr Ile Ser Gly Met Gly Cys
20

<210> SEQ ID NO 222
<211> LENGTH: 39
<212> TYPE: PRT
<213> ORGANISM: Ornithorhynchus anatinus

<400> SEQUENCE: 222

Leu Leu His Asp His Pro Asn Pro Arg Lys Tyr Lys Pro Ala Asn Lys
1 5 10 15

Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly
20 25 30

Ser Thr Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 223

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<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Ornithorhynchus anatinus

<400> SEQUENCE: 223

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Thr Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 224
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 224

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ala
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 225
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 225

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Gln Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 226
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 226

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Ala Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 227
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 227

Ser Leu Arg Arg Ser Ser Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly
1 5 10 15

Ser Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 228
<211> LENGTH: 27

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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 228

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys Asn Ser Phe Arg Tyr
20 25

<210> SEQ ID NO 229
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
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<400> SEQUENCE: 229

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1 5 10 15

Cys Asn Ser Phe Arg Tyr
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<400> SEQUENCE: 230

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1 5

<210> SEQ ID NO 231
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<210> SEQ ID NO 232
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-continued

<400> SEQUENCE: 232

Glu Xaa Xaa Tyr Xaa Gln Gly
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<210> SEQ ID NO 233
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<400> SEQUENCE: 233

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<400> SEQUENCE: 234

Gly Ser Asp Arg
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<210> SEQ ID NO 235
<211> LENGTH: 4
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<213> ORGANISM: Artificial Sequence
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<400> SEQUENCE: 235

Ile Thr Asp Arg
1

<210> SEQ ID NO 236
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
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<400> SEQUENCE: 236

Pro Gly Asp Arg
1

<210> SEQ ID NO 237
<211> LENGTH: 8
<212> TYPE: PRT
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<400> SEQUENCE: 237

Ala Phe Leu Gly Pro Gly Asp Arg
1 5

What is claimed is:

[1. A variant of C-type natriuretic peptide (CNP) selected from the group consisting of:

(SEQ ID NO: 179)
GDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMS GLGC (Gly-CNP53);

-continued

(SEQ ID NO: 185)
PDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMS GLGC (Pro-CNP53);

(SEQ ID NO: 190)
MDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMS GLGC (Met-CNP53)

-continued

(SEQ ID NO: 180)
DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNS
GLGC [CNP-53(M48N)].

]

2. A pharmaceutical composition comprising a CNP variant of claim **[1]** *15*, and a pharmaceutically acceptable excipient, carrier or diluent.

3. The composition of claim **2**, which is a lyophilized formulation prepared from a formulation that comprises a citric acid/citrate buffer or an acetic acid/acetate buffer having a pH from about 4 to about 6.

4. The composition of claim **3**, wherein the lyophilized formulation is prepared from a formulation that further comprises (a) an isotonicity-adjusting agent or a bulking agent or (b) an antioxidant.

5. A method of treating a bone-related disorder or skeletal dysplasia, comprising administering a CNP variant to a subject in need thereof, wherein the CNP variant is a CNP variant according to claim **[1]** *15*, and wherein the bone-related disorder or skeletal dysplasia is selected from the group consisting of achondroplasia, hypochondroplasia, short stature, dwarfism and homozygous achondroplasia.

[6. A method for recombinant production of a CNP variant, comprising culturing in a medium a host cell comprising a first polynucleotide encoding a CNP variant polypeptide linked to a second polynucleotide encoding a cleavable peptide or protein under conditions that result in expression of a fusion polypeptide encoded by the polynucleotides, wherein the fusion polypeptide comprises the CNP variant polypeptide directly linked to the cleavable peptide or protein or indirectly linked thereto via a linker, wherein the CNP variant is selected from the group consisting of:

(SEQ ID NO: 179)
GDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMS
GLGC (Gly-CNP53);

(SEQ ID NO: 185)
PDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMS
GLGC (Pro-CNP53);

(SEQ ID NO: 190)
MDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMS
GLGC (Met-CNP53);

(SEQ ID NO: 180)
DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNS
GLGC [CNP-53(M48N)].

]

7. The method of claim **[6]** *16*, wherein the cleavable peptide or protein is selected from the group consisting of histidine tags, human transcription factor TAF12, TAF12 fragments, TAF12 histone fold domain, mutants of TAF12 and fragments thereof, TAF12(C/A), TAF12(D/E), TAF12 (4D/4E), TAF12(6D/6E), TAF12(10D/10E), TAF12(C/A & D/E), TAF12(C/A & 4D/4E), TAF12(C/A & 6D/6E), TAF12 (C/A & 10D/10E), ketosteroid isomerase, maltose-binding protein, β-galactosidase, glutathione-S-transferase, thioredoxin, chitin-binding domain, BMP-2, BMP-2 mutants, BMP-2(C/A), and mutants and fragments thereof.

8. The method of claim **[6]** *16*, wherein the host cell is bacterial.

9. The method of claim **[6]** *16*, wherein the fusion polypeptide is expressed as a soluble protein or as an inclusion body.

10. The method of claim **[6]** *16*, further comprising isolating the expressed fusion polypeptide from the host cell or culture medium.

11. The method of claim **10**, further comprising contacting the isolated fusion polypeptide with a cleaving agent selected from the group consisting of formic acid, cyanogen bromide (CNBr), hydroxylamine, protein self cleavage, Factor Xa, enterokinase, ProTEV, and SUMO protease.

[12. A CNP variant produced according to the method of claim **6**, wherein the CNP variant is selected from the group consisting of:

(SEQ ID NO: 179)
GDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMS
GLGC (Gly-CNP53));

(SEQ ID NO: 185)
PDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMS
GLGC (Pro-CNP53));

(SEQ ID NO: 190)
MDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMS
GLGC (Met-CNP53));

(SEQ ID NO: 180)
DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSG
LGC [CNP-53(M48N)]).

]

13. A method of increasing long bone growth, comprising administering a CNP variant according to claim **[1]** *15* to a subject in need thereof, where the administration increases long bone growth.

14. A CNP variant according to claim **[1]** *15* useful for increasing long bone growth or treating achondroplasia, hypochondroplasia, short stature, dwarfism, or homozygous achondroplasia in a subject in need thereof.

15. A variant of C-type natriuretic peptide (CNP) selected from the group consisting of:

GDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSM
SGLGC (SEQ ID NO: 179) (Gly-CNP53);

PDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSM
SGLGC (SEQ ID NO: 185) (Pro-CNP53);

MDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSM
SGLGC (SEQ ID NO: 190) (Met-CNP53);

DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNS
GLGC (SEQ ID NO: 180) [CNP-53(M48N)];

a CNP53 variant comprising a K4R mutation in the CNP22 portion of the peptide;
a CNP53 variant comprising a furin cleavage site mutation;

PEG-GDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDR
IGSMSGLGC (SEQ ID NO: 179) (PEG-Gly-CNP53);

PEG-PDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDR
IGSMSGLGC (SEQ ID NO: 185) (PEG-Pro-CNP53);

PEG-MDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDR
IGSMSGLGC (SEQ ID NO: 190) (PEG-Met-CNP53); and

PEG-DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRI
GSNSGLGC (SEQ ID NO: 180) [PEG-CNP-53(M48N)].

16. A method for recombinant production of a CNP variant, comprising culturing in a medium a host cell comprising a first polynucleotide encoding a CNP variant polypeptide linked to a second polynucleotide encoding a cleavable peptide or protein under conditions that result in expression of a fusion polypeptide encoded by the polynucle-

otides, wherein the fusion polypeptide comprises the CNP variant polypeptide directly linked to the cleavable peptide or protein or indirectly linked thereto via a linker, wherein the CNP variant is selected from the group consisting of:

GDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSM SGLGC (SEQ ID NO: 179) (Gly-CNP53);

PDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSM SGLGC (SEQ ID NO: 185) (Pro-CNP53);

MDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSM SGLGC (SEQ ID NO: 190) (Met-CNP53);

DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNS GLGC (SEQ ID NO: 180) [CNP-53(M48N)];

a CNP53 variant comprising a K4R mutation in the CNP22 portion of the peptide; and

a CNP53 variant comprising a furin cleavage site mutation.

17. A CNP variant produced according to the method of claim 16, wherein the CNP variant is selected from the group consisting of:

GDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSM SGLGC (SEQ ID NO: 179) (Gly-CNP53);

PDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSM SGLGC (SEQ ID NO: 185) (Pro-CNP53);

MDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSM SGLGC (SEQ ID NO: 190) (Met-CNP53);

DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNS GLGC (SEQ ID NO: 180) [CNP-53(M48N)];

a CNP53 variant comprising a K4R mutation in the CNP22 portion of the peptide; and

a CNP53 variant comprising a furin cleavage site mutation.

* * * * *

US008598121B2

(12) **United States Patent**
Wendt et al.

(10) **Patent No.: US 8,598,121 B2**
(45) **Date of Patent: Dec. 3, 2013**

(54) **VARIANTS OF C-TYPE NATRIURETIC PEPTIDE**

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(21) Appl. No.: **13/466,672**

(22) Filed: **May 8, 2012**

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(52) **U.S. Cl.**

USPC **514/12.4**; 514/16.7; 514/21.3; 435/69.4; 435/69.7; 435/70.1; 530/324

(58) **Field of Classification Search**

None

See application file for complete search history.

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(57) **ABSTRACT**

The present disclosure provides variants of C-type natriuretic peptide (CNP), pharmaceutical compositions comprising CNP variants, and methods of making CNP variants. The CNP variants are useful as therapeutic agents for the treatment of diseases responsive to CNP, including but not limited to bone-related disorders, such as skeletal dysplasias (e.g., achondroplasia), and vascular smooth muscle disorders (e.g., restenosis and arteriosclerosis).

14 Claims, 61 Drawing Sheets

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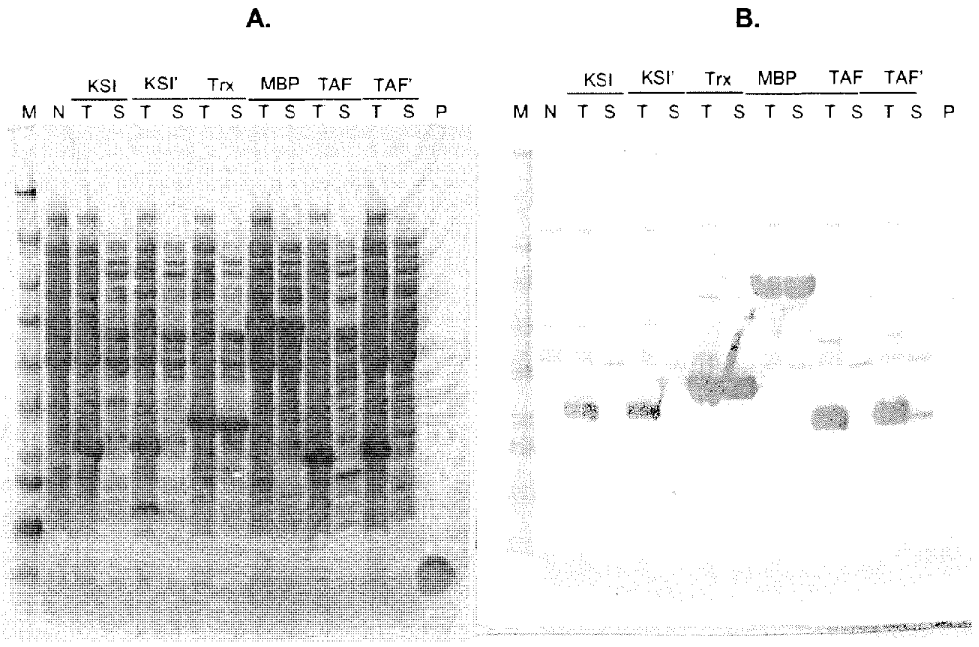
* cited by examiner

FIGURE 1



Coomassie blue stain

Western blot with anti-CNP antibody

FIGURE 2

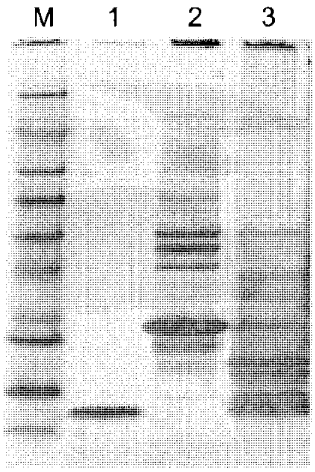


FIGURE 3

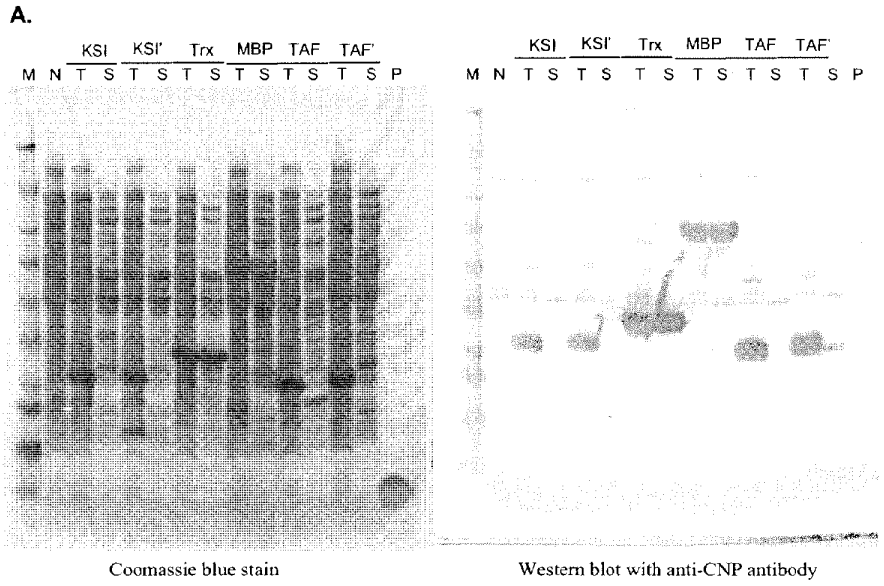

A.
KSI KSI' Trx MBP TAF TAF'
M N T S T S T S T S T S T S P
KSI KSI' Trx MBP TAF TAF'
M N T S T S T S T S T S T S P
Coomassie blue stain
Western blot with anti-CNP antibody
B.
BMP-Pro-CNP37 TAF-Pro-CNP37
M C Tu T S P Tu T S P
BMP-Pro-CNP37 TAF-Pro-CNP37
M C Tu T S P Tu T S P
Coomassie blue stain
Western blot with anti-CNP antibody

FIGURE 3 continued

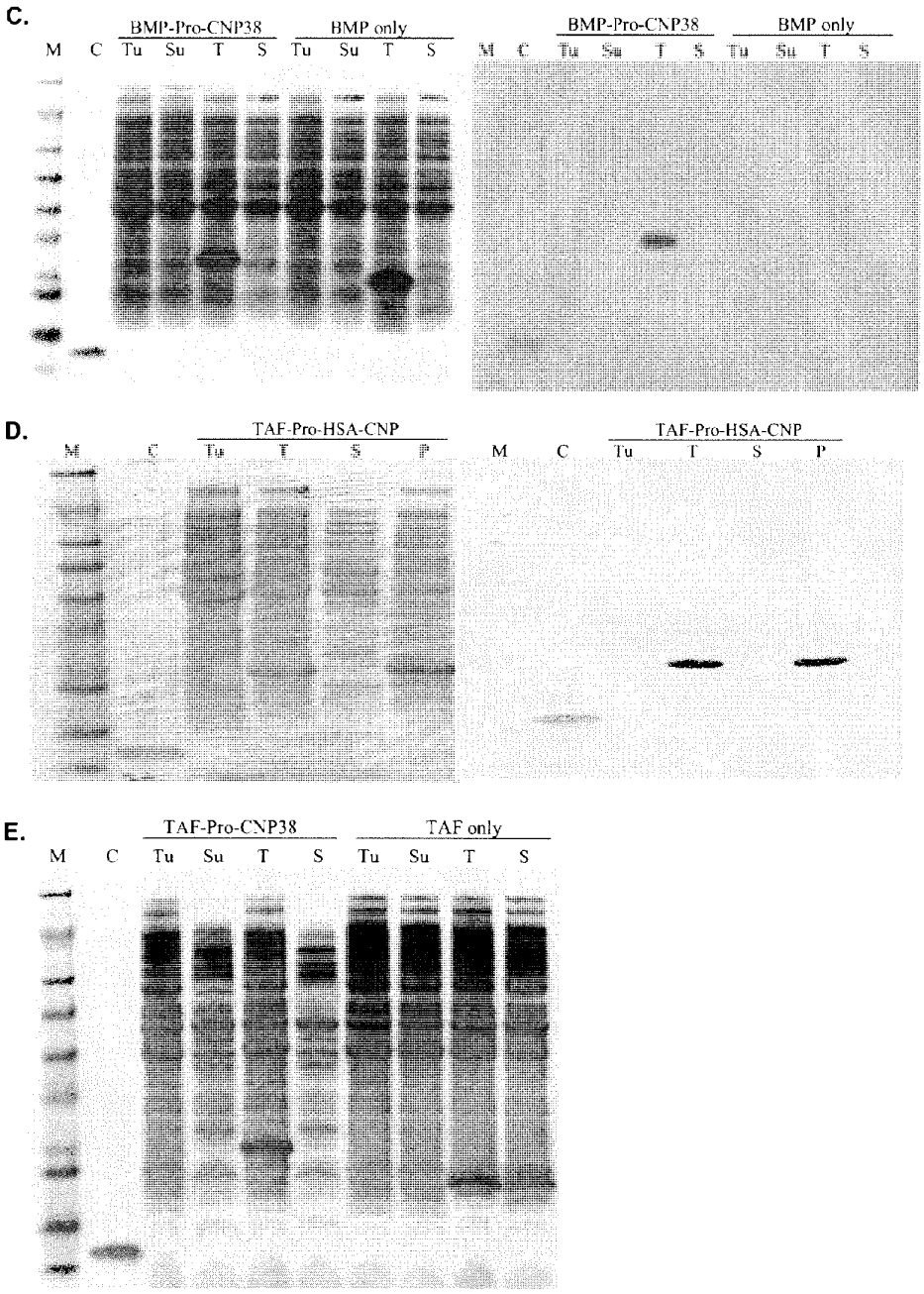


FIGURE 4

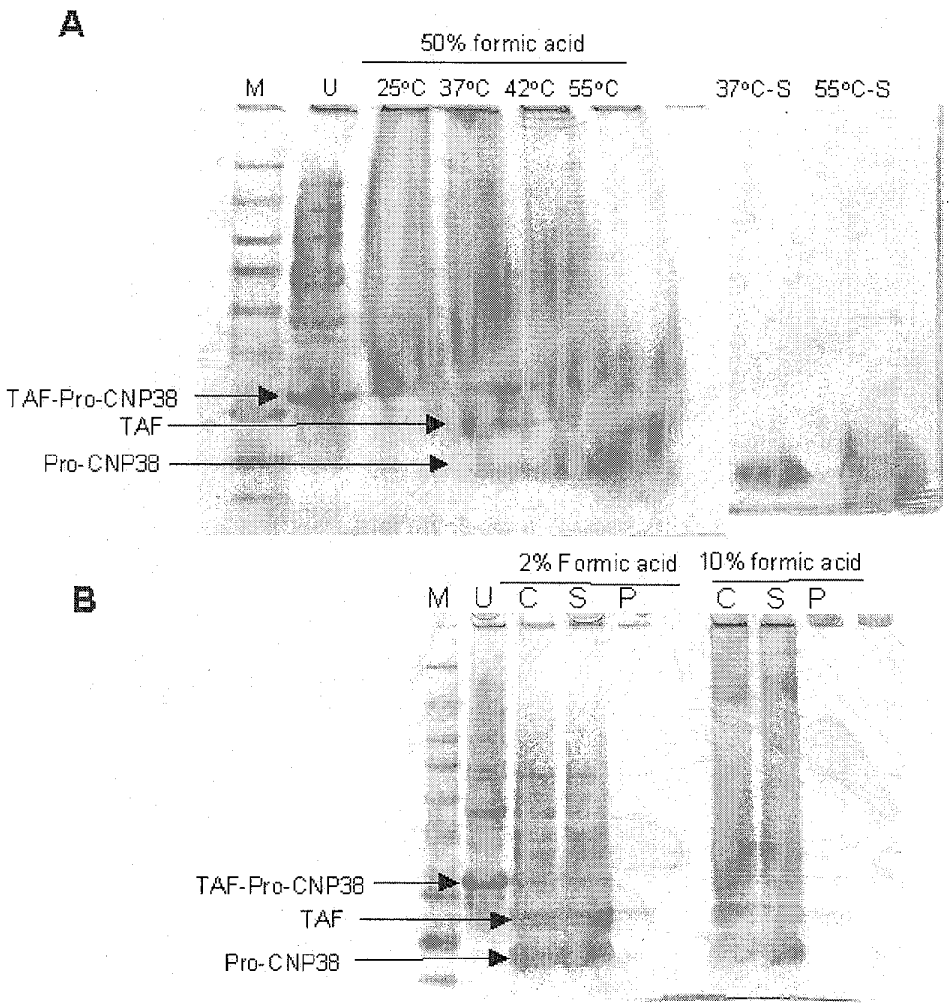

A
50% formic acid
M
U
25°C
37°C
42°C
55°C
37°C-S
55°C-S
TAF-Pro-CNP38
TAF
Pro-CNP38
B
2% Formic acid
10% formic acid
M
U
C
S
P
C
S
P
TAF-Pro-CNP38
TAF
Pro-CNP38

FIGURE 4C

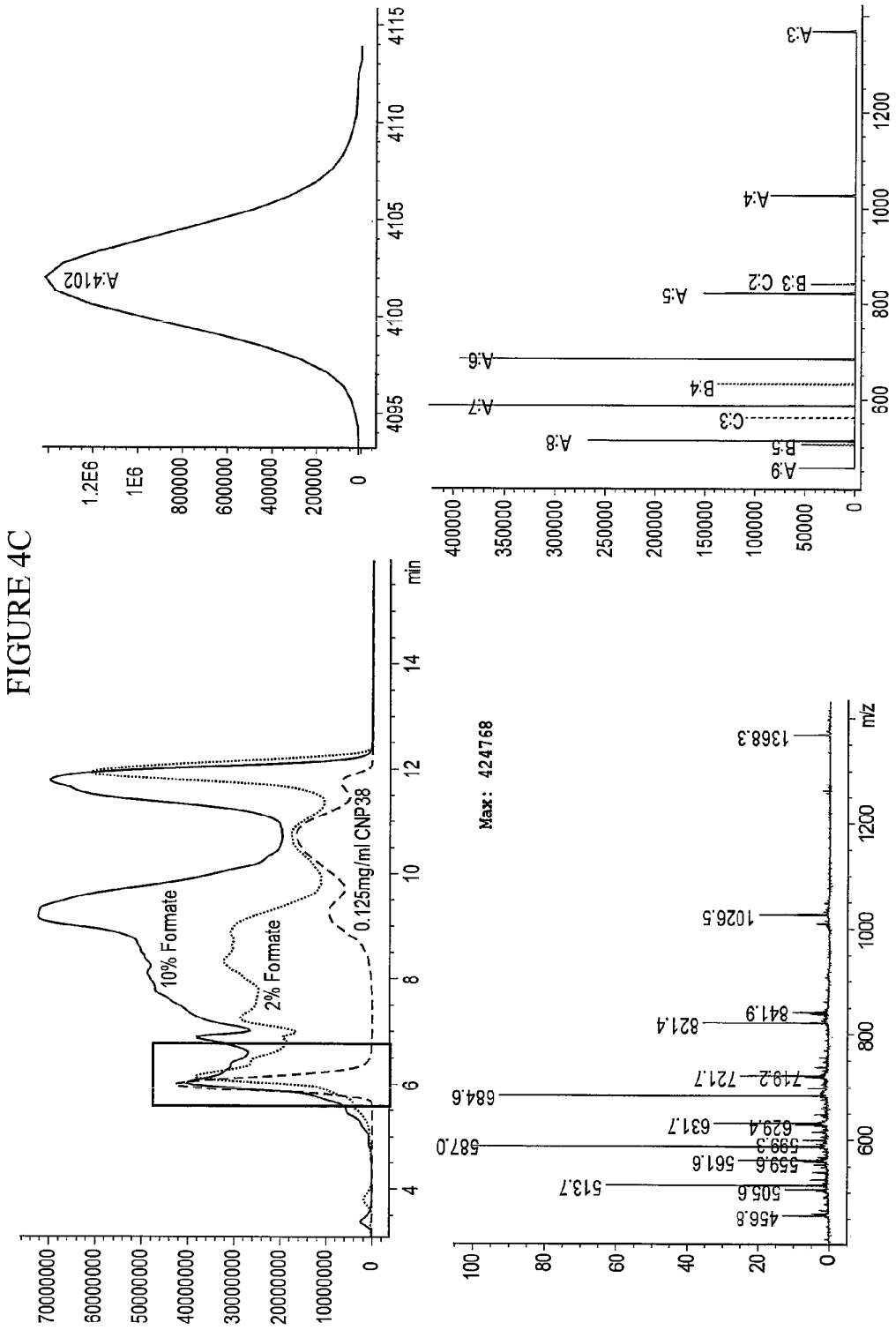

10% Formate
2% Formate
0.125mg/ml CNP38
min
A:4102
Max: 424768
m/z
A:3
A:4
B:3 C:2
A:5
A:6
A:7
B:4
C:3
A:8
B:5
A:9
1368.3
1026.5
841.9
821.4
721.7
719.2
684.6
587.0
631.7
629.4
599.3
561.6
559.6
513.7
505.6
456.8

FIGURE 5

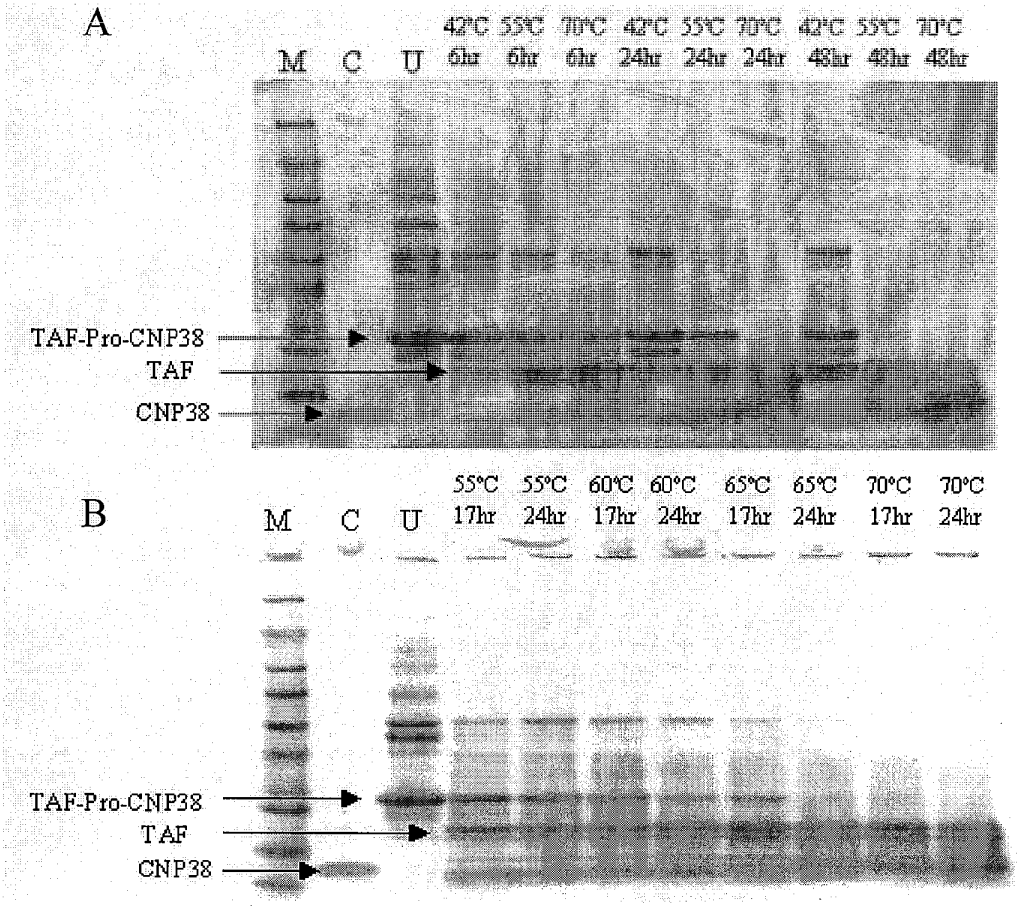

A
M
C
U
42°C 6hr
55°C 6hr
70°C 6hr
42°C 24hr
55°C 24hr
70°C 24hr
42°C 48hr
55°C 48hr
70°C 48hr
TAF-Pro-CNP38
TAF
CNP38
B
M
C
U
55°C 17hr
55°C 24hr
60°C 17hr
60°C 24hr
65°C 17hr
65°C 24hr
70°C 17hr
70°C 24hr
TAF-Pro-CNP38
TAF
CNP38

FIGURE 5C

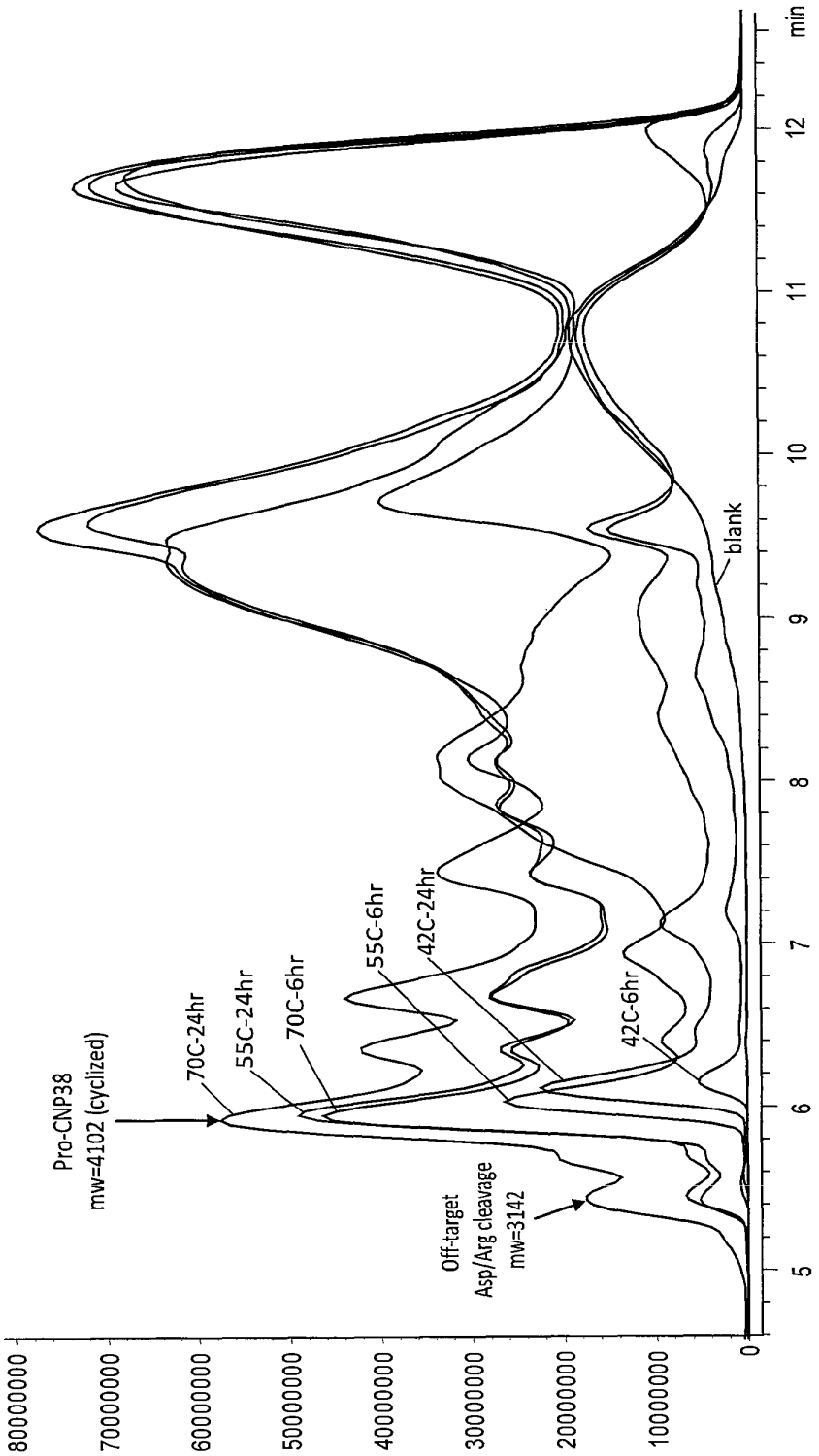

Pro-CNP38
mw=4102 (cyclized)
70C-24hr
55C-24hr
70C-6hr
55C-6hr
42C-24hr
42C-6hr
blank
Off-target
Asp/Arg cleavage
mw=3142
80000000
70000000
60000000
50000000
40000000
30000000
20000000
10000000
0
5
6
7
8
9
10
11
12
min

FIGURE 6

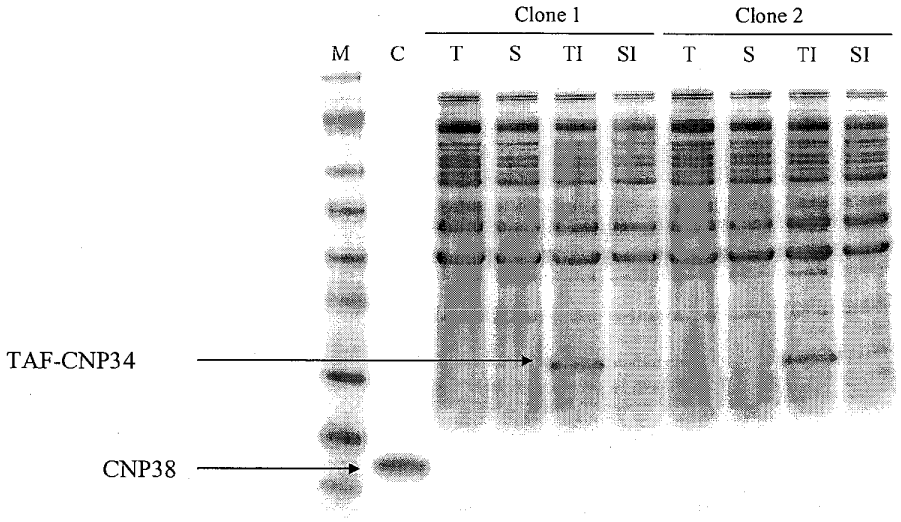


FIGURE 7

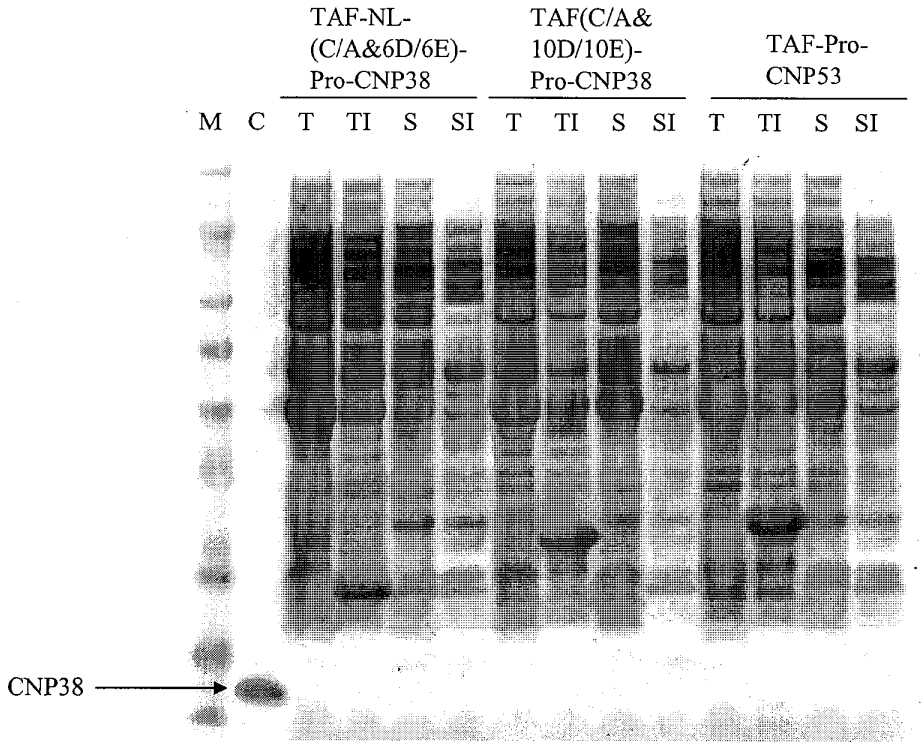


FIGURE 8

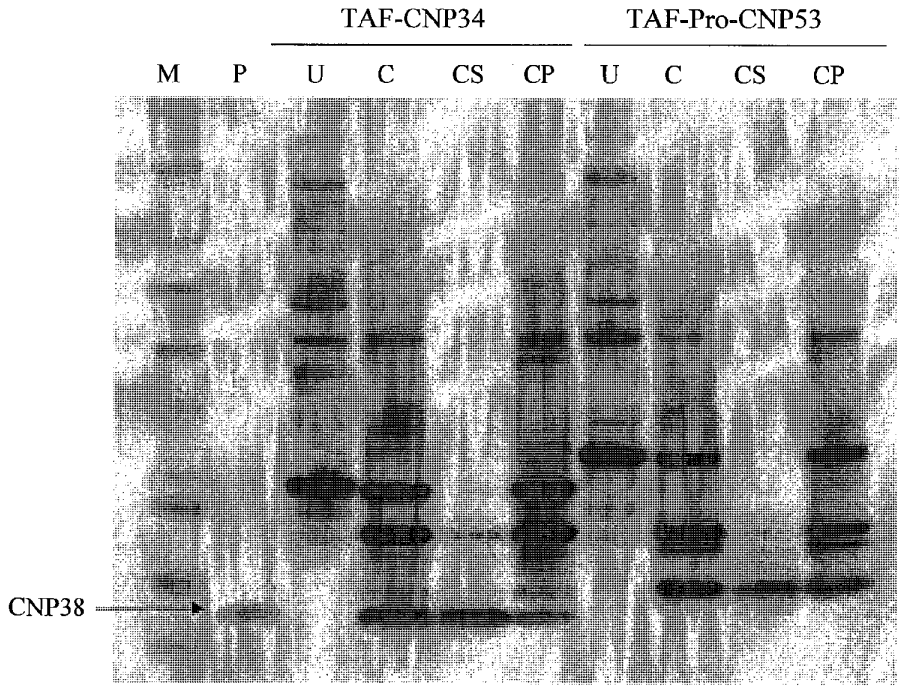


FIGURE 9

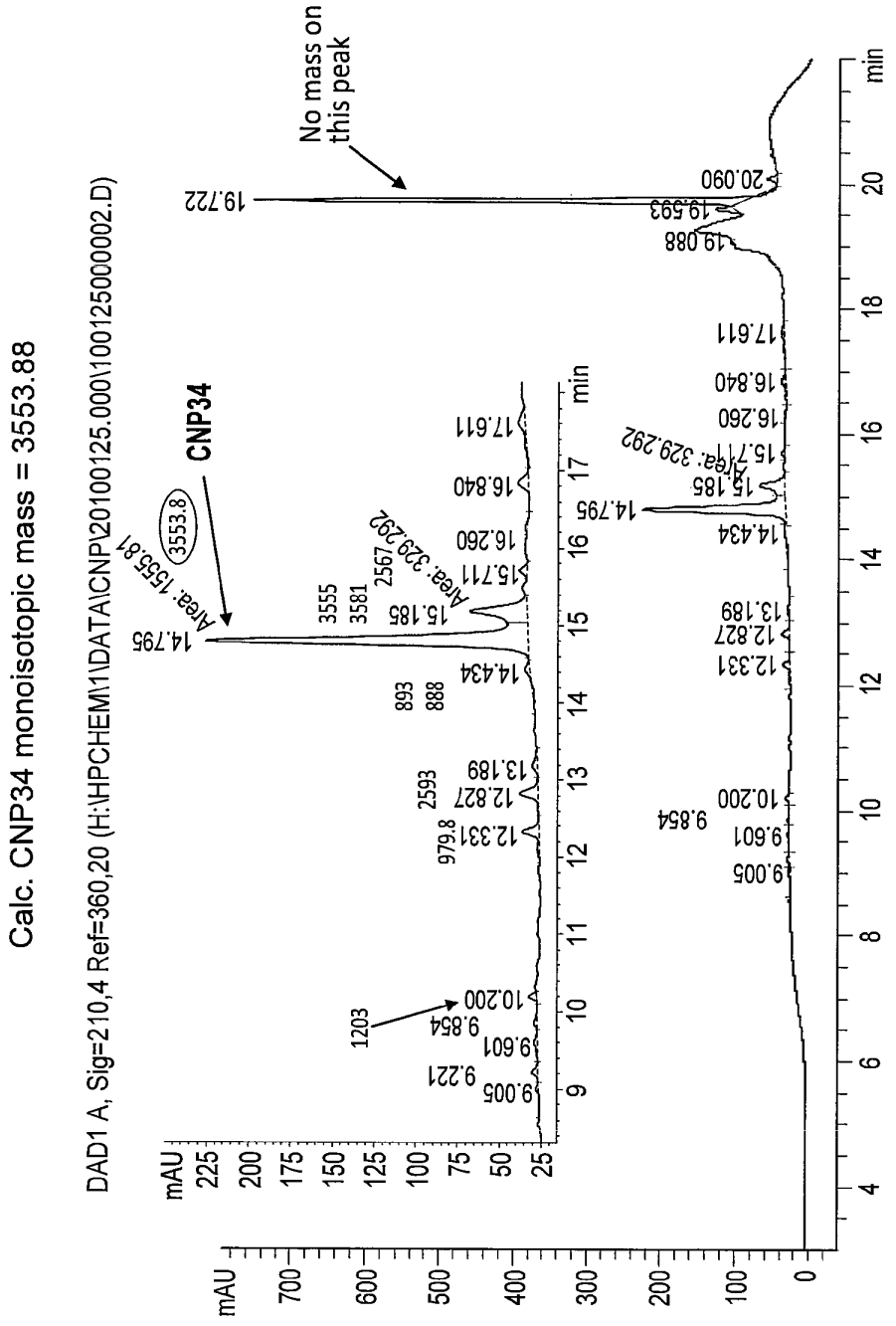

Calc. CNP34 monoisotopic mass = 3553.88
DAD1 A, Sig=210,4 Ref=360,20 (H:\HPCHEM\1\DATA\CNP\20100125.000\100125000002.D)
No mass on this peak
CNP34
3553.8
Area: 1555.81
Area: 329.292
mAU
min

FIGURE 10

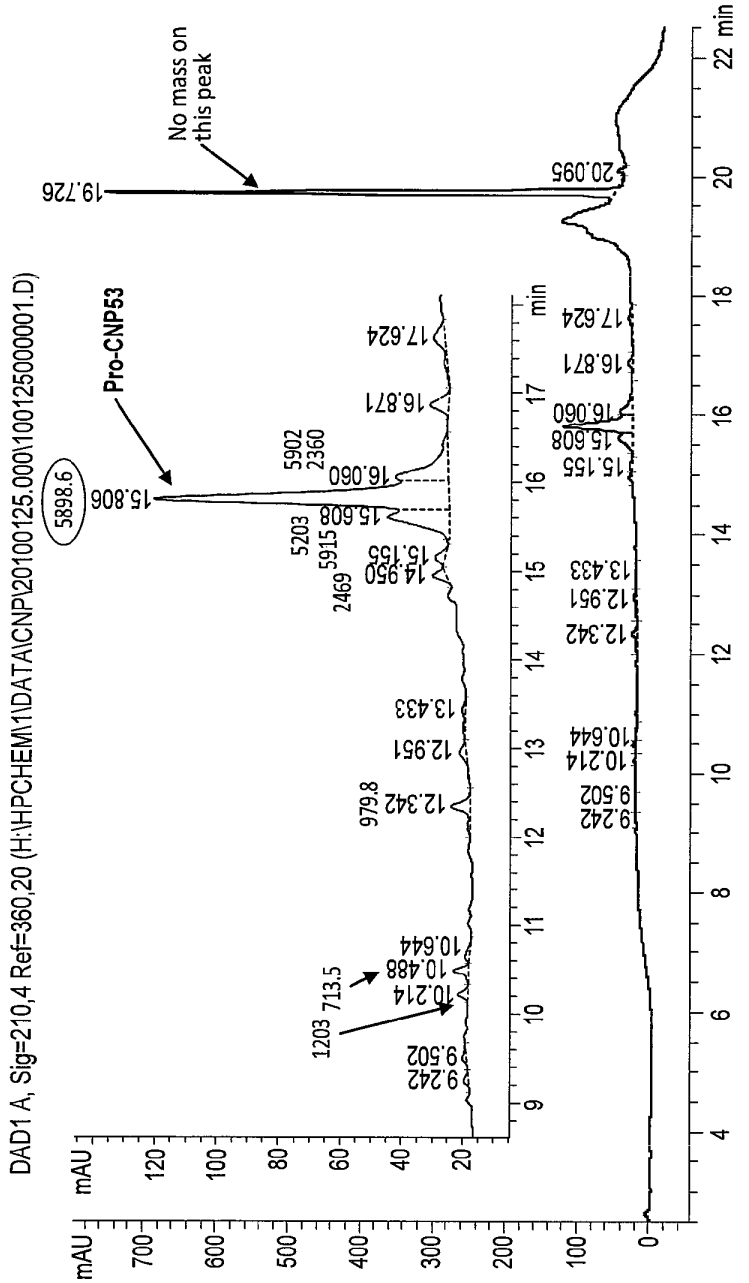

Calc. Pro-CNP53 monoisotopic mass = 5898.14
DAD1 A, Sig=210,4 Ref=360,20 (H:\HPCHEM\1\DATA\CNP\20100125.000\100125000001.D)
5898.6
Pro-CNP53
No mass on this peak
19.726
20.095
15.806
16.060
16.871
17.624
15.608
15.155
14.950
13.433
12.951
12.342
10.644
10.488
10.214
9.502
9.242
713.5
979.8
5902
2360
5203
5915
2469
1203
mAU
min

FIGURE 11

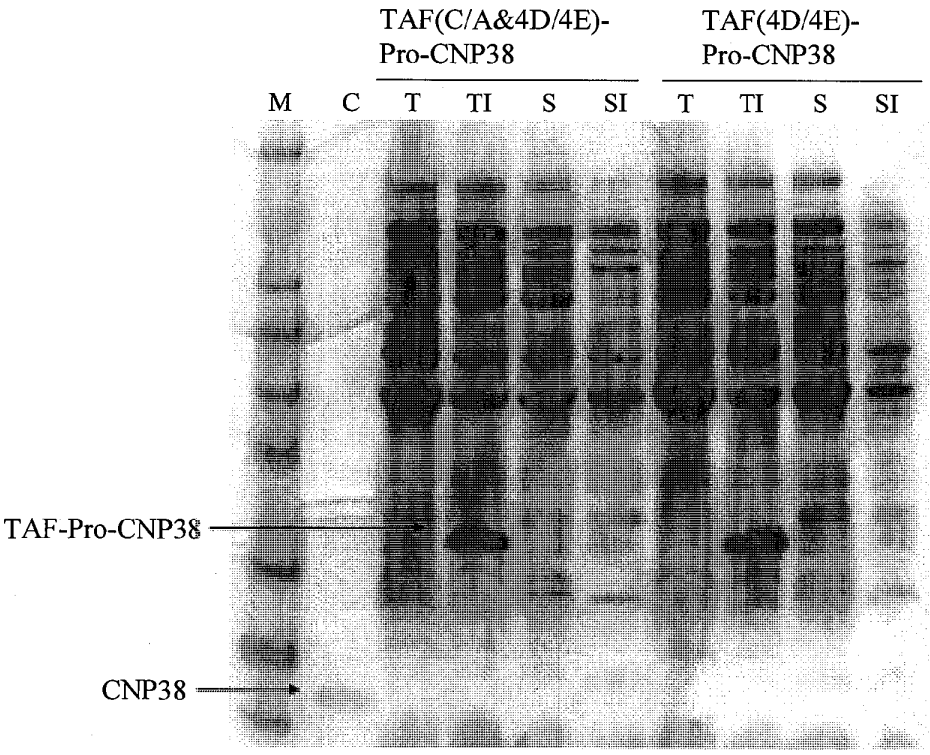

TAF(C/A&4D/4E)-Pro-CNP38
TAF(4D/4E)-Pro-CNP38
M
C
T
TI
S
SI
T
TI
S
SI
TAF-Pro-CNP38
CNP38

FIGURE 12

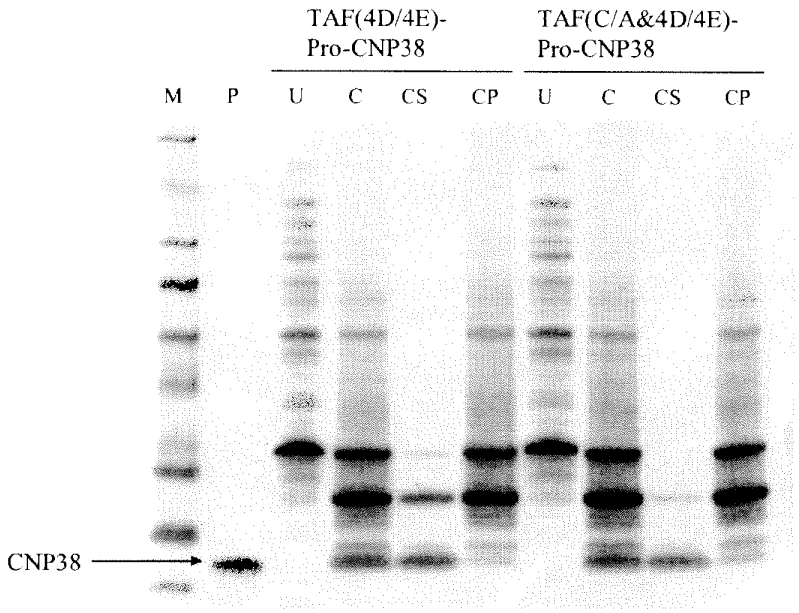


FIGURE 13

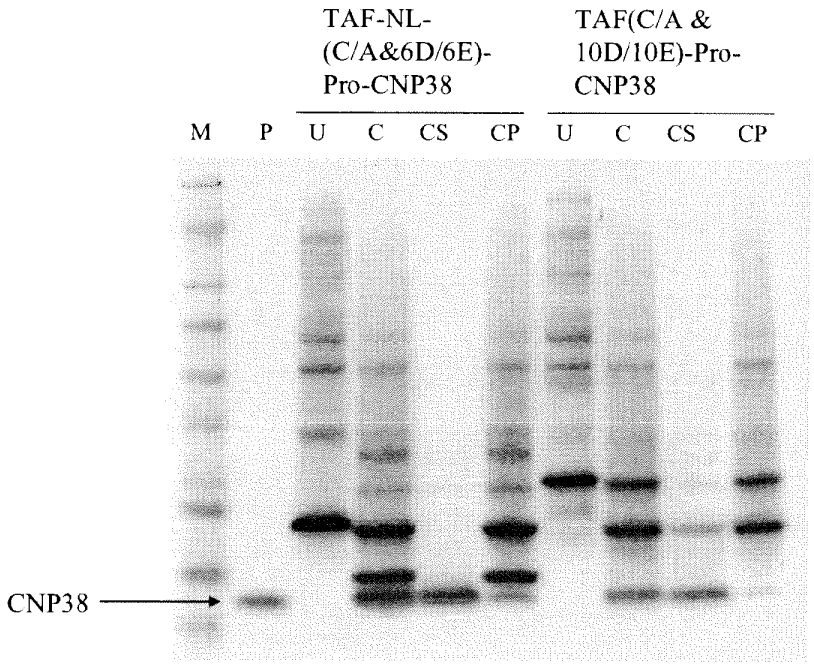


FIGURE 14

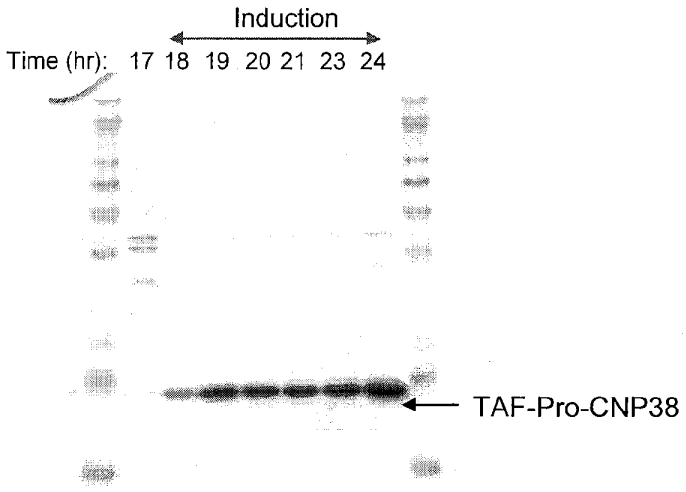


FIGURE 15

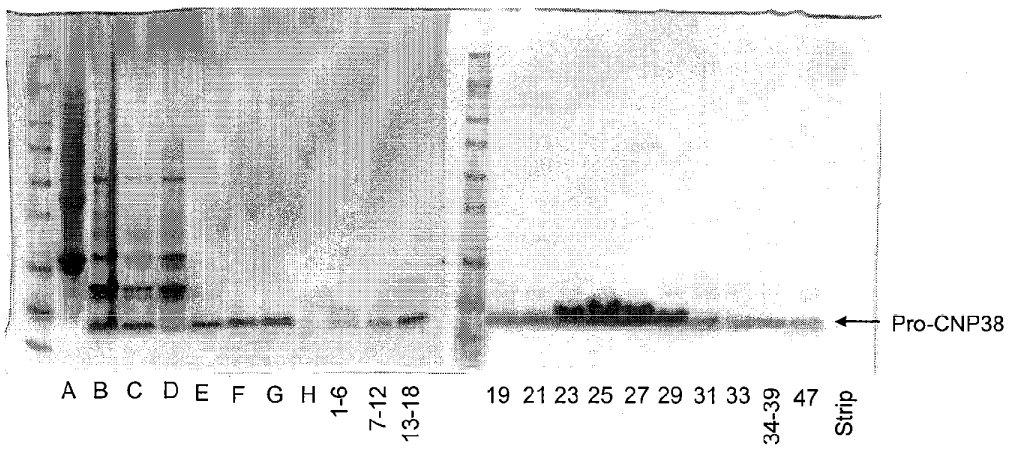


FIGURE 16

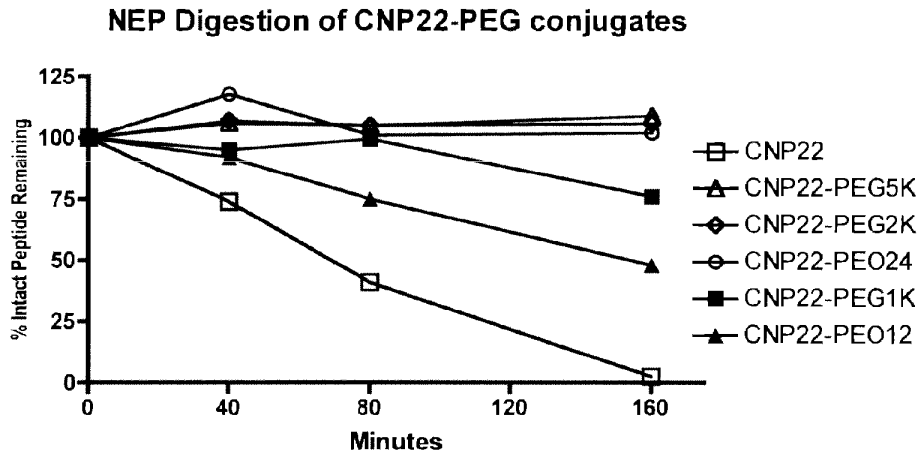


FIGURE 17

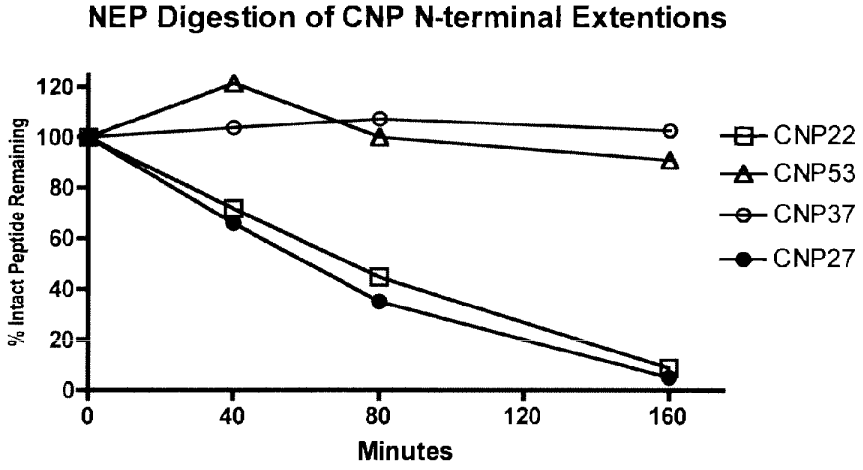


FIGURE 18

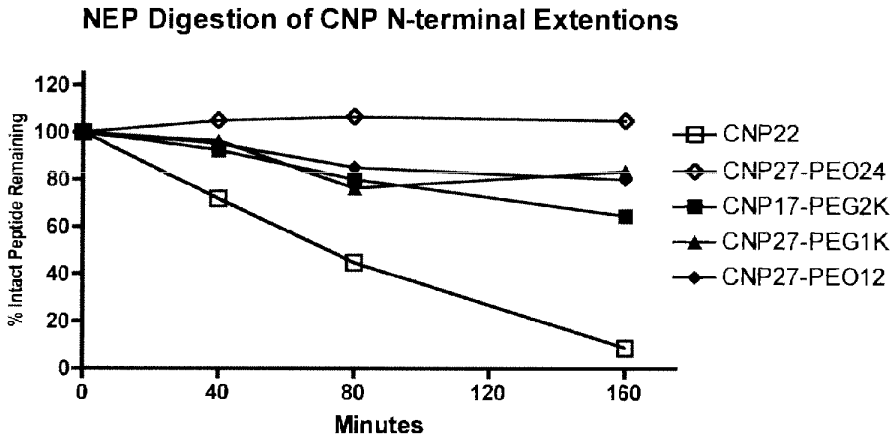


FIGURE 19

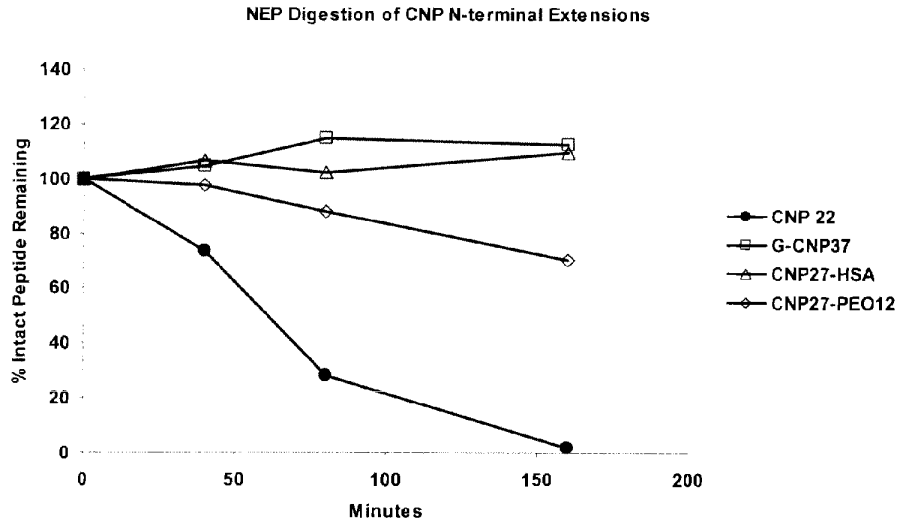


FIGURE 20

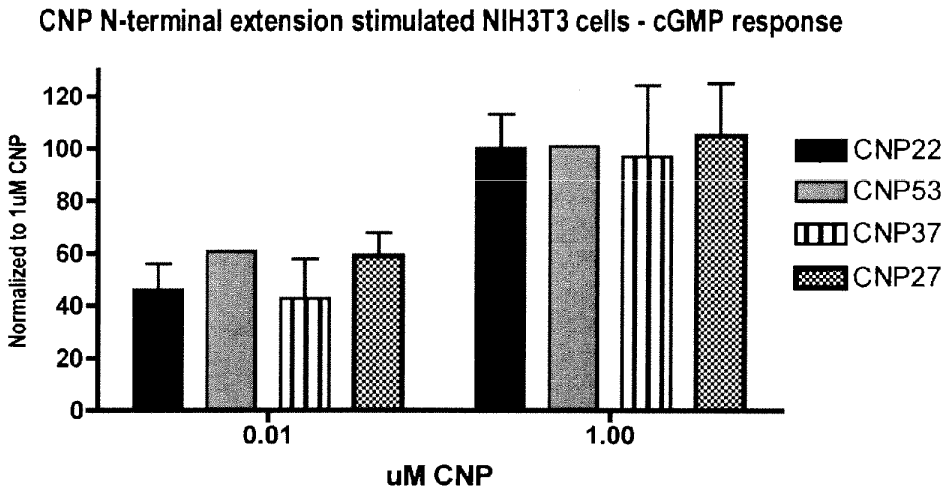


FIGURE 21

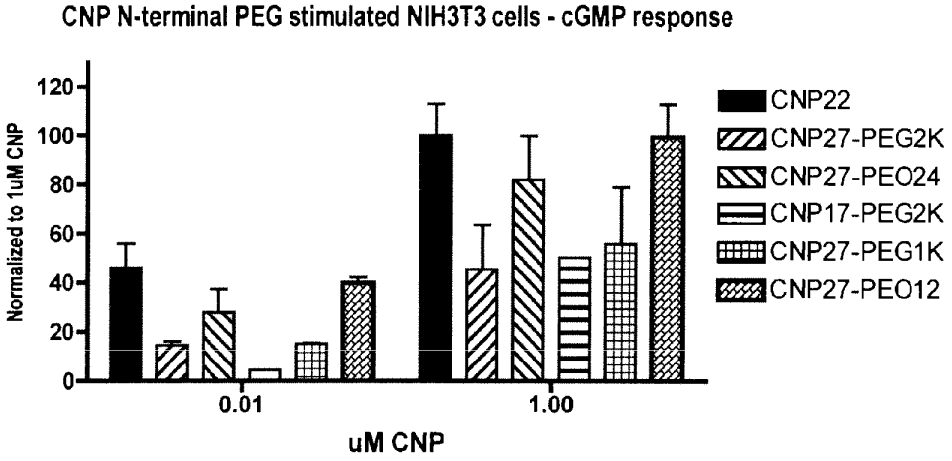


FIGURE 22

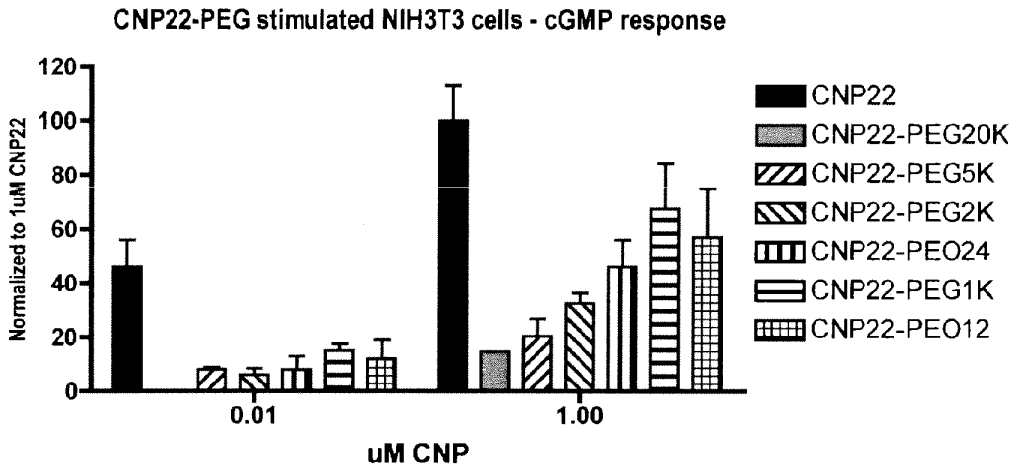


FIGURE 23

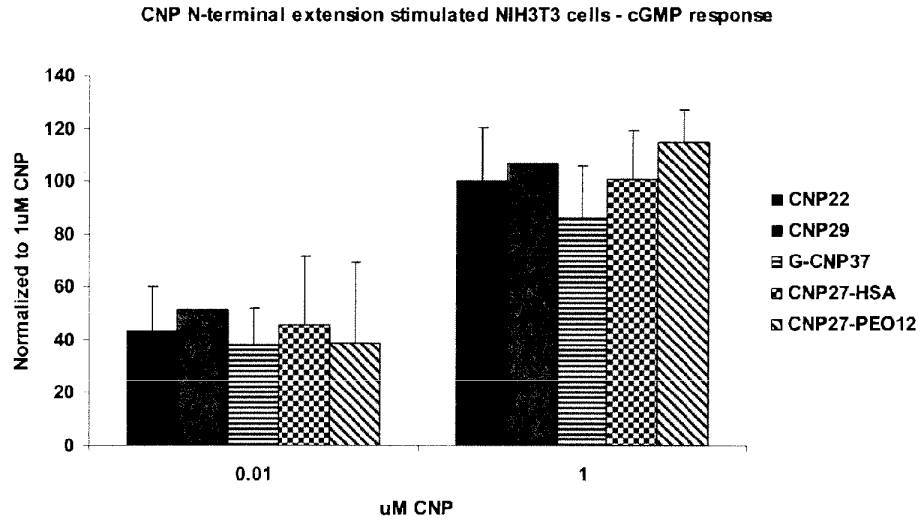
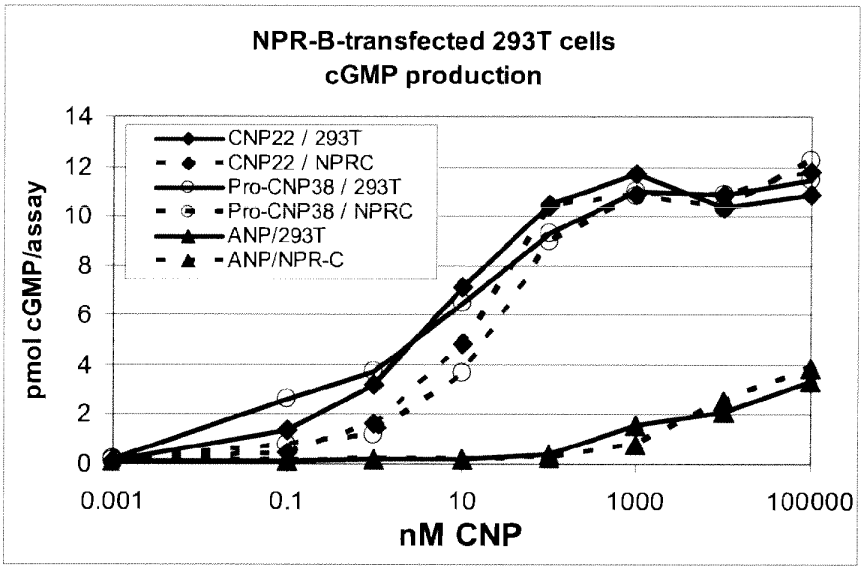


FIGURE 24

A.


NPR-B-transfected 293T cells
cGMP production
pmol cGMP/assay
nM CNP
CNP22 / 293T
CNP22 / NPRC
Pro-CNP38 / 293T
Pro-CNP38 / NPRC
ANP/293T
ANP/NPR-C
0
2
4
6
8
10
12
14
0.001
0.1
10
1000
100000

B.

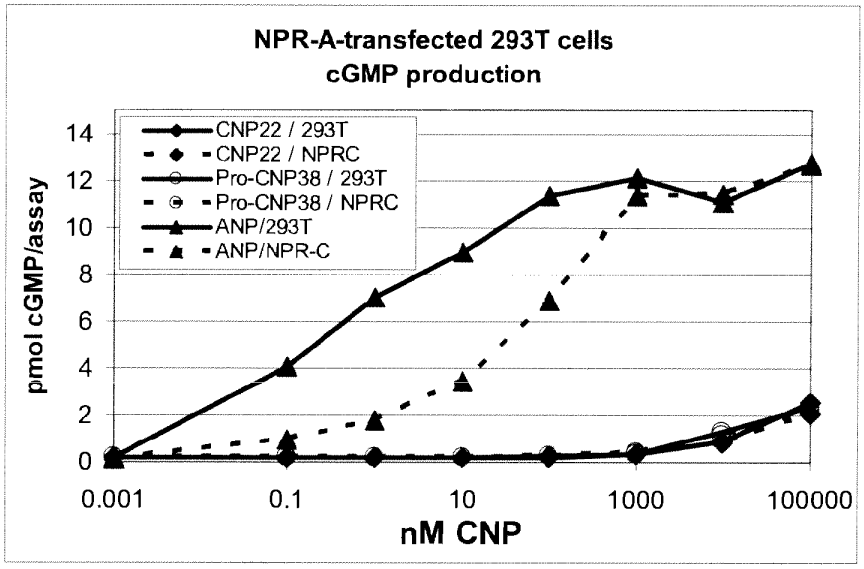
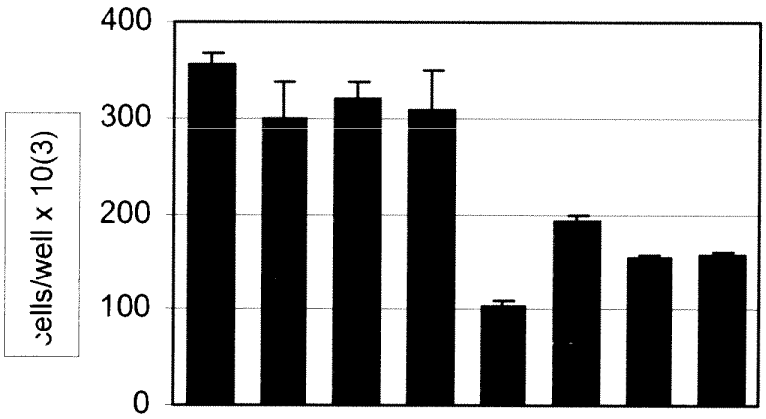

NPR-A-transfected 293T cells
cGMP production
pmol cGMP/assay
nM CNP
CNP22 / 293T
CNP22 / NPRC
Pro-CNP38 / 293T
Pro-CNP38 / NPRC
ANP/293T
ANP/NPR-C
0
2
4
6
8
10
12
14
0.001
0.1
10
1000
100000

FIGURE 25



Bars from left to right:

1 control (no CNP22, no FGF2)
2 CNP22 (0.2 uM) continuous
3 CNP22 (0.2 uM) 1 hr once daily
4 CNP22 (0.2 uM) 2 hr once daily
5 FGF2 (5 ng/mL) continuous
6 FGF2 + CNP22 (0.2 uM) continuous
7 FGF2 + CNP22 (0.2 uM) 1 hr daily
8 FGF2 + CNP22 (0.2 uM) 2 hr daily

FIGURE 26

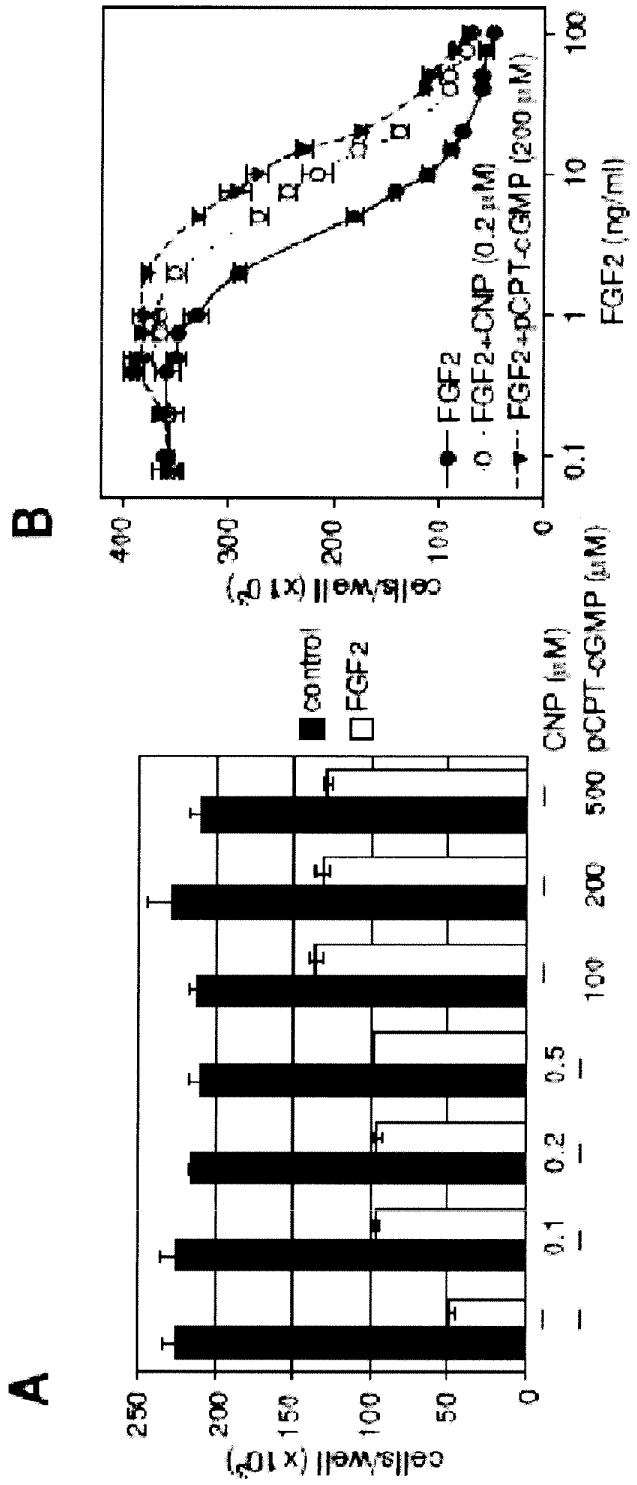

A
cells/well (x 10^2)
250
200
150
100
50
0
control
FGF2
—
0.1
0.2
0.5
—
100
200
500
CNP (μM)
pCPT-cGMP (μM)
B
cells/well (x 10^2)
400
300
200
100
0
FGF2
FGF2+CNP (0.2 μM)
FGF2+pCPT-cGMP (200 μM)
0.1
1
10
100
FGF2 (ng/ml)

FIGURE 27

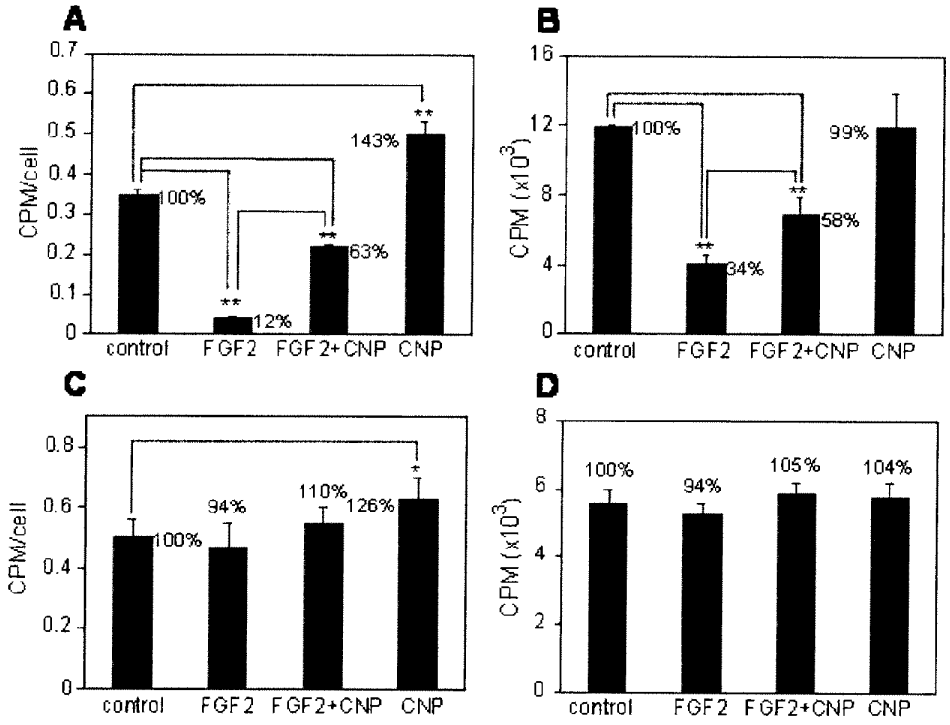

A
CPM/cell
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
100%
12%
63%
143%
**
control
FGF2
FGF2+CNP
CNP
B
CPM (x10^3)
16
12
8
4
0
100%
34%
58%
99%
**
control
FGF2
FGF2+CNP
CNP
C
CPM/cell
0.8
0.6
0.4
0.2
0
100%
94%
110%
126%
*
control
FGF2
FGF2+CNP
CNP
D
CPM (x10^3)
8
6
4
2
0
100%
94%
105%
104%
control
FGF2
FGF2+CNP
CNP

FIGURE 28

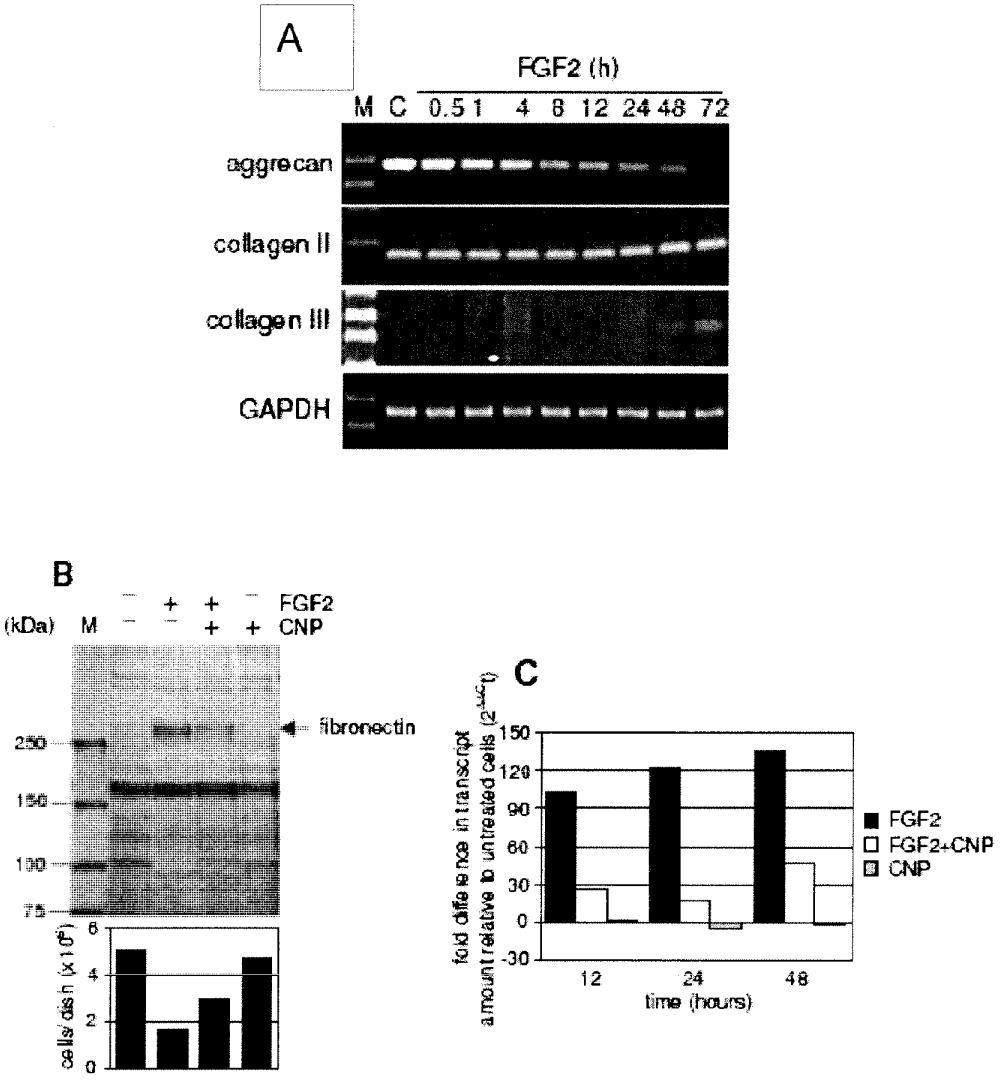

A
FGF2 (h)
M C 0.5 1 4 8 12 24 48 72
aggrecan
collagen II
collagen III
GAPDH
B
(kDa) M
FGF2
CNP
fibronectin
250
150
100
75
cells/dish (x10⁵)
C
fold difference in transcript amount relative to untreated cells (2^-ΔΔCt)
time (hours)
FGF2
FGF2+CNP
CNP

FIGURE 29

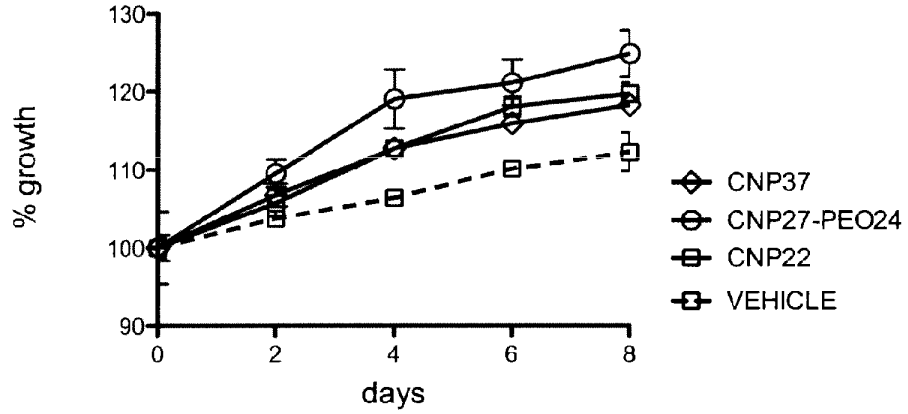


FIGURE 30

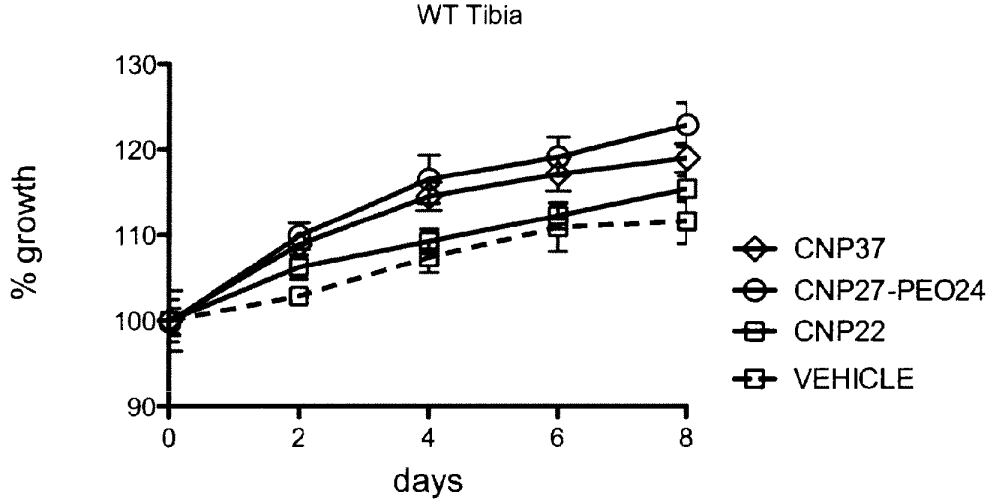


FIGURE 31

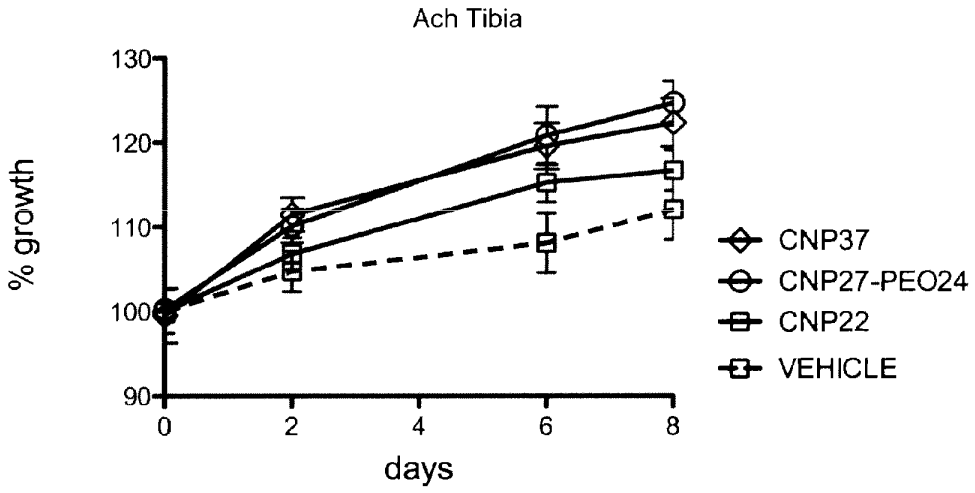


FIGURE 32

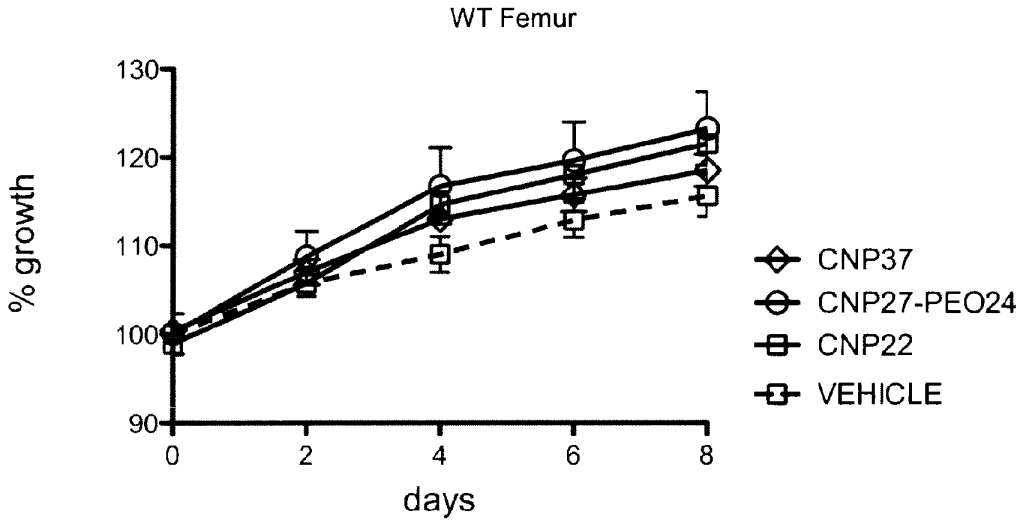


FIGURE 33

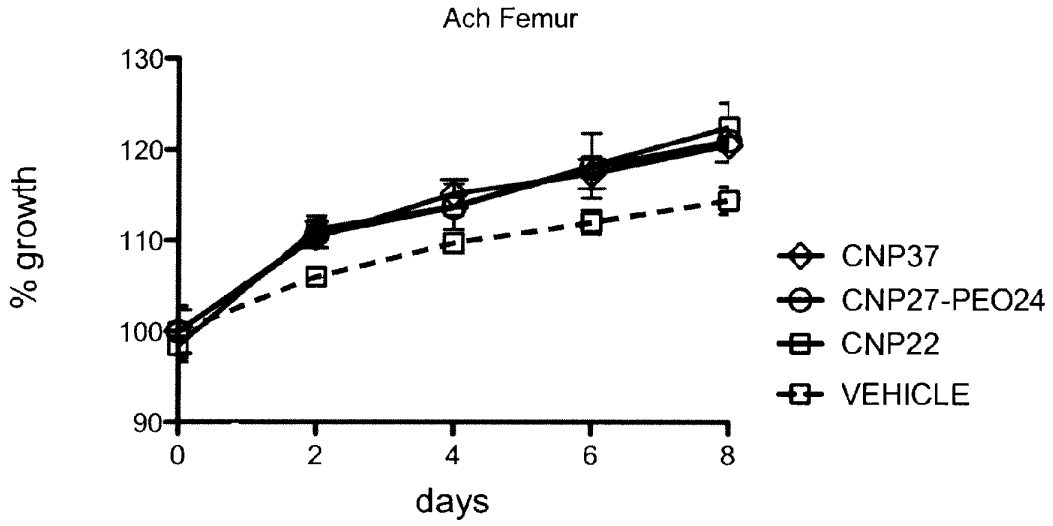

Ach Femur
% growth
130
120
110
100
90
0
2
4
6
8
days
CNP37
CNP27-PEO24
CNP22
VEHICLE

FIGURE 34

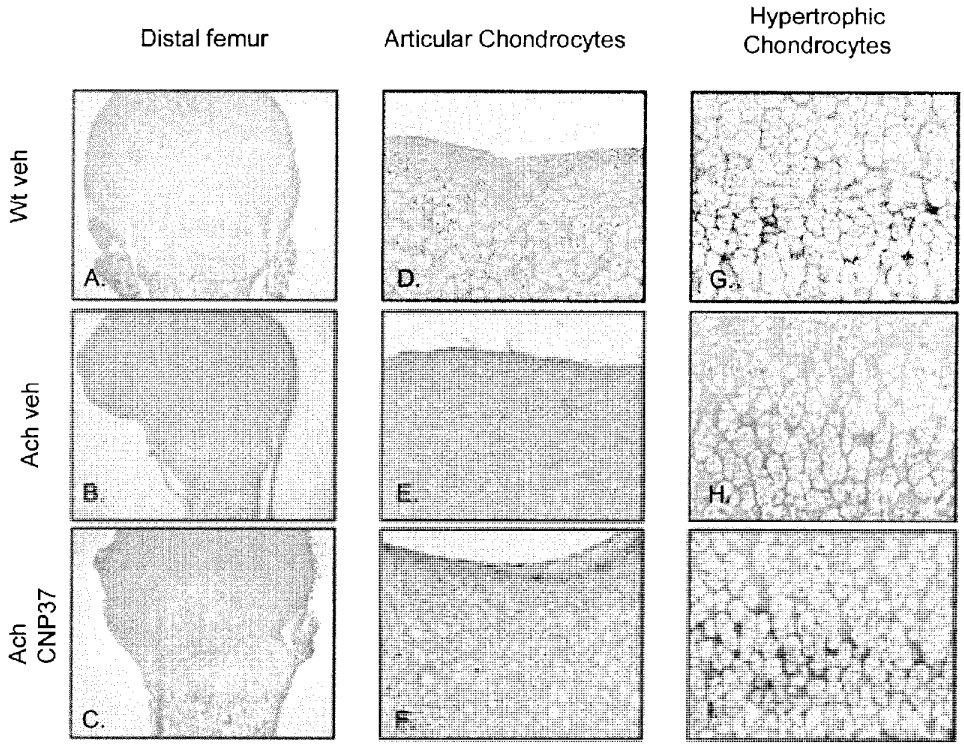

Distal femur
Articular Chondrocytes
Hypertrophic Chondrocytes
Wt veh
Ach veh
Ach CNP37
A.
B.
C.
D.
E.
F.
G.
H.
I.

FIGURE 35

Cellularity of Proliferating Columns

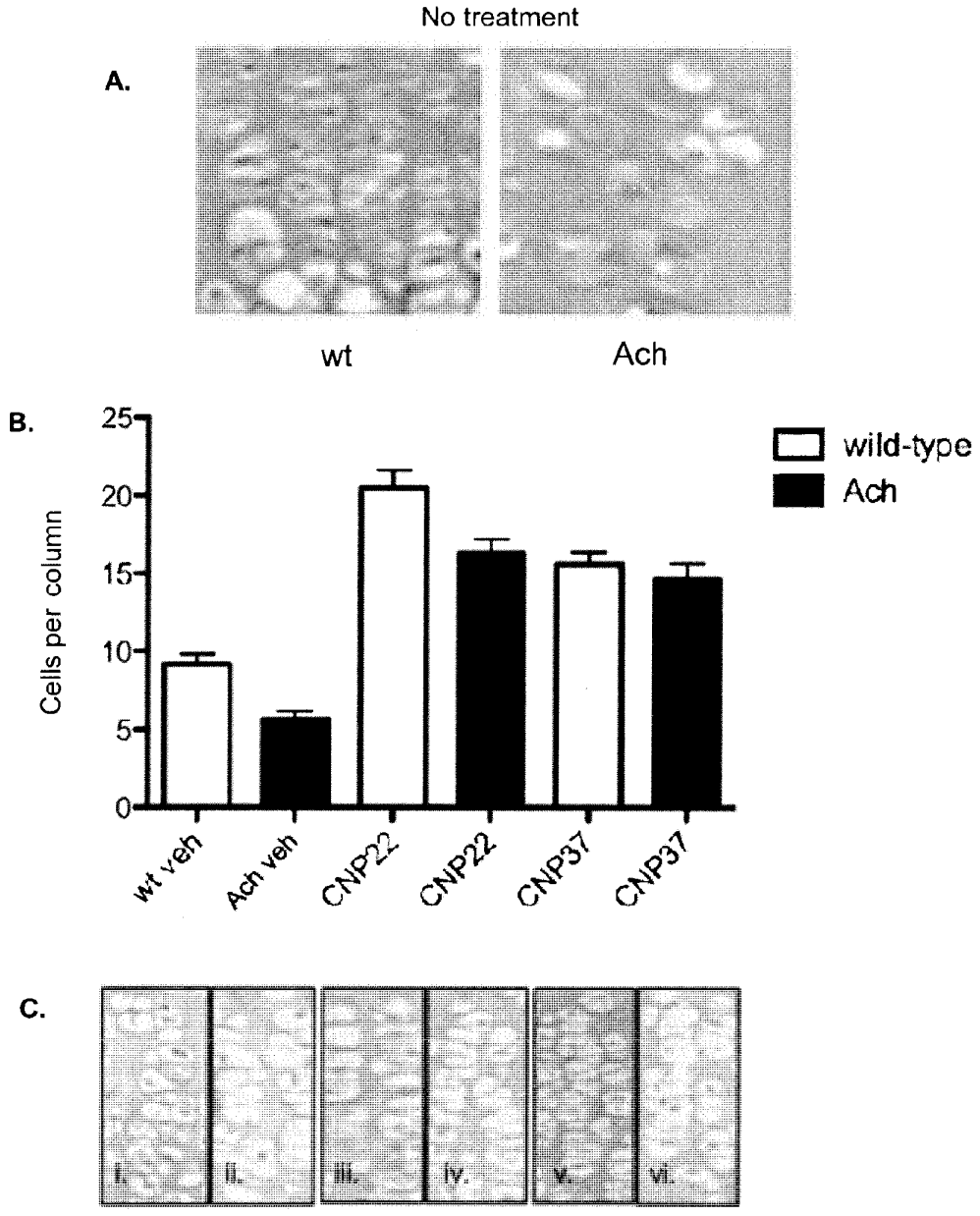


FIGURE 36

Chondrocyte Hypertrophy

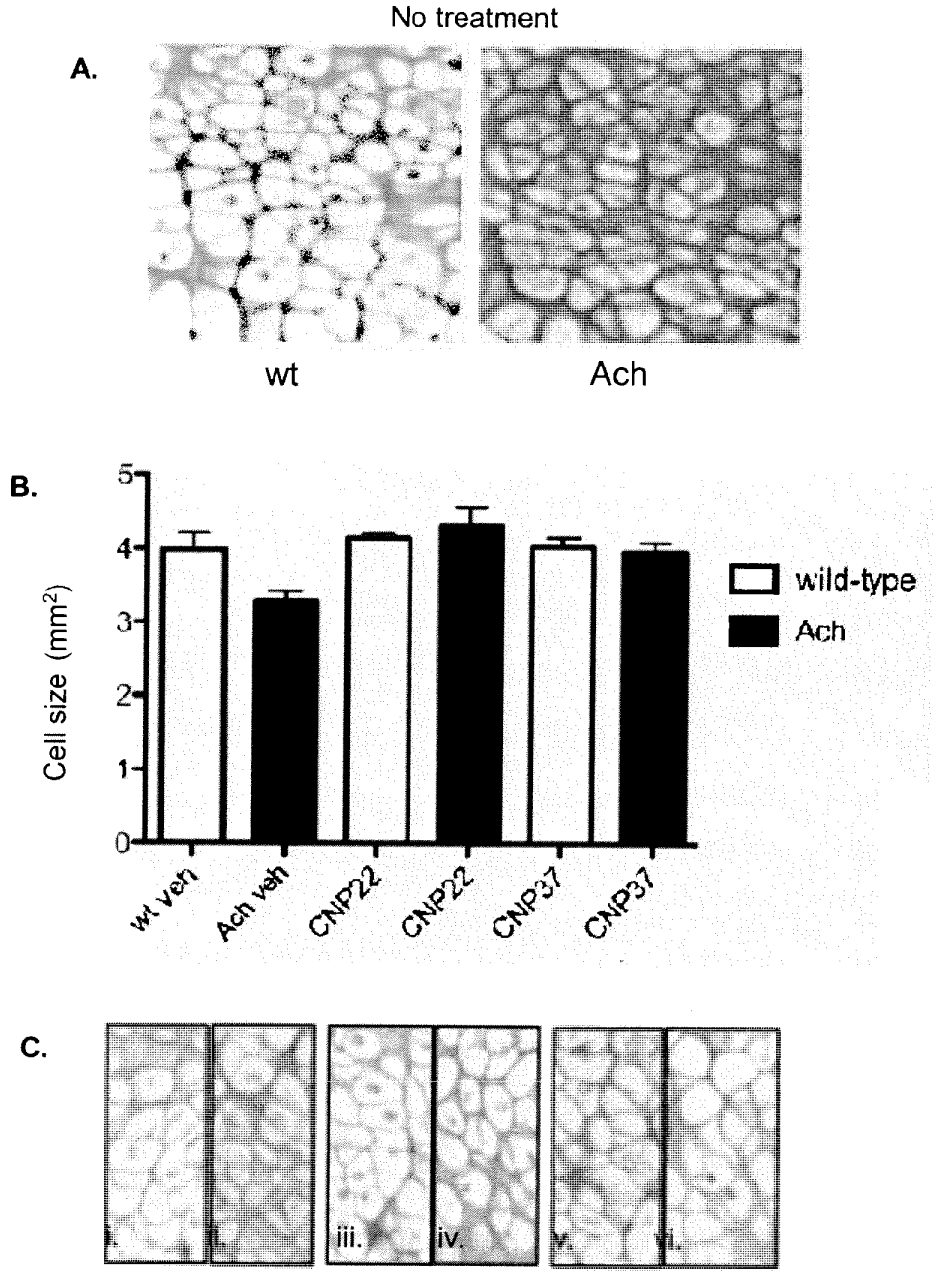


FIGURE 37

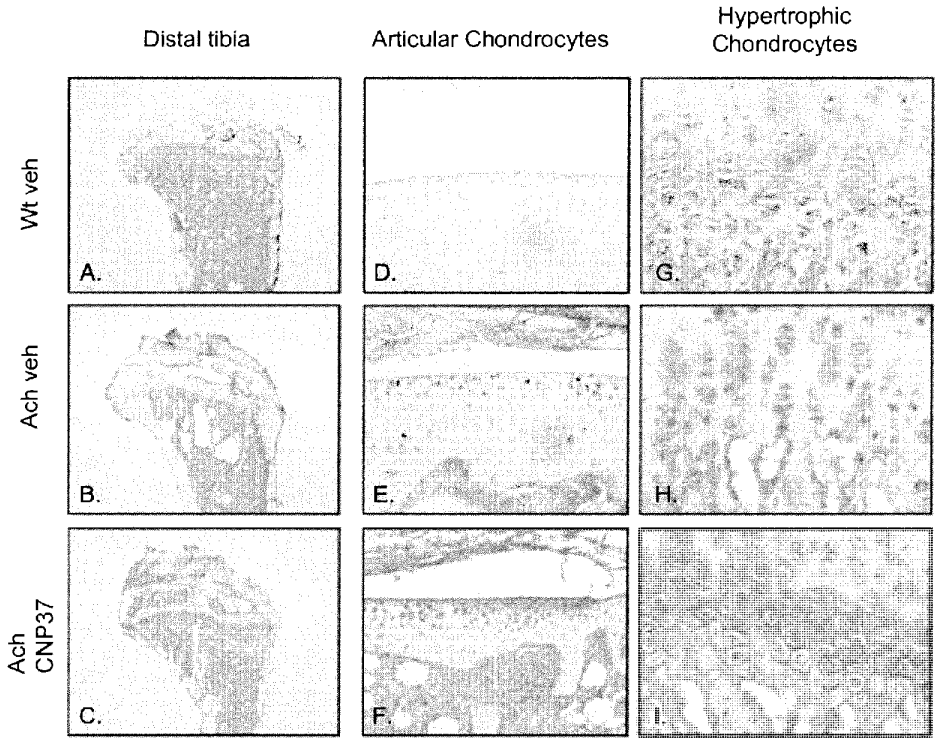
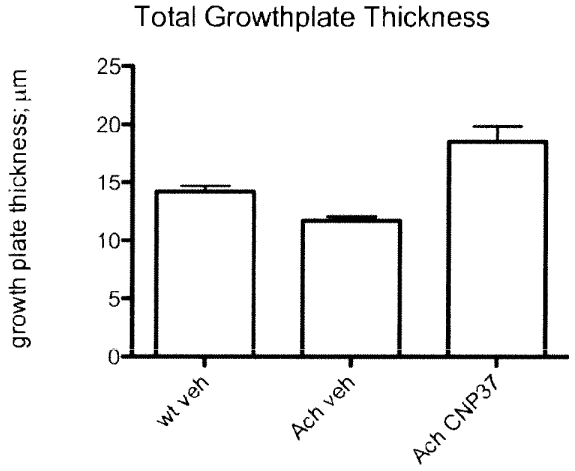
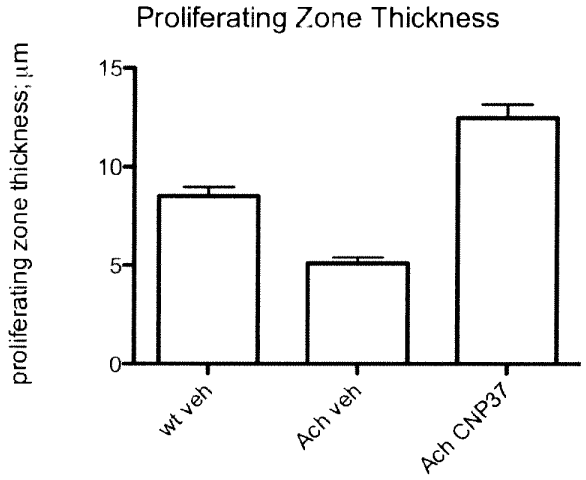

Distal tibia
Articular Chondrocytes
Hypertrophic Chondrocytes
Wt veh
Ach veh
Ach CNP37
A.
B.
C.
D.
E.
F.
G.
H.
I.

FIGURE 38

A.



B.



C.

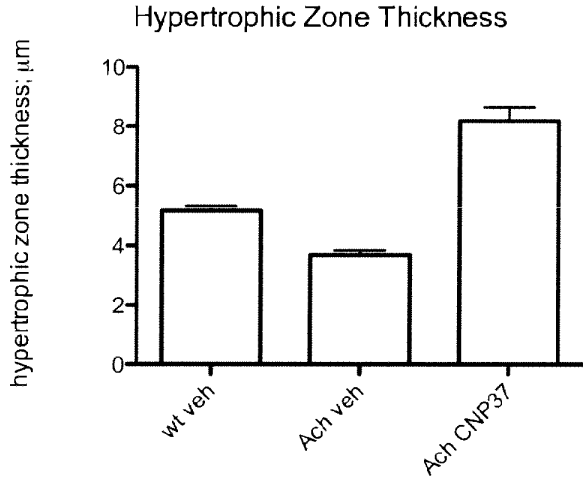


FIGURE 39

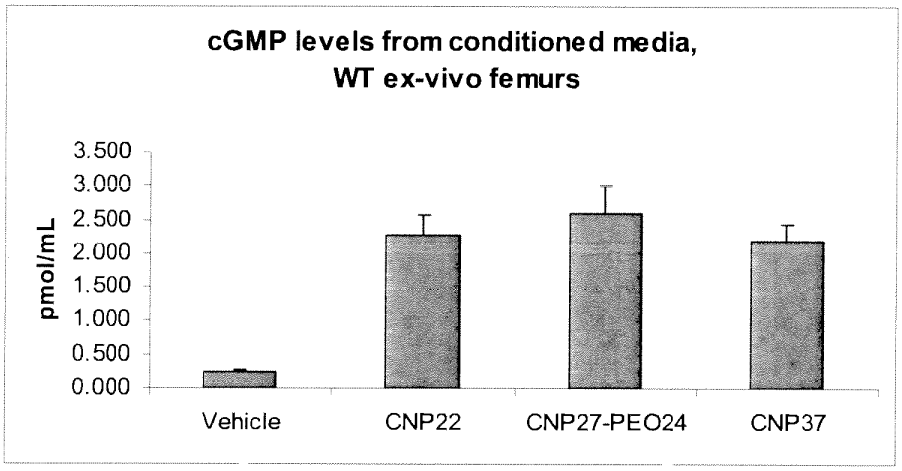


FIGURE 40

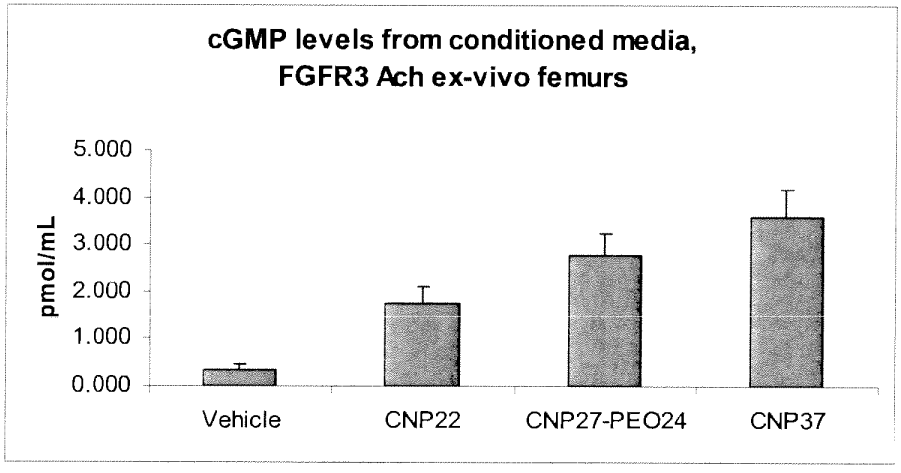


FIGURE 41

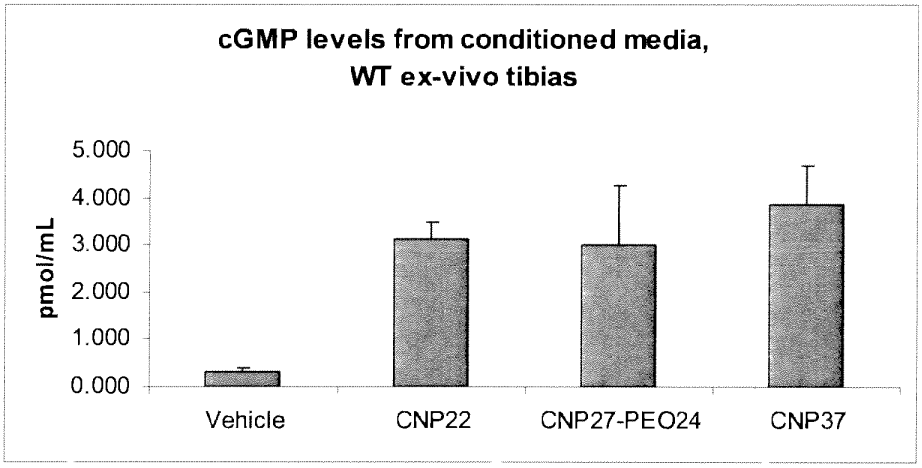


FIGURE 42

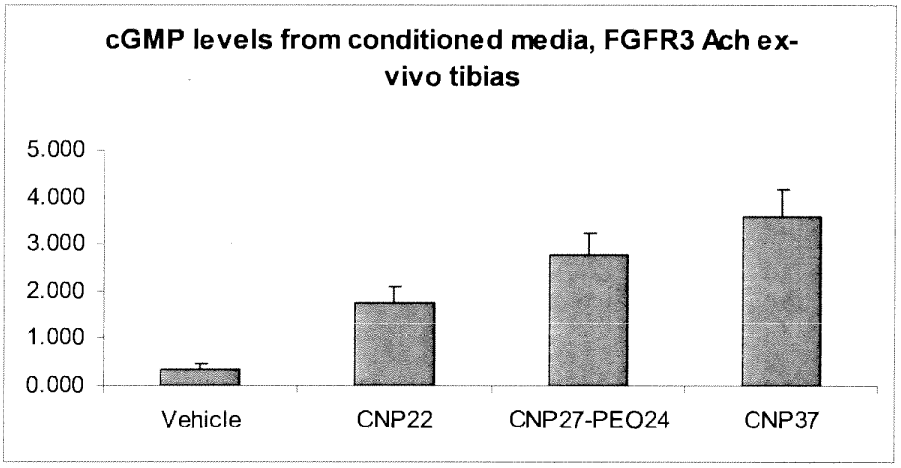


FIGURE 43

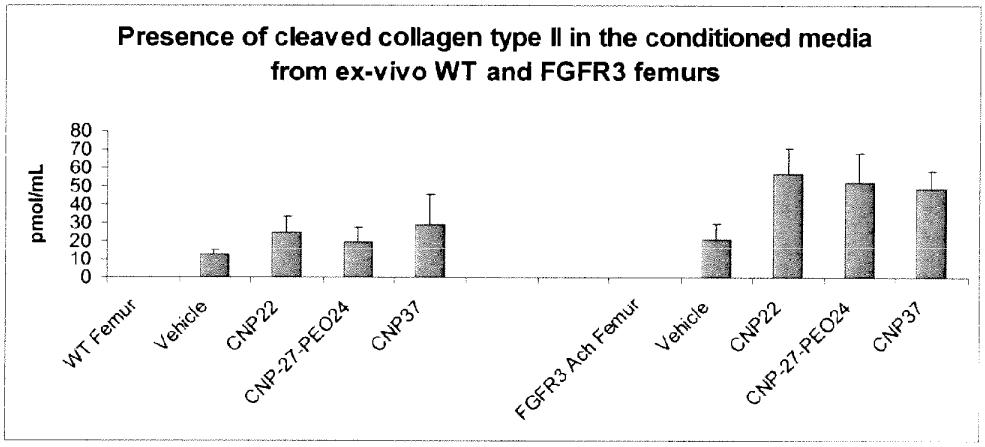


FIGURE 44

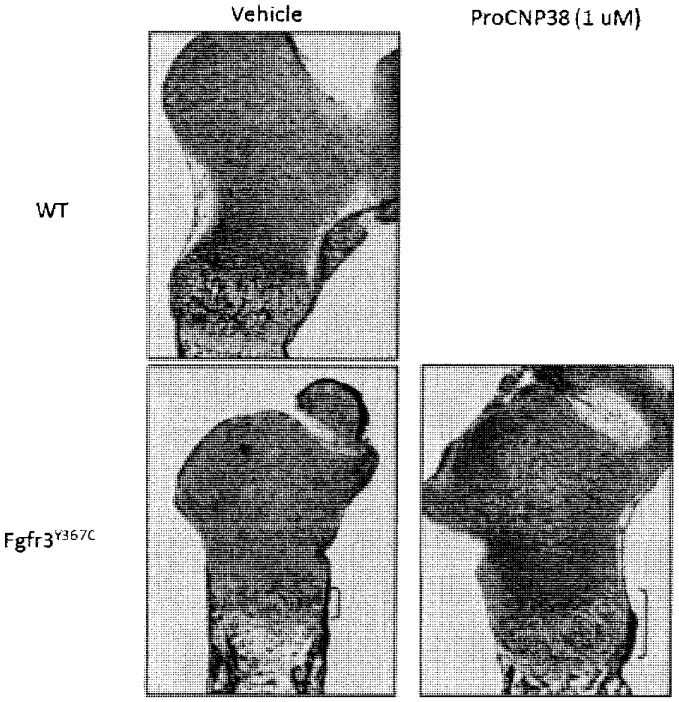


FIGURE 45

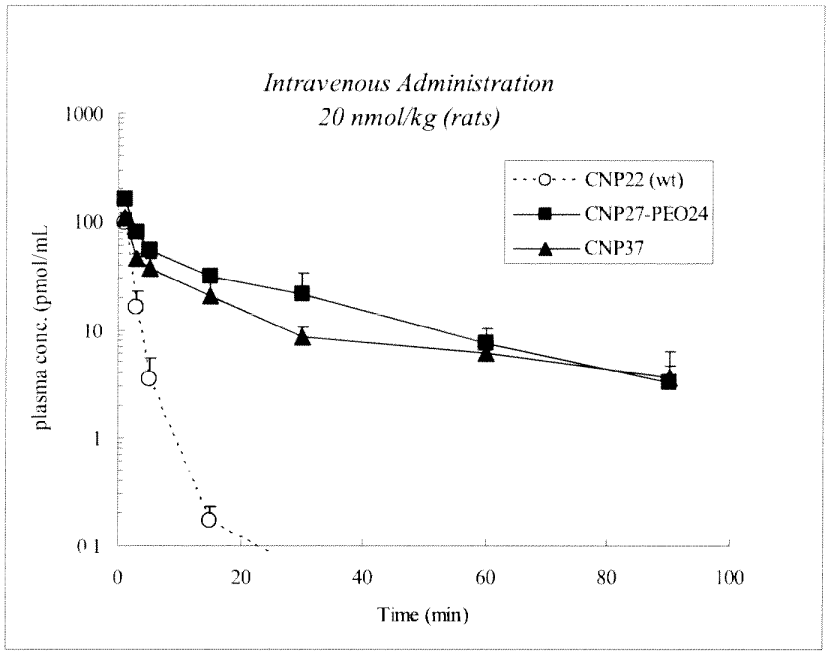


FIGURE 46

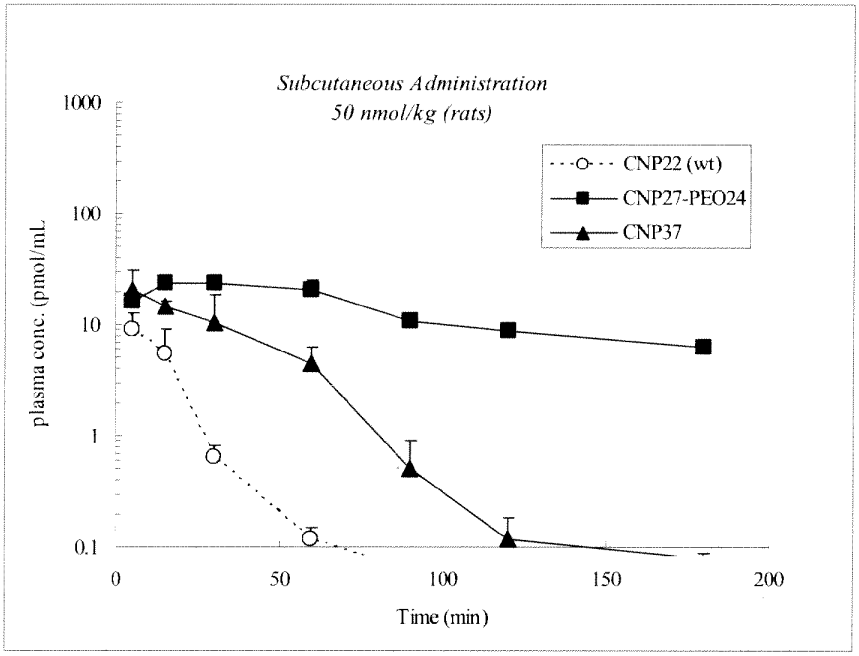


FIGURE 47

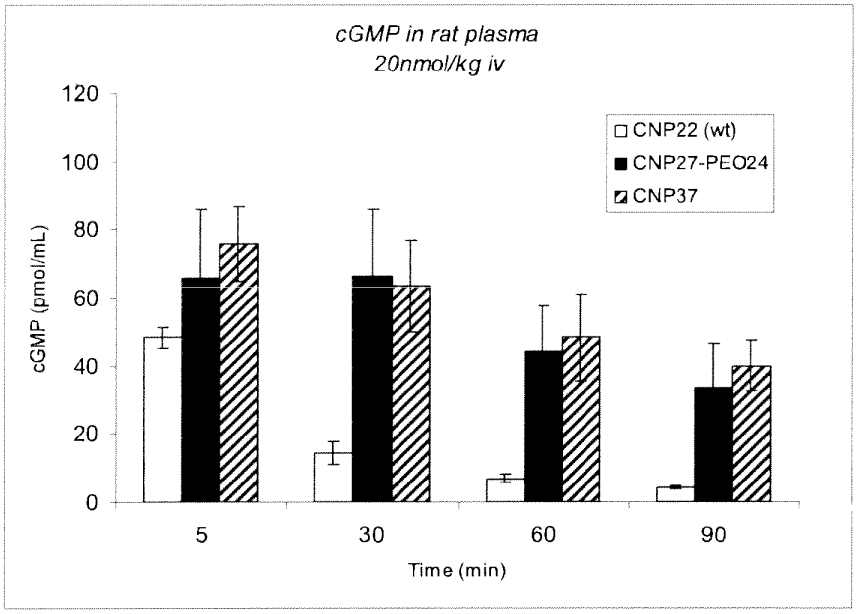


FIGURE 48

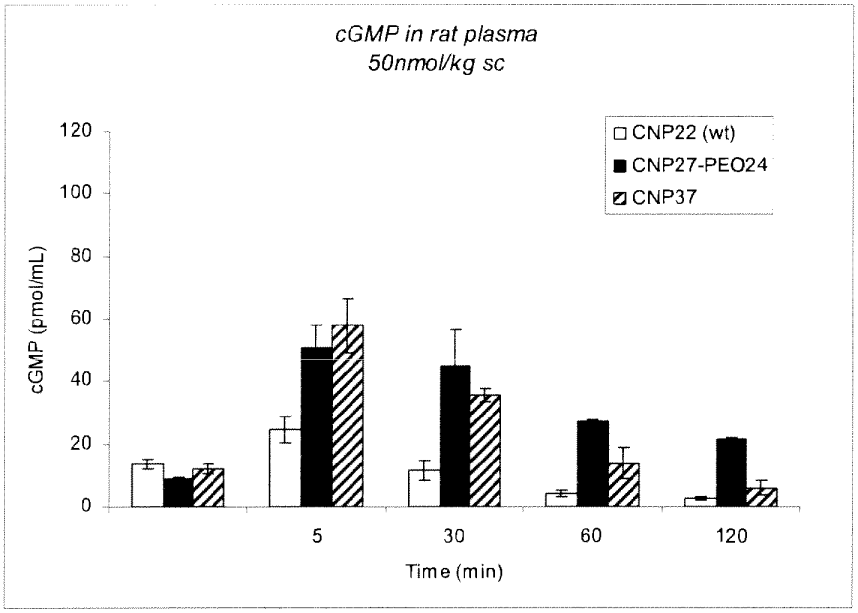


FIGURE 49

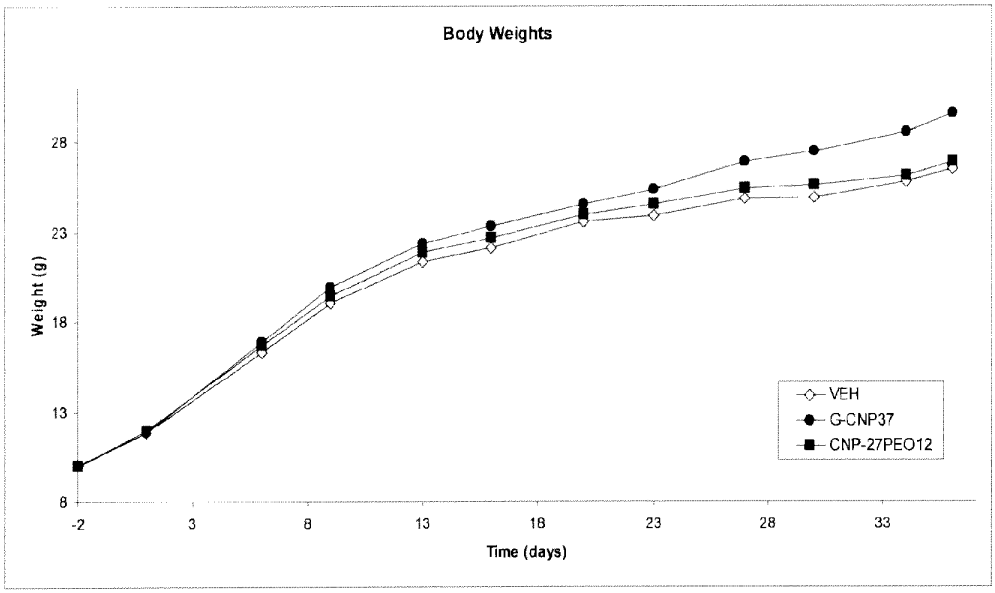


FIGURE 50

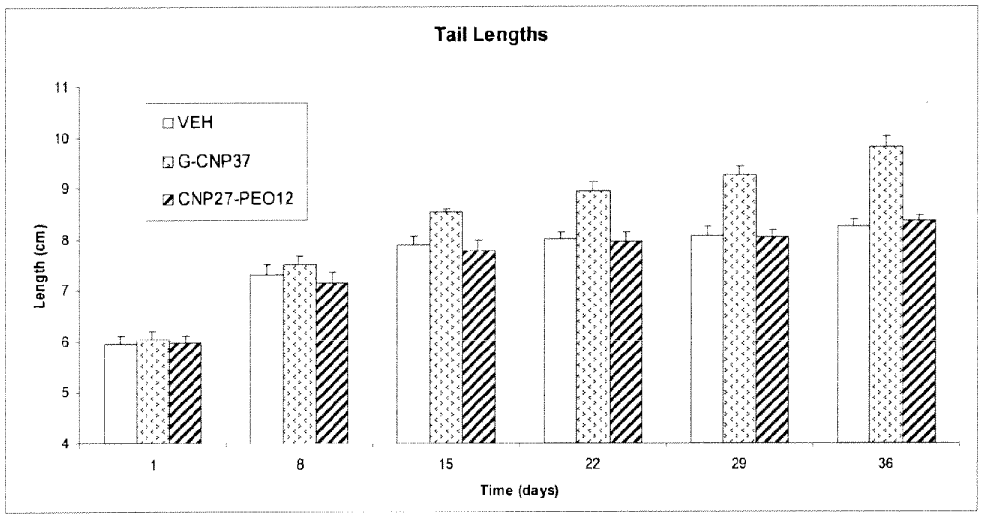


FIGURE 51

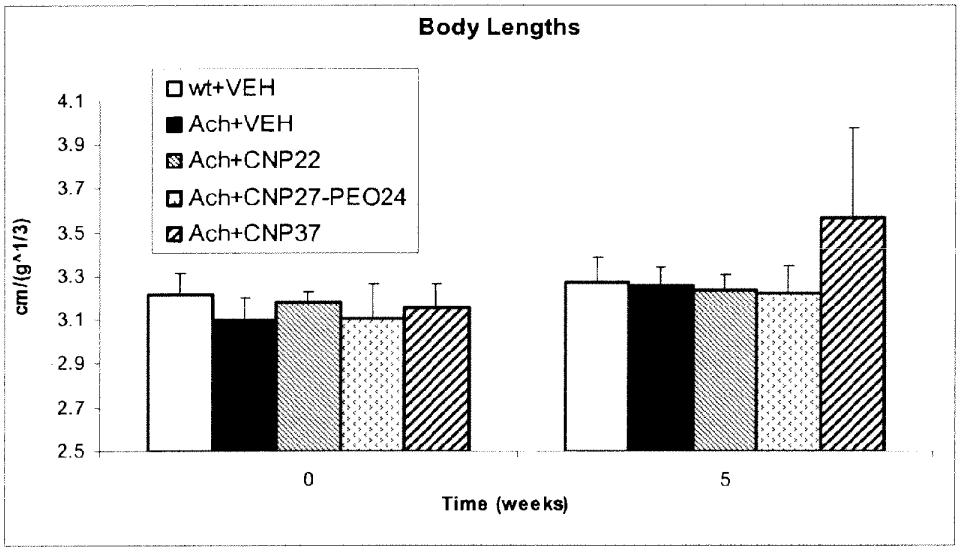


FIGURE 52

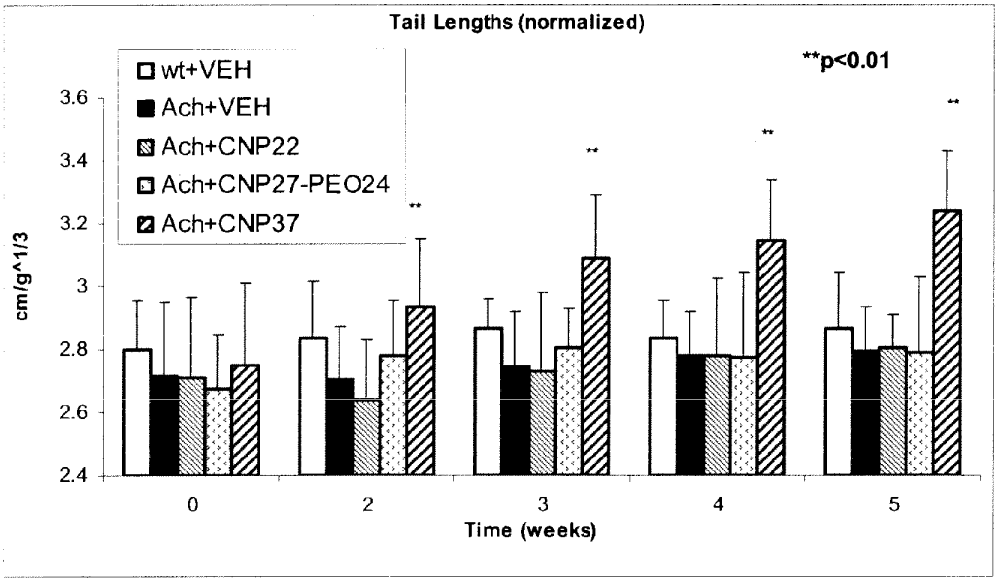
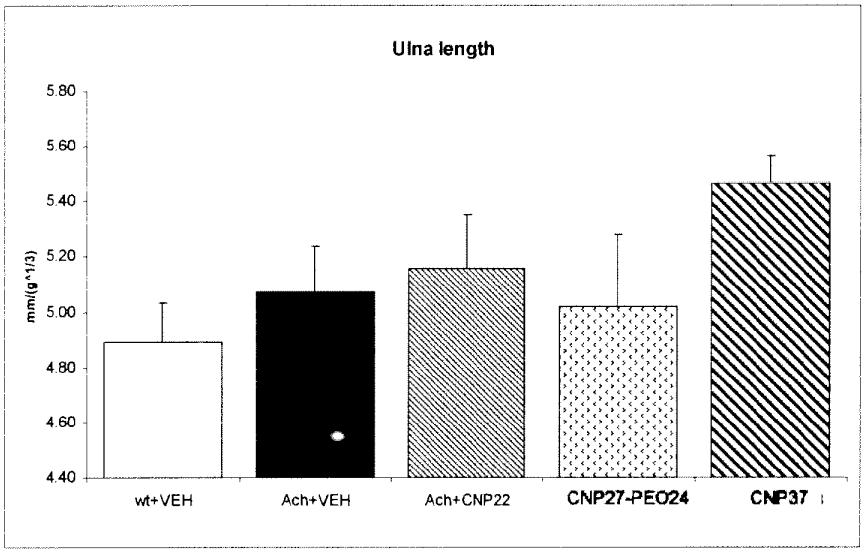


FIGURE 53

A.


Ulna length
mm/(g^1/3)
5.80
5.60
5.40
5.20
5.00
4.80
4.60
4.40
wt+VEH
Ach+VEH
Ach+CNP22
CNP27-PEO24
CNP37

B.

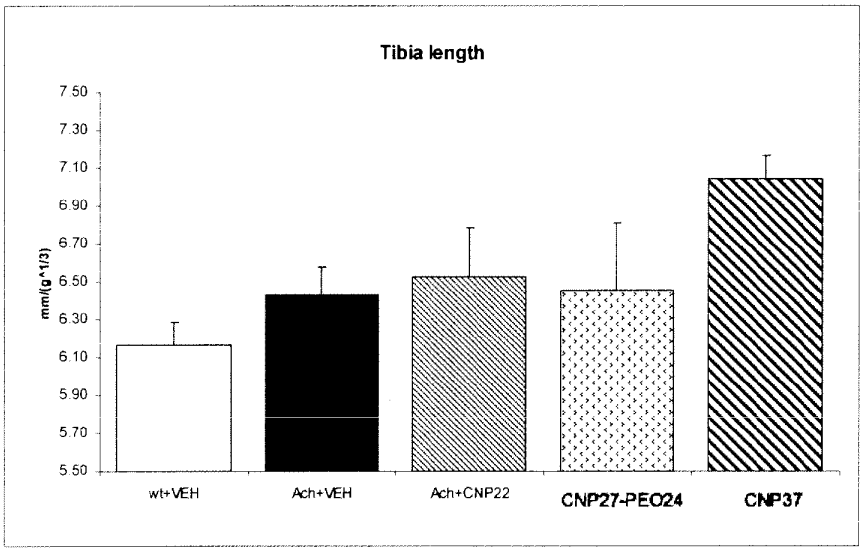
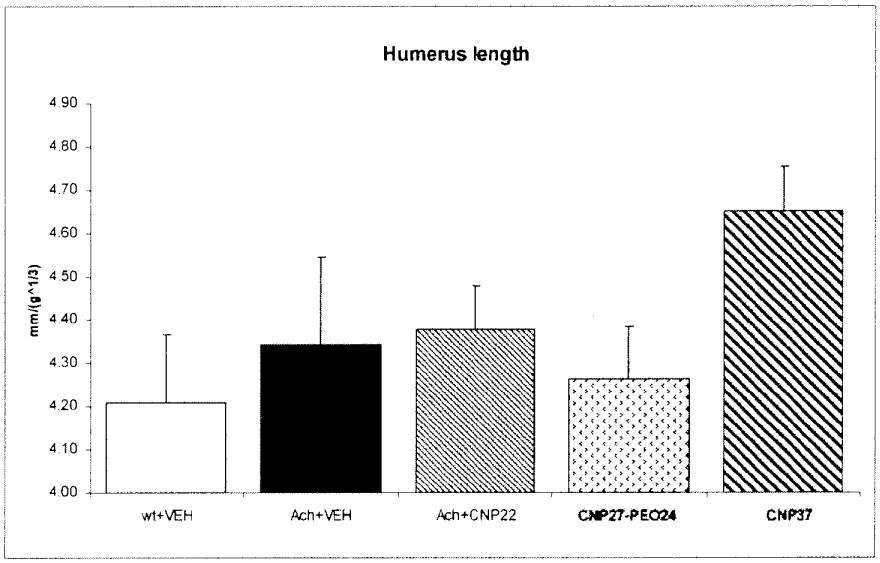

Tibia length
mm/(g^1/3)
7.50
7.30
7.10
6.90
6.70
6.50
6.30
6.10
5.90
5.70
5.50
wt+VEH
Ach+VEH
Ach+CNP22
CNP27-PEO24
CNP37

FIGURE 54

A.


Humerus length
mm/(g^1/3)
4.90
4.80
4.70
4.60
4.50
4.40
4.30
4.20
4.10
4.00
wt+VEH
Ach+VEH
Ach+CNP22
CNP27-PEO24
CNP37

B.

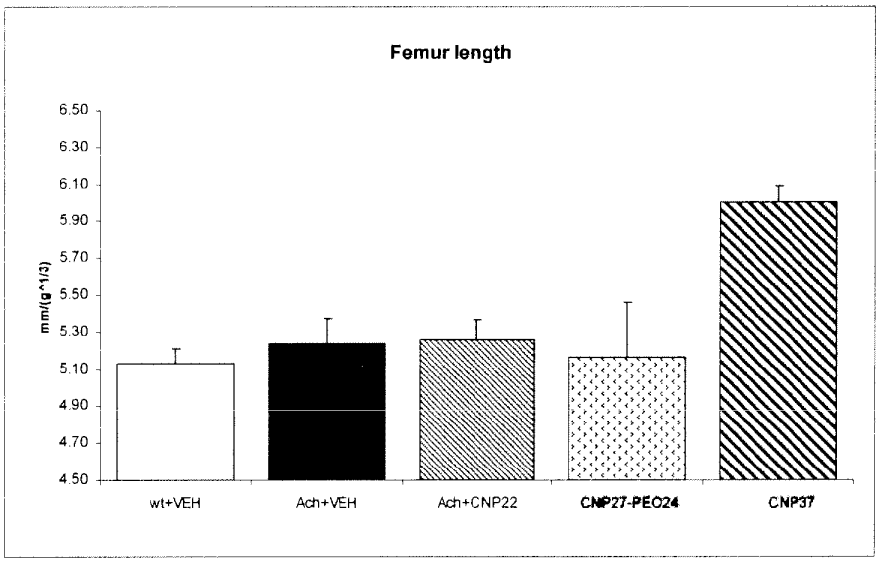

Femur length
mm/(g^1/3)
6.50
6.30
6.10
5.90
5.70
5.50
5.30
5.10
4.90
4.70
4.50
wt+VEH
Ach+VEH
Ach+CNP22
CNP27-PEO24
CNP37

FIGURE 55

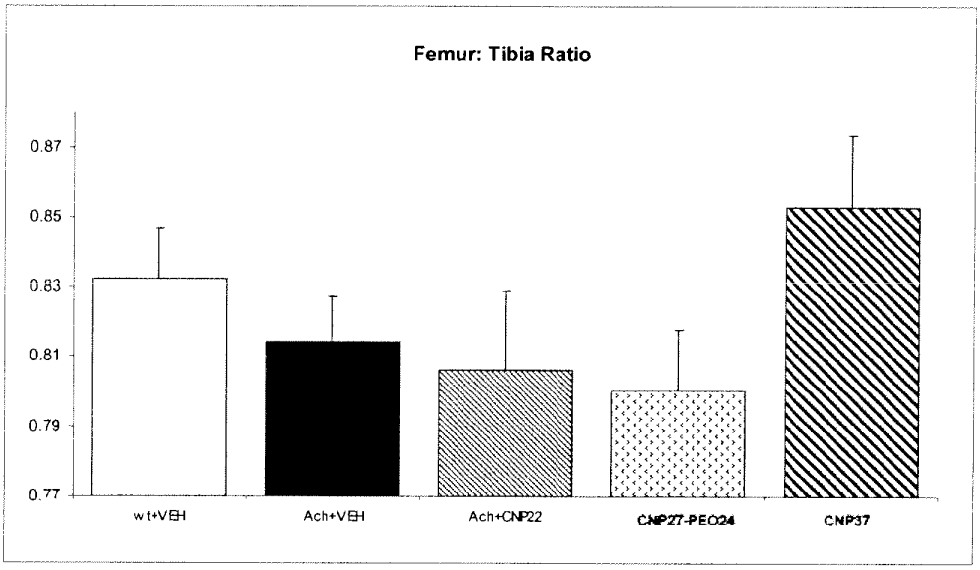


FIGURE 56

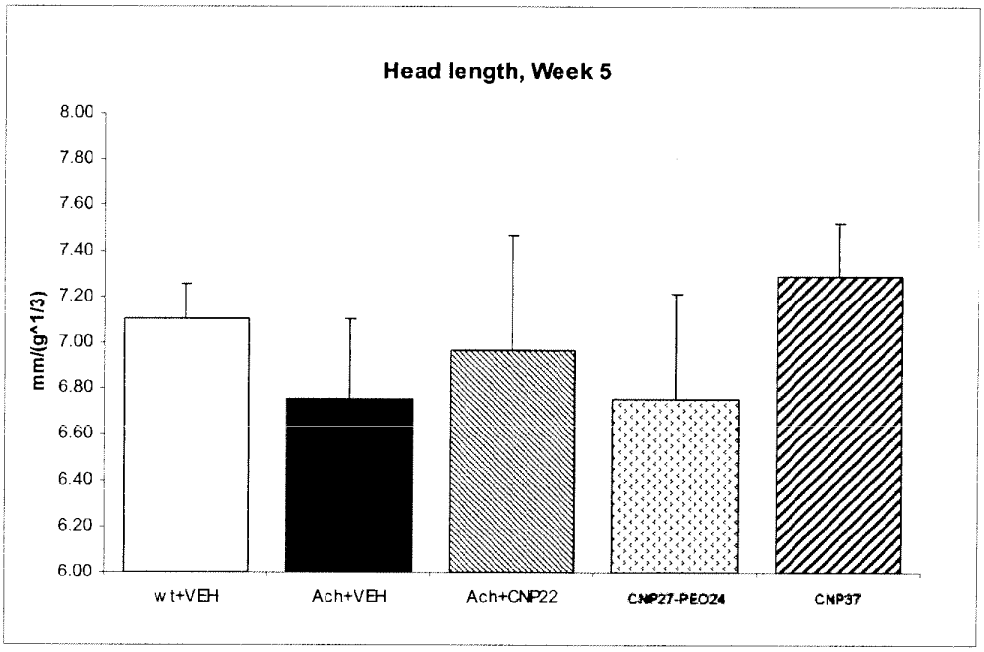


FIGURE 57

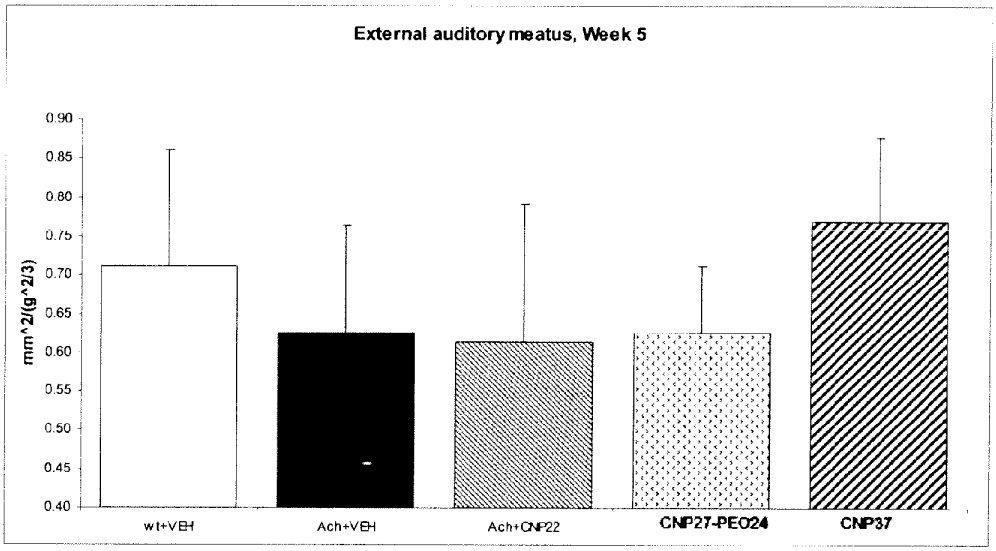


FIGURE 58

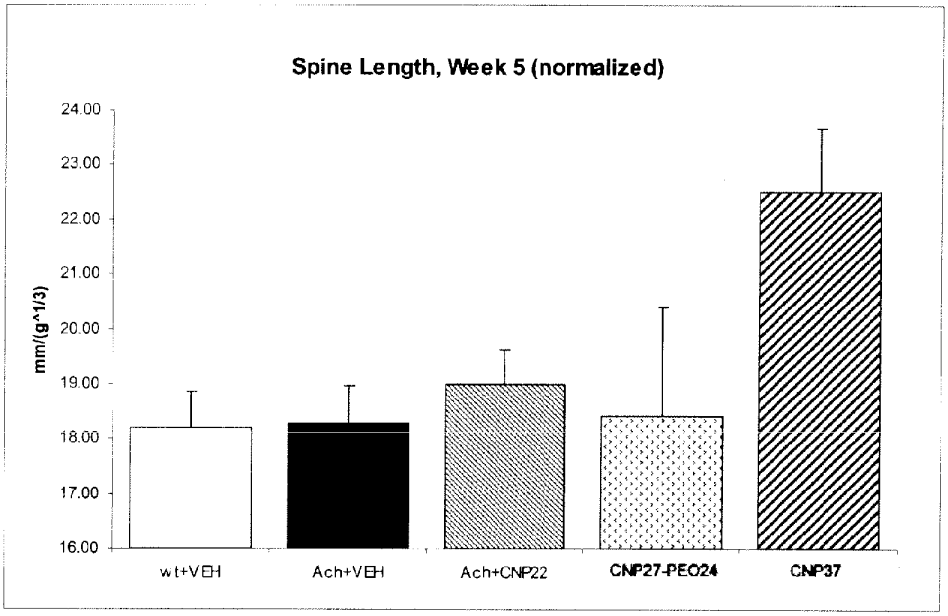


FIGURE 59

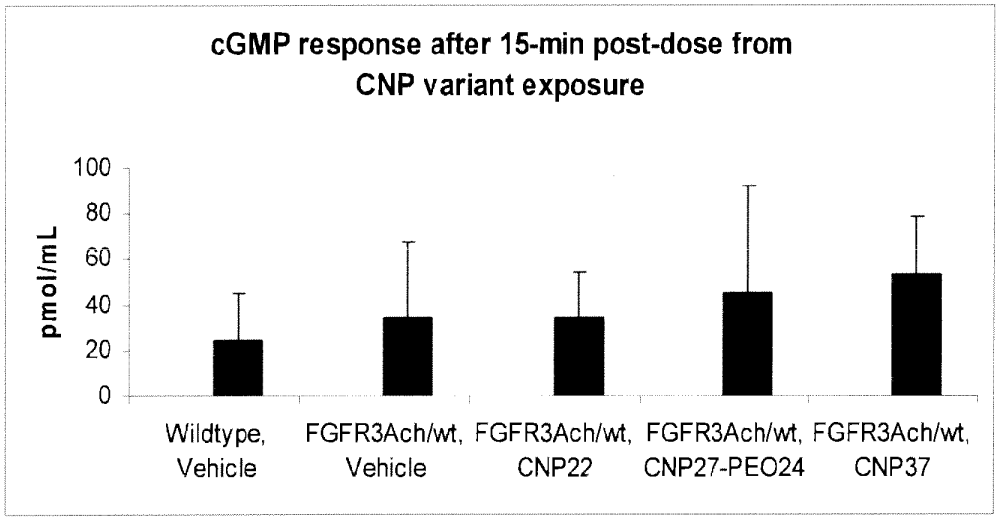


FIGURE 60

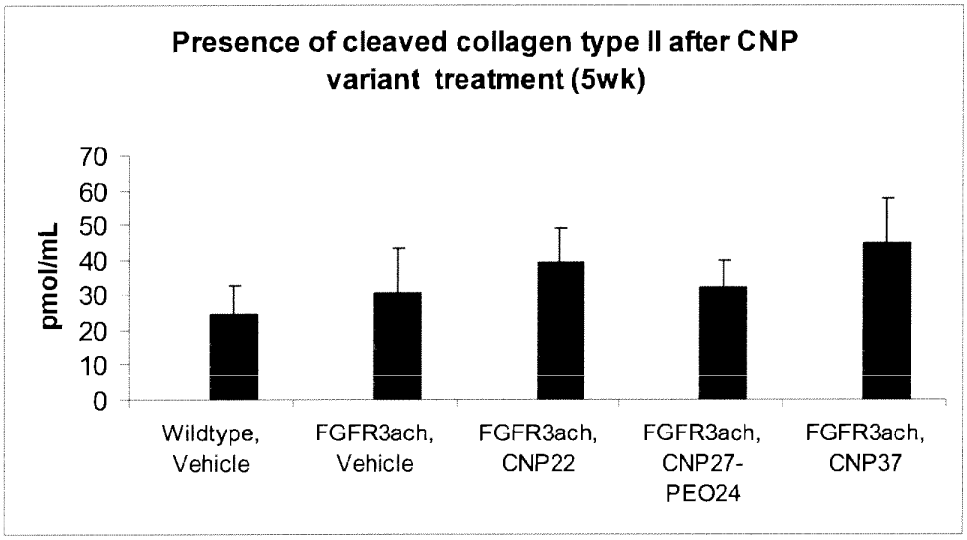


FIGURE 61

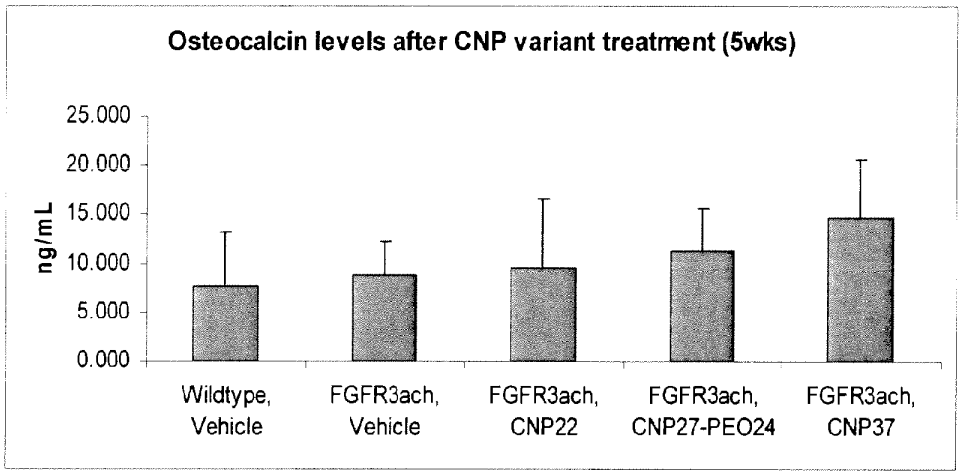


FIGURE 62

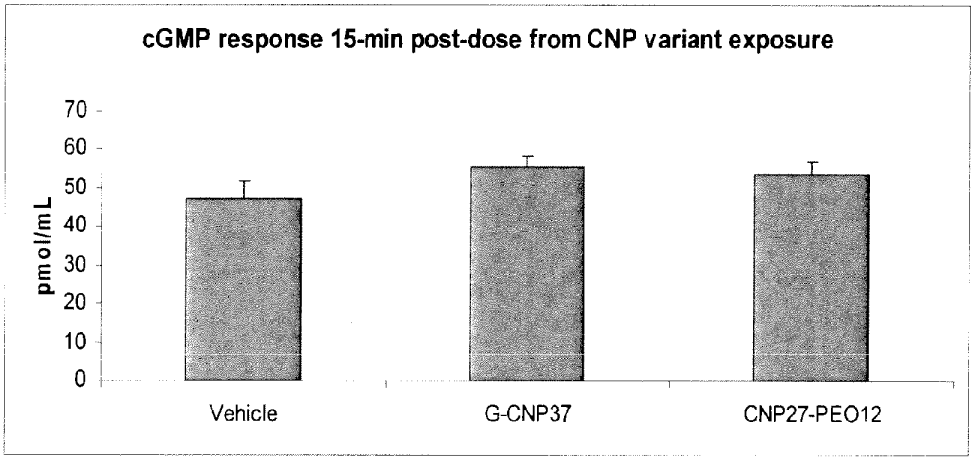


FIGURE 63

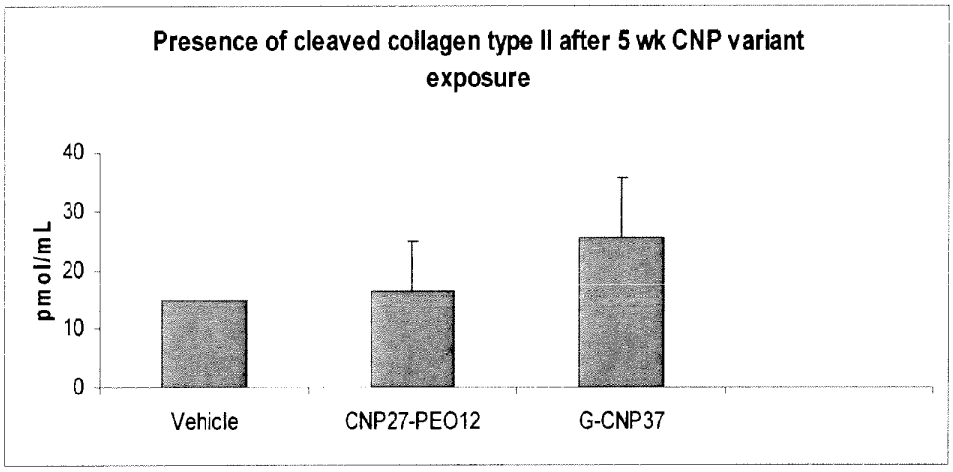
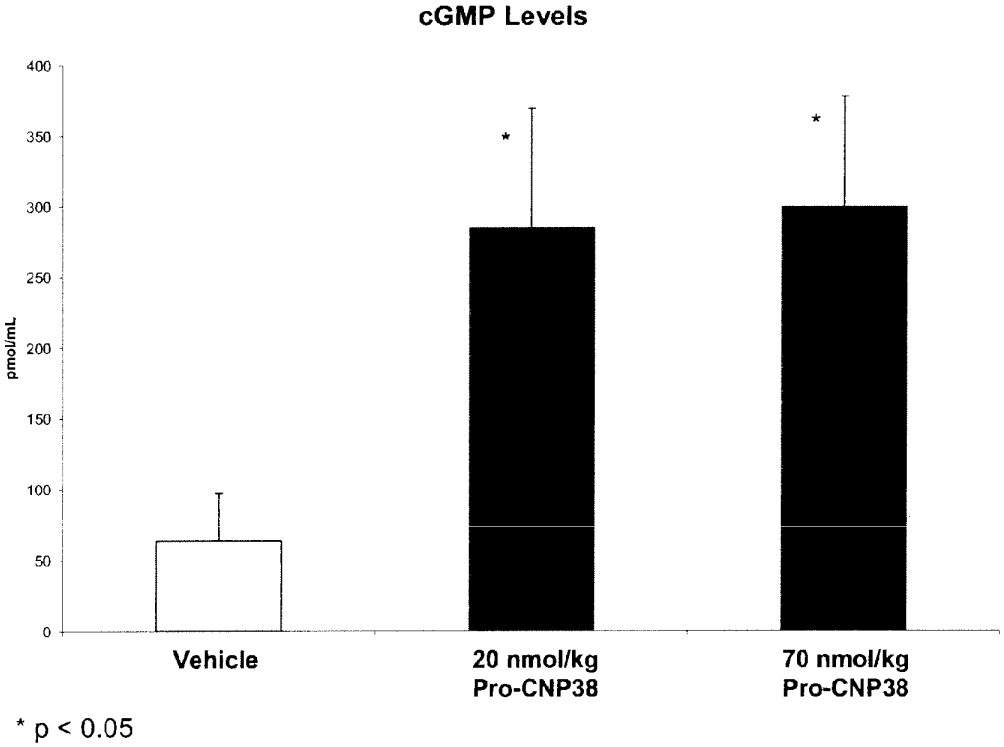


FIGURE 64



* $p < 0.05$

FIGURE 65

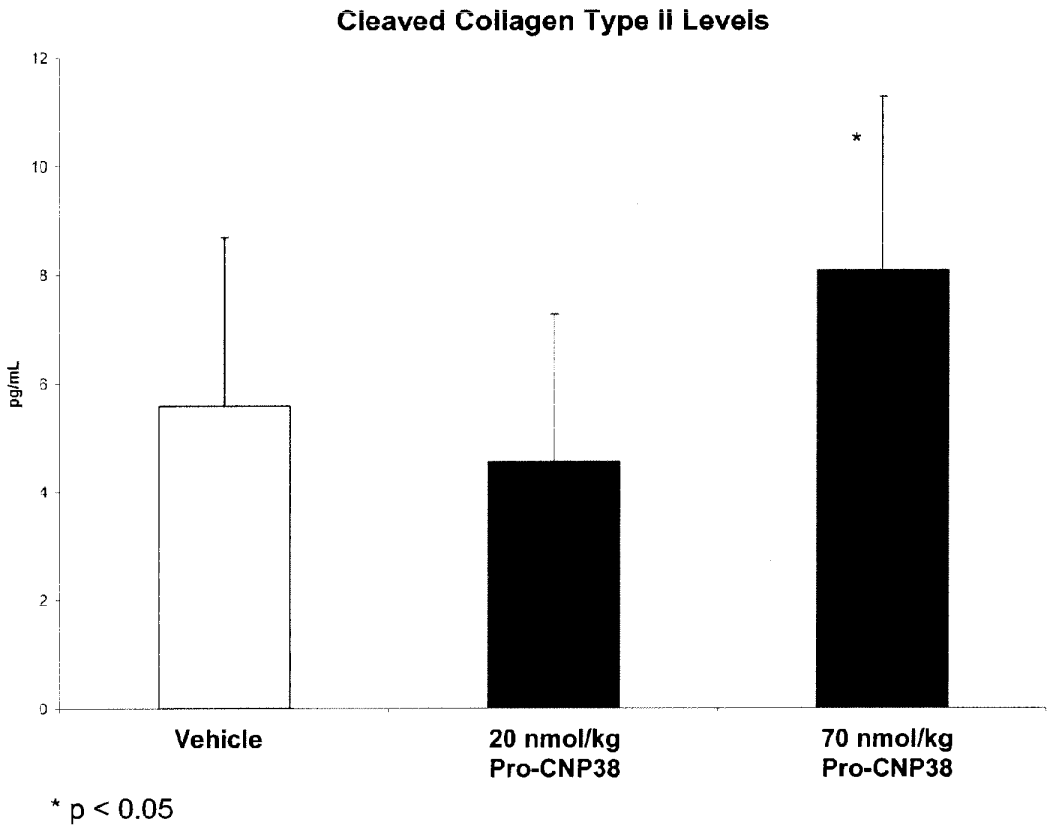

Cleaved Collagen Type II Levels
pg/mL
12
10
8
6
4
2
0
*
Vehicle
20 nmol/kg Pro-CNP38
70 nmol/kg Pro-CNP38
* p < 0.05

FIGURE 66

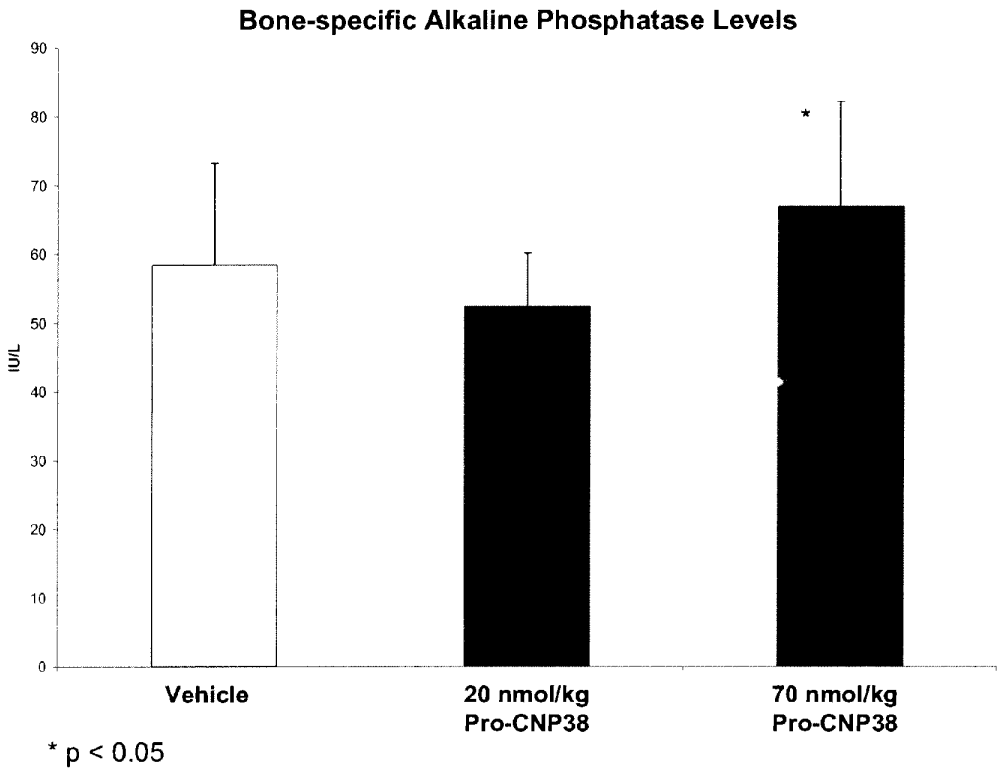

Bone-specific Alkaline Phosphatase Levels
IU/L
90
80
70
60
50
40
30
20
10
0
*
Vehicle
20 nmol/kg Pro-CNP38
70 nmol/kg Pro-CNP38
* p < 0.05

FIGURE 67

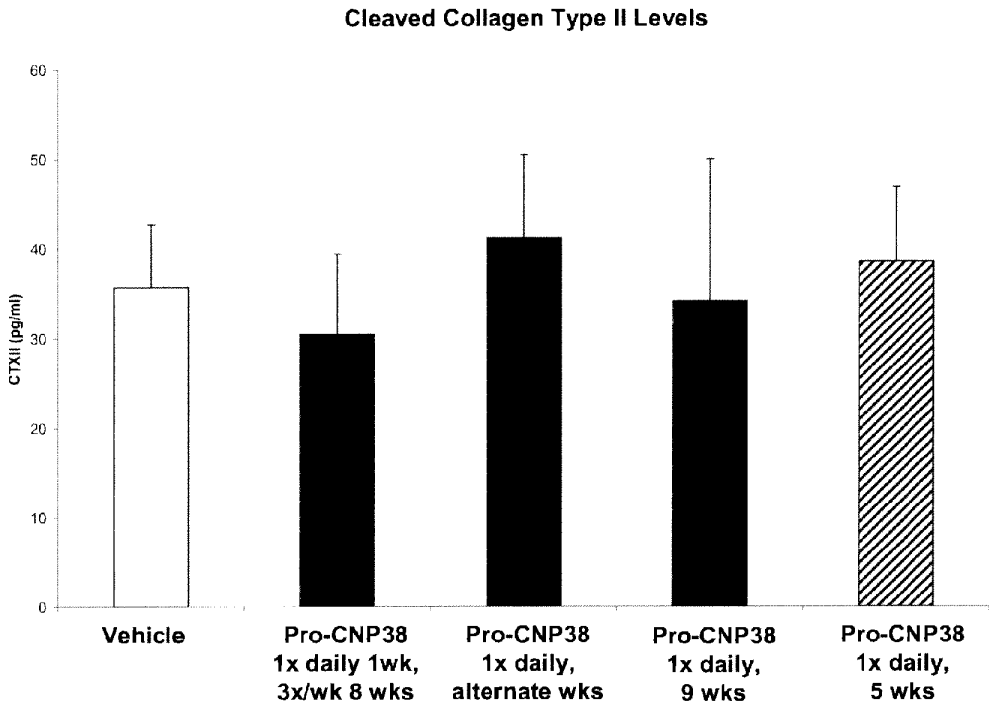

Cleaved Collagen Type II Levels
CTXII (pg/ml)
60
50
40
30
20
10
0
Vehicle
Pro-CNP38 1x daily 1wk, 3x/wk 8 wks
Pro-CNP38 1x daily, alternate wks
Pro-CNP38 1x daily, 9 wks
Pro-CNP38 1x daily, 5 wks

FIGURE 68

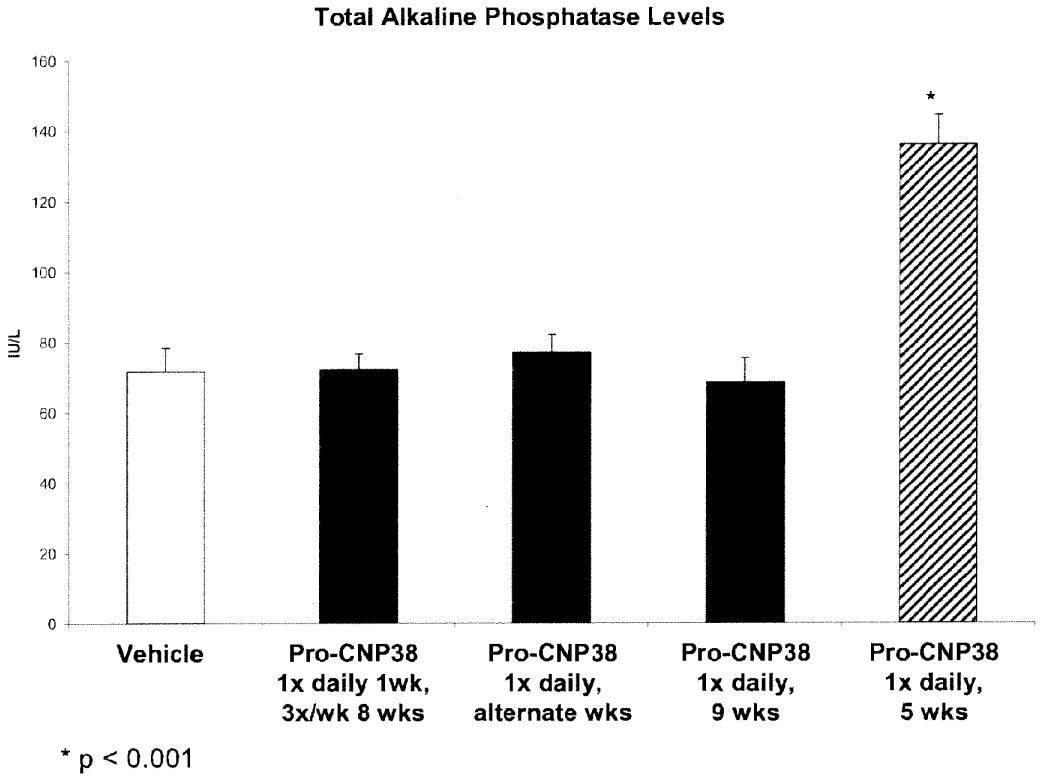

Total Alkaline Phosphatase Levels
IU/L
160
140
120
100
80
60
40
20
0
*
Vehicle
Pro-CNP38 1x daily 1wk, 3x/wk 8 wks
Pro-CNP38 1x daily, alternate wks
Pro-CNP38 1x daily, 9 wks
Pro-CNP38 1x daily, 5 wks
* p < 0.001

FIGURE 69

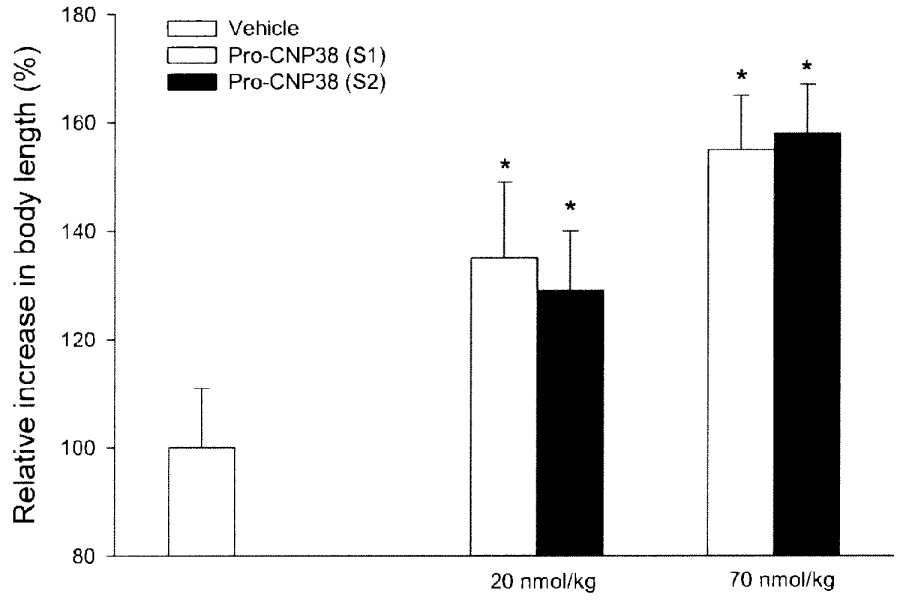
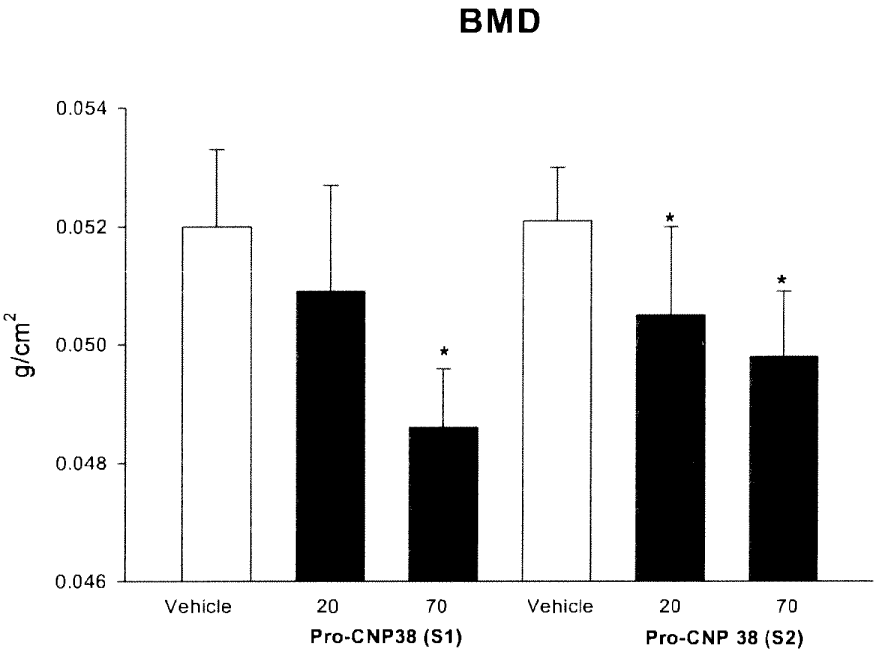

Vehicle
Pro-CNP38 (S1)
Pro-CNP38 (S2)
Relative increase in body length (%)
180
160
140
120
100
80
*
*
*
*
20 nmol/kg
70 nmol/kg

FIGURE 70

A.


BMD
0.054
0.052
0.050
0.048
0.046
g/cm2
*
*
*
Vehicle
20
70
Vehicle
20
70
Pro-CNP38 (S1)
Pro-CNP 38 (S2)

B.

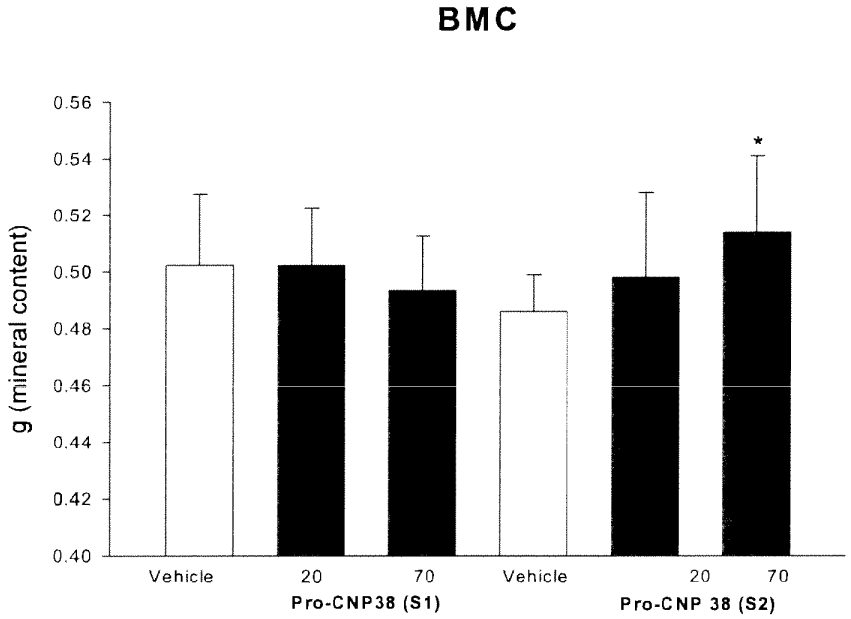

BMC
0.56
0.54
0.52
0.50
0.48
0.46
0.44
0.42
0.40
g (mineral content)
*
Vehicle
20
70
Vehicle
20
70
Pro-CNP38 (S1)
Pro-CNP 38 (S2)

FIGURE 71

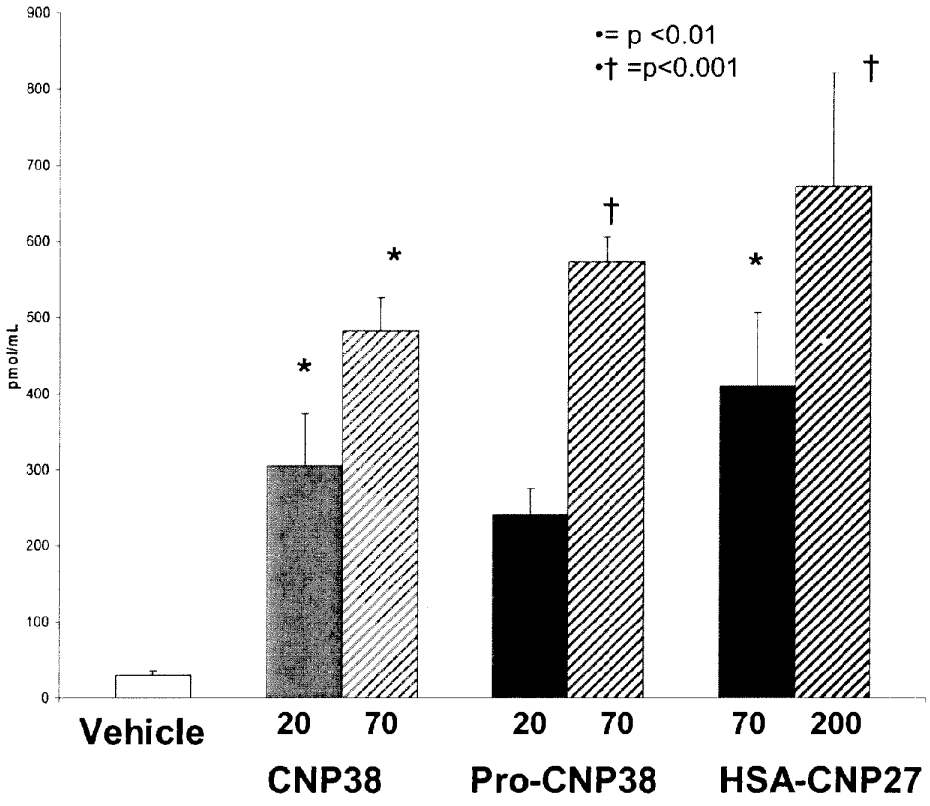

•= p <0.01
•† =p<0.001
pmol/mL
900
800
700
600
500
400
300
200
100
0
*
*
*
†
†
Vehicle
20
70
20
70
70
200
CNP38
Pro-CNP38
HSA-CNP27

FIGURE 72

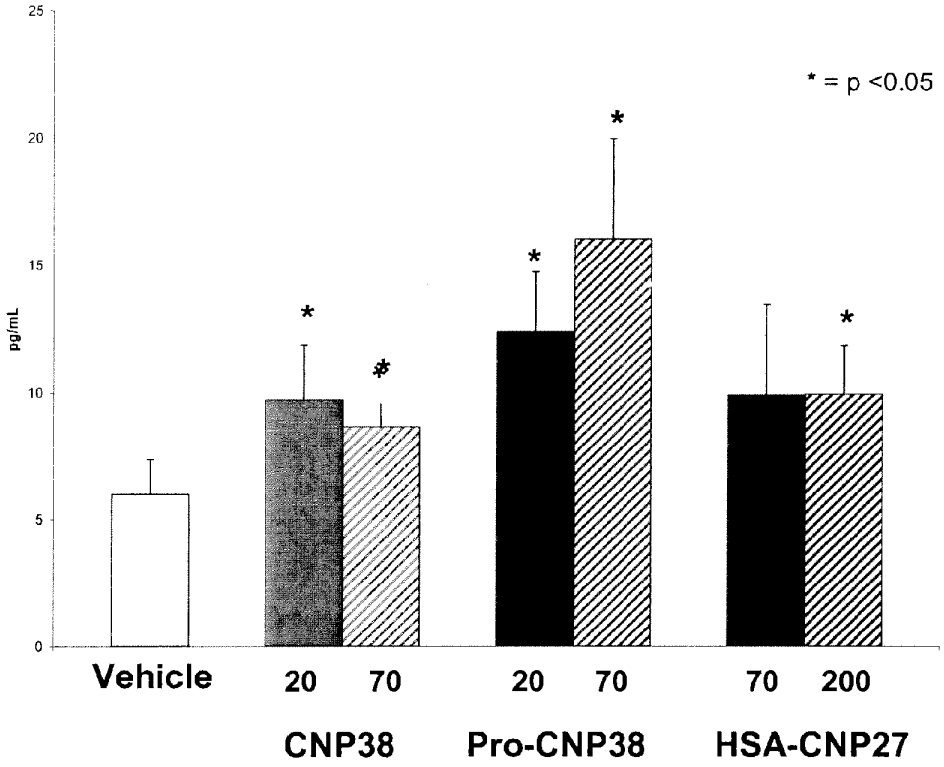

* = p <0.05
pg/mL
25
20
15
10
5
0
*
*
*
*
*
Vehicle
20
70
20
70
70
200
CNP38
Pro-CNP38
HSA-CNP27

FIGURE 73

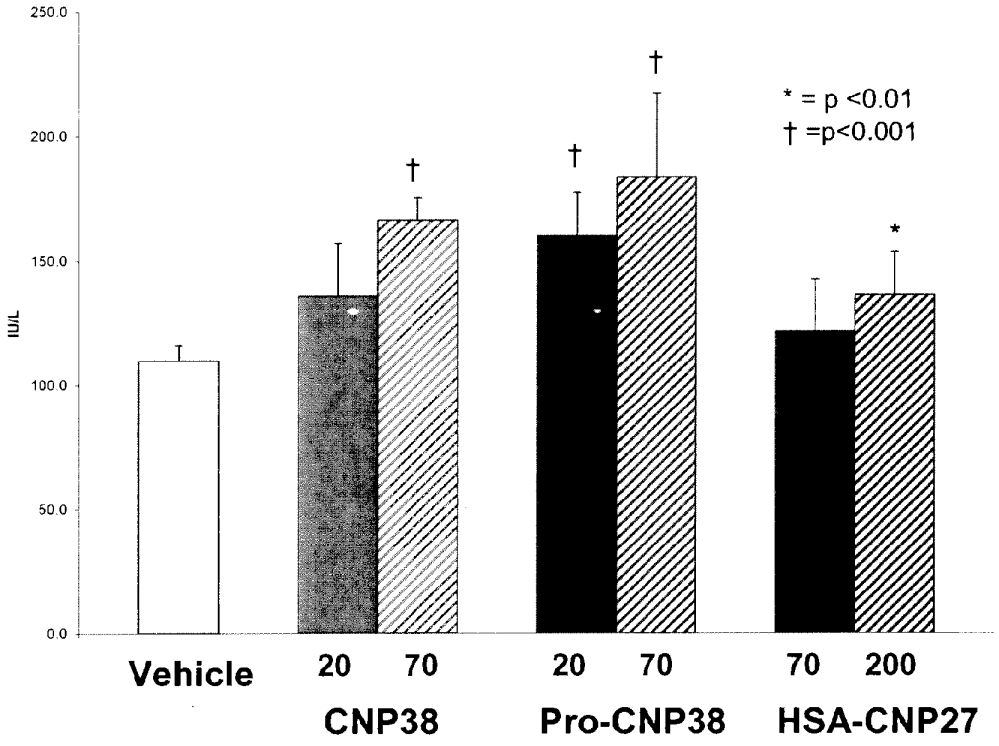
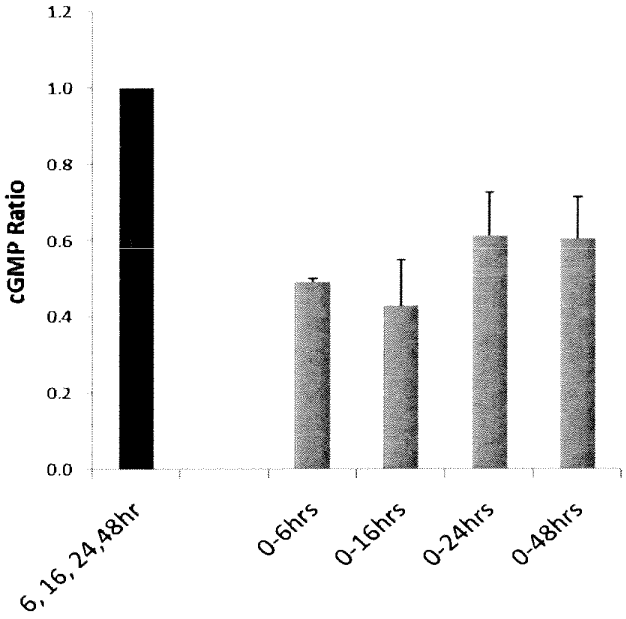

250.0
200.0
150.0
100.0
50.0
0.0
IU/L
* = p <0.01
† =p<0.001
†
†
†
*
Vehicle
20
70
20
70
70
200
CNP38
Pro-CNP38
HSA-CNP27

FIGURE 74

A.


1.2
1.0
0.8
0.6
0.4
0.2
0.0
cGMP Ratio
6, 16, 24, 48hr
0-6hrs
0-16hrs
0-24hrs
0-48hrs

B.

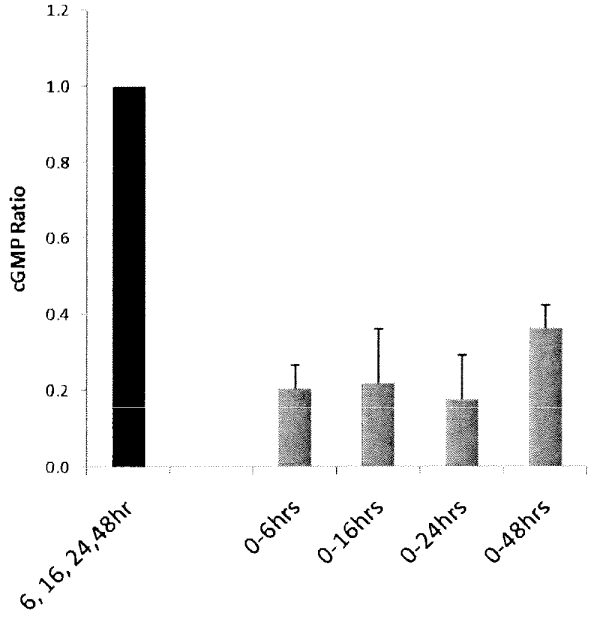
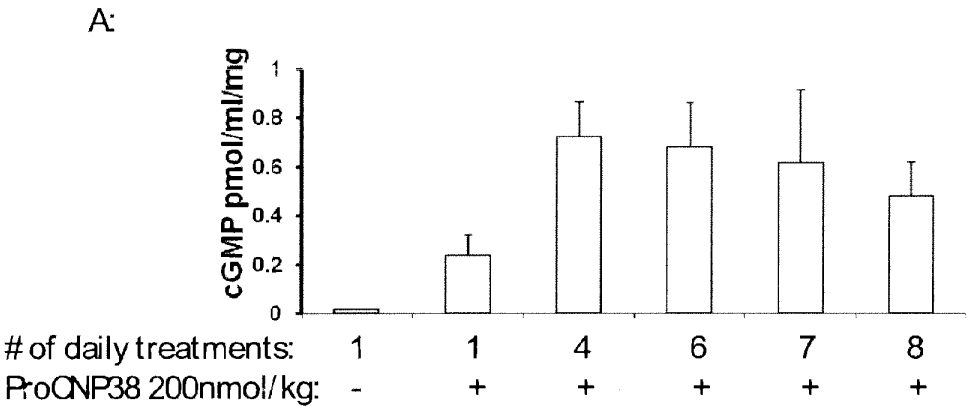

1.2
1.0
0.8
0.6
0.4
0.2
0.0
cGMP Ratio
6, 16, 24, 48hr
0-6hrs
0-16hrs
0-24hrs
0-48hrs

FIGURE 75


A:
cGMP pmol/ml/mg
1
0.8
0.6
0.4
0.2
0
# of daily treatments: 1 1 4 6 7 8
ProCNP38 200nmol/kg: - + + + + +

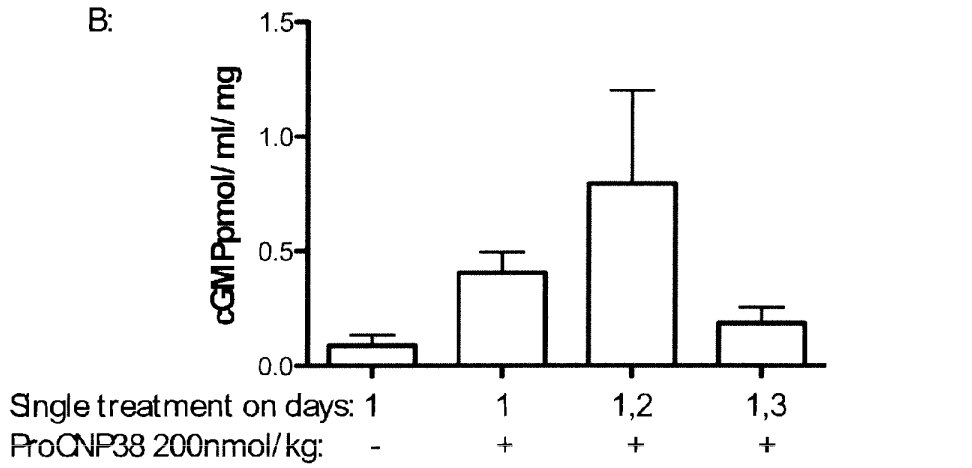

B:
cGMPpmol/ml/mg
1.5
1.0
0.5
0.0
Single treatment on days: 1 1 1,2 1,3
ProCNP38 200nmol/kg: - + + +

FIGURE 76

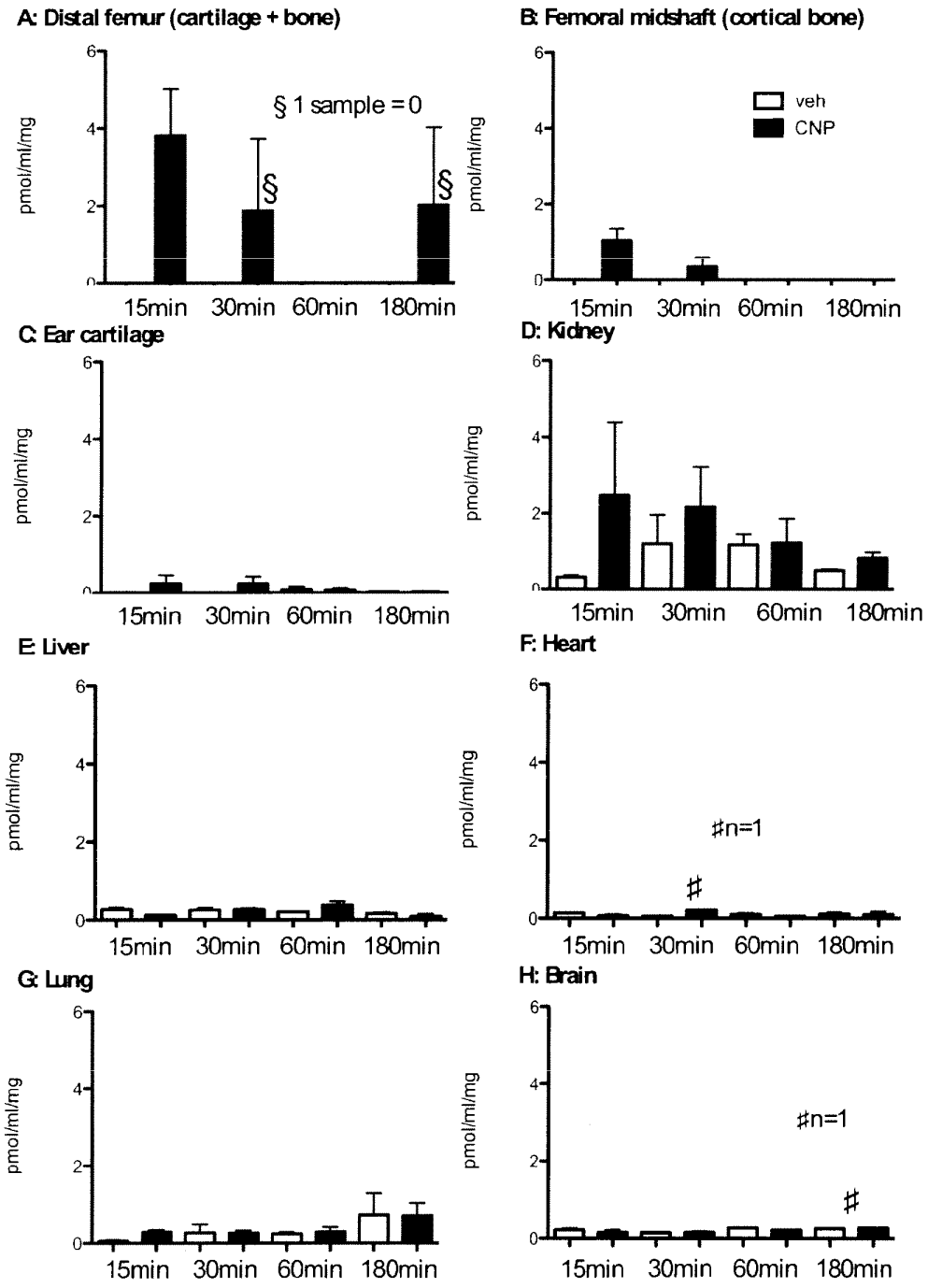

A: Distal femur (cartilage + bone)
§ 1 sample = 0
§
§
pmol/ml/mg
15min 30min 60min 180min
B: Femoral midshaft (cortical bone)
veh
CNP
C: Ear cartilage
D: Kidney
E: Liver
F: Heart
#n=1
#
G: Lung
H: Brain
#n=1
#

FIGURE 77

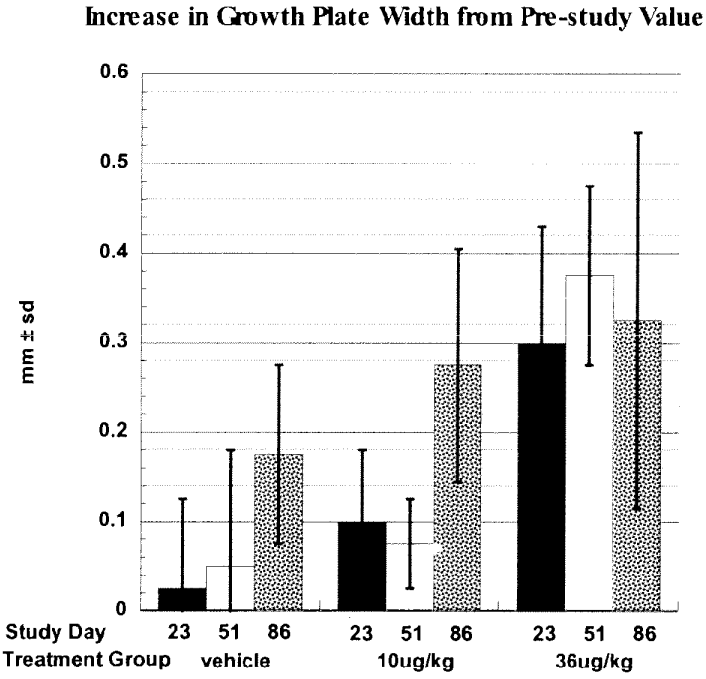


FIGURE 79

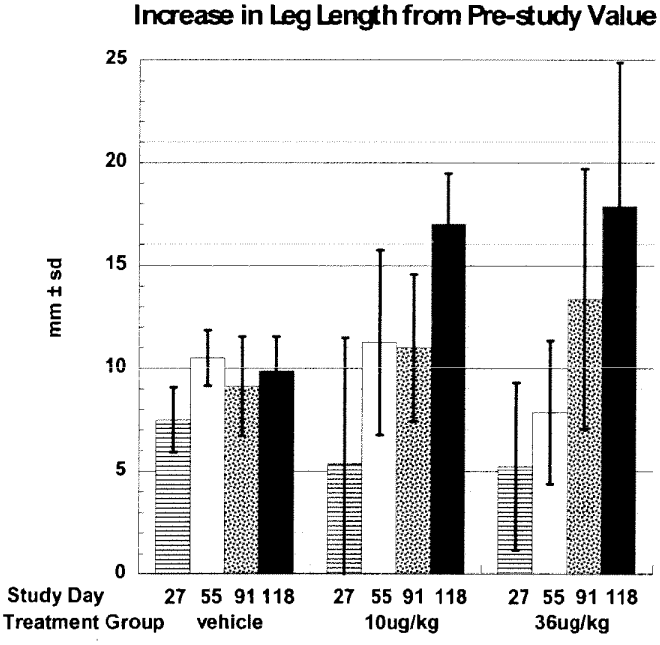
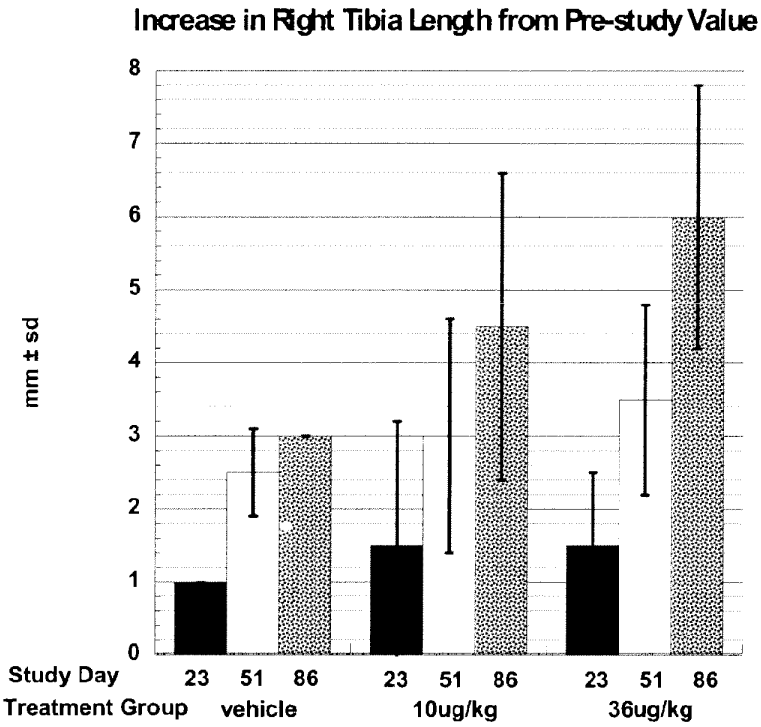


FIGURE 78

A.


Increase in Right Tibia Length from Pre-study Value
mm ± sd
0
1
2
3
4
5
6
7
8
Study Day
23 51 86 23 51 86 23 51 86
Treatment Group
vehicle
10ug/kg
36ug/kg

B.

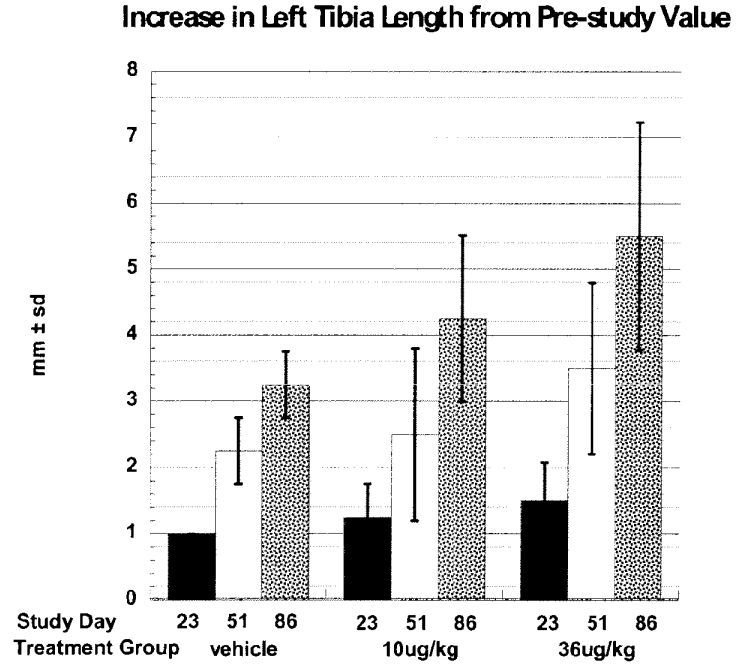

Increase in Left Tibia Length from Pre-study Value
mm ± sd
0
1
2
3
4
5
6
7
8
Study Day
23 51 86 23 51 86 23 51 86
Treatment Group
vehicle
10ug/kg
36ug/kg

FIGURE 80

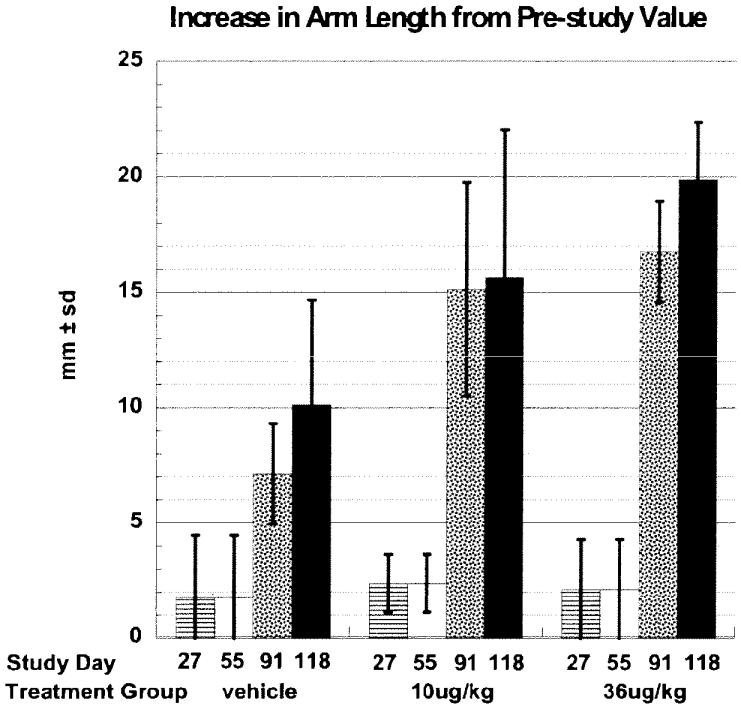


FIGURE 81

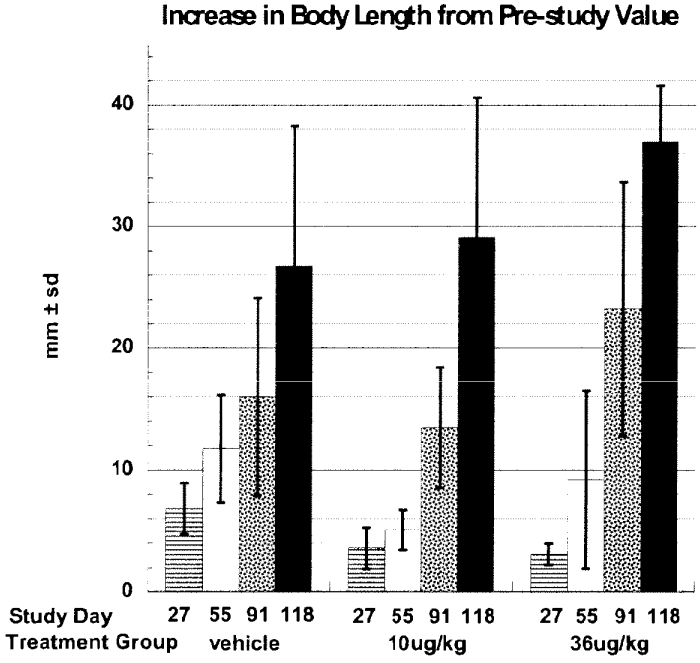


FIGURE 82

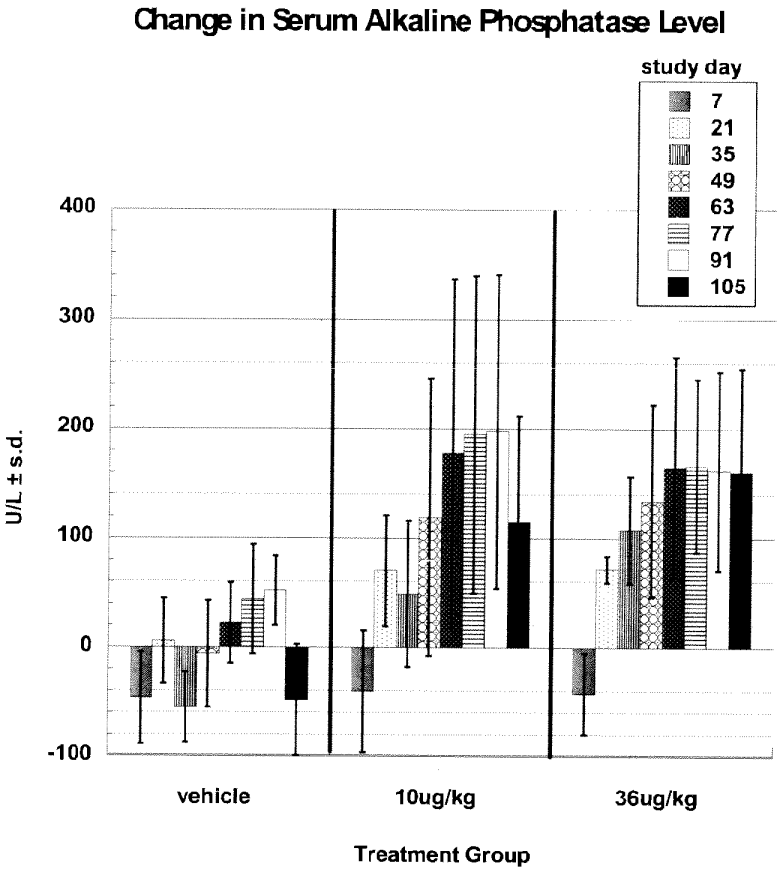
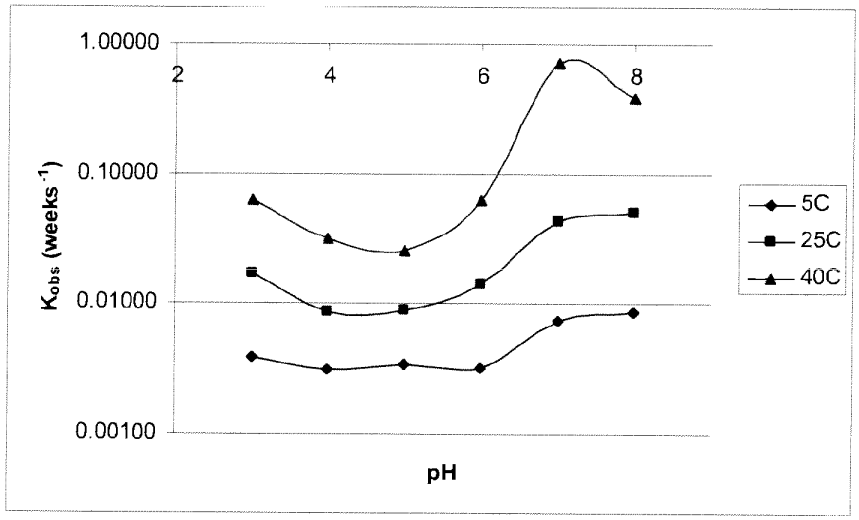


FIGURE 83



VARIANTS OF C-TYPE NATRIURETIC PEPTIDE

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application claims the priority and benefit of U.S. Provisional Application No. 61/254,563, filed on Oct. 23, 2009, and U.S. Provisional Application No. 61/180,112, filed on May 20, 2009, the disclosure of each of which is incorporated herein by reference in its entirety.

FIELD OF THE DISCLOSURE

The field of the disclosure relates, in general, to variants of C-type natriuretic peptide (CNP), compositions comprising CNP variants, methods of making CNP variants, and methods of using CNP variants to treat disorders responsive to CNP, including but not limited to bone-related disorders such as skeletal dysplasias (e.g., achondroplasia) and vascular smooth muscle disorders.

BACKGROUND OF THE DISCLOSURE

The natriuretic peptide family consists of three structurally related peptides: atrial natriuretic peptide (ANP) (Genbank Accession No. NP_006163, for the ANP precursor protein, NPPA), brain natriuretic peptide (BNP) (GenBank Accession No. NP_002512, for the BNP precursor protein, NPPB), and C-type natriuretic peptide (CNP) (Biochem. Biophys. Res. Commun., 168: 863-870 (1990) (GenBank Accession No. NP_077720, for the CNP precursor protein, NPPC) (J. Hypertens., 10: 907-912 (1992)). These small, single chain peptides (ANP, BNP, CNP) have a 17-amino acid loop structure (Levin et al., N. Engl. J. Med., 339: 863-870 (1998)) and have important roles in multiple biological processes. ANP and BNP bind to and activate the natriuretic peptide receptor A (NPR-A), also termed guanalyl cyclase A (GC-A), resulting in higher intracellular cyclic guanosine monophosphate (cGMP) levels. Likewise, CNP interacts with NPR-B (GC-B) to stimulate the generation of cGMP (J. Hypertens., 10: 1111-1114 (1992)). A third type of receptor, NPR-C, binds each of the natriuretic peptides with high affinity and functions primarily to capture the peptides from the extracellular compartment and deposit the peptides into lysosomes, where they are degraded (Science, 238: 675-678 (1987)). ANP and BNP are produced primarily within the muscle cells of the heart, and are believed to have important roles in cardiovascular homeostasis (Science, 252: 120-123 (1991)). CNP is expressed more widely, including in the central nervous system, reproductive tract, bone and endothelium of blood vessels (Hypertension, 49: 419-426 (2007)).

In humans, CNP is initially produced from the natriuretic peptide precursor C(NPPC) gene as a single chain 126-amino acid pre-pro polypeptide (Biochem. Biophys. Res. Commun., 168: 863-870 (1990)). Removal of the signal peptide yields pro-CNP, and further cleavage by the endoprotease furin generates an active 53-amino acid peptide (CNP-53), which is secreted and cleaved again by an unknown enzyme to produce the mature 22-amino acid peptide (CNP-22) (Wu, J. Biol. Chem. 278: 25847-852 (2003)). CNP-53 and CNP-22 differ in their distribution, with CNP-53 predominating in tissues, while CNP-22 is mainly found in plasma and cerebrospinal fluid (J. Alfonzo, Recept. Signal. Transduct. Res., 26: 269-297 (2006)). The predominant CNP form in cartilage is unknown. Both CNP-53 and CNP-22 bind similarly to NPR-B. Furthermore, they both induce cGMP production in a dose-dependent and similar fashion (V T Yeung, Peptides, 17: 101-106 (1996)).

Natural CNP gene and polypeptide have been previously described. U.S. Pat. No. 5,352,770 discloses isolated and purified CNP-22 from porcine brain identical in sequence to human CNP and its use in treating cardiovascular indications. U.S. Pat. No. 6,034,231 discloses the human gene and polypeptide of proCNP (126 amino acids) and the human CNP-53 gene and polypeptide.

Clearance of CNP from the extracellular space occurs through the action of membrane-bound neutral endopeptidase (NEP), which rapidly degrades CNP (Biochem. J., 291 (Pt 1): 83-88 (1993)), and through NPR-C, which binds to and deposits CNP into lysosomes, where CNP is degraded. CNP has been shown to have an in vivo half-life of 2.6 min in the normal human (J. Clin. Endocrinol. Metab., 78: 1428-35 (1994)). The low plasma concentration of CNP (J. Bone Moner. Res., 19 (Suppl. 1) S20 (2004)) and its co-expression with NPR-B in a number of tissues suggests that CNP functions primarily through an autocrine/paracrine mechanism.

As stated above, CNP binds to and activates natriuretic peptide receptor B (NPR-B), also termed guanylyl cyclase B (GC-B), resulting in higher intracellular cyclic guanosine monophosphate (cGMP) levels. Downstream signaling mediated by cGMP generation influences a diverse array of biological processes that include endochondral ossification. Accordingly, elevated or depressed levels of any of the components in this pathway may lead to aberrant bone growth. For example, knockout of either CNP or NPR-B in mouse models results in animals having a dwarfed phenotype with shorter long bones and vertebrae. Mutations in human NPR-B that block proper CNP signaling have been identified and result in dwarfism (Olney, et al., J. Clin. Endocrinol. Metab. 91(4): 1229-1232 (2006); Bartels, et al., Am. J. Hum. Genet. 75: 27-34 (2004)). In contrast, mice engineered to produce elevated levels of CNP display elongated long bones and vertebrae.

Achondroplasia is a result of an autosomal dominant mutation in the gene for fibroblast growth factor receptor 3 (FGFR-3), which causes an abnormality of cartilage formation. FGFR-3 normally has a negative regulatory effect on chondrocyte growth, and hence bone growth. In achondroplasia, the mutated form of FGFR-3 is constitutively active, which leads to severely shortened bones. Both chondrocyte proliferation and differentiation appear to be disturbed, leading to remarkably short growth plate cartilage (P. Krejci et al., J. Cell Sci. 118: 5089-5100 (2005)). Endochondral ossification is the process that governs longitudinal long-bone growth. There are four zones of the growth plate—resting, proliferative, hypertrophic and zone of calcification. In the growth plate, NPR-B is expressed by proliferative cells while NPR-C is expressed by hypertrophic cells (Yamashite et al., J. Biochem. 127: 177-179 (2000)). In normal endochondral bone growth, chondrocytes organize in columns and proliferate in the proliferative zone of the growth plate. These columns are disorganized in achondroplasia patients. Additionally, the hypertrophic zone is where the cells become large and eventually apoptose (lyse), leading to osteocyte invasion and mineralization. The hypertrophic chondrocytes and the overall size of the zone are much smaller in achondroplasia patients than in normal patients. CNP is an agonist for NPR-B, a positive regulator of chondrocyte and bone growth. Downstream signaling of CNP/NPR-B inhibits the FGFR-3 pathway at the level of mitogen-activated protein kinase (MAP K). Inhibition at MAP K promotes proliferation and differ-

entiation of the chondrocytes in the proliferative and hypertrophic zones of the growth plate, resulting in bone growth.

In humans activating mutations of FGFR-3 are the primary cause of genetic dwarfism. Mice having activated FGFR-3 serve as a model of achondroplasia, the most common form of the skeletal dysplasias, and overexpression of CNP rescues these animals from dwarfism. Accordingly, CNP and functional variants of CNP are potential therapeutics for treatment of the various skeletal dysplasias.

Therapeutic use of CNP is currently limited by its short plasma half-life, which has been shown to be 2.6 minutes in vivo in humans (J. Clin. Endocrinol. Metab., 78: 1428-35 (1994)). To increase CNP concentration above intrinsic levels (about 5 pM) typically found in human plasma, continuous infusion has been necessary in all human and animal studies using systemically administered CNP. A CNP variant having a longer in vivo serum half-life and exhibiting similar or improved activity to that of wild-type CNP is important for a sustainable therapeutic strategy. Two mechanisms by which the half-life of CNP is reduced in human plasma are degradation by neutral endopeptidase (NEP) and clearance by natriuretic peptide receptor C(NPR-C) (Growth Horm. & IGF Res., 16: S6-S14 (2006)). Modifications of peptides reportedly can improve resistance to endopeptidase and exopeptidase cleavage (Amino Acids, 30: 351-367 (2006); Curr. Opin. Biotech., 17: 638-642 (2006)).

The biological activities of various analogs and derivatives of CNP have been evaluated. By substituting S-methyl Cys in place of both Cys_6 and Cys_{22}, cyclization of the peptide via a Cys6-Cys22 disulfide linkage was reportedly shown to be important for the activity of CNP in stimulating cGMP formation (Biochem. Biophys. Res. Comm., 183: 964-969 (1992), also using alanine scanning to identify amino acids important for CNP functionality). A significant additional enhancement of activity reportedly results from the combined presence of the amino acids Leu_9, Lys_{10}, and Leu_{11}. U.S. Pat. No. 5,434,133 describes CNP analogs comprising CNP-22 with substitutions at amino acid position 6, 7, 9, 11, or 22, wherein the amino acid is selected from Cys or Pmp (pentacyclomercaptopropionic acid) at position 6, Phe, 4-chloro-Phe, 4-fluoro-Phe, 4-nitro-Phe, or Cha (3-cyclohexyl-Ala) at position 7, Gly, Val, Aib, or tLeu at position 9, Leu or Ile at position 11, and Cys or Pmp at position 22.

U.S. Patent Publication No. 2004/0138134 (now U.S. Pat. No. 7,276,481) describes CNP variants comprising amino acids Cys_6 to Cys_{22} of CNP-22 ("CNP-17") which include at least one substitution for another natural amino acid at position 9, 10, 11, 16, 17, 19, or 20, CNP variants with insertions and deletions, such as addition of a His residue at the reported primary site of NEP cleavage, between Cys_6 and Phe_7, and methods of using such variants for increasing the size of a bone growth plate in abnormal bone and elongation of an abnormal bone. However, no significant gains in activity as measured by cGMP production were obtained for these variants, and activity was diminished for nearly all of the variants, as observed in an in vitro cell-based method (Example 7). Further no supportive data, such as for example in vitro stability or in vivo determination of improved pharmacokinetics (PK) were provided to substantiate the asserted NEP resistance and NPR-C resistance of the CNP analogs. U.S. Pat. No. 6,743,425 discloses substances for treating achondroplasia which activate NPR-B/GC-B and are peptides or low molecular weight compounds, including the C-type natriuretic peptides CNP-22 and CNP-53. PCT Publication No. WO 94/20534 discloses a chimera of CNP-22 and the 5-amino acid C-terminus of ANP designated as the vasonatrin peptide (VNP), a limited number of amino acid substitutions and cyclic chimeric peptides that result from formation of a disulfide or double bond.

Approaches for improving the half-life of other natriuretic peptide family members include decreasing the affinity of ANP for NPR-C (U.S. Pat. No. 5,846,932), utilizing pentapeptide antagonists of NPR-C (WO 00/61631), and co-administering NEP inhibitors such as thiorphan and candoxatril (Clin. Exp. Pharma. Physiol., 25: 986-991 (1997), Hyperten., 30: 184-190 (1997)). WO 2004/047871 describes conjugates of BNP and BNP variants to polyalkylene glycol moieties, sugar moieties, polysorbate moieties, polycationic moieties, and other hydrophilic polymer moieties that reportedly exhibit improved half-life in circulation and reportedly are useful for the treatment of acute congestive heart failure.

There have been no published reports, however, on a successful strategy for making CNP resistant to NEP while retaining its functionality.

SUMMARY OF THE DISCLOSURE

The present disclosure relates to variants of C-type natriuretic peptide (CNP) which are useful in the treatment of bone-related disorders (e.g., achondroplasia) and vascular smooth muscle disorders. The disclosure encompasses CNP variants having increased serum half-life, e.g. as a result of reduced ability to bind to neutral endopeptidase (NEP), greater resistance to proteolysis by NEP and/or reduced affinity to the clearance natriuretic peptide receptor C(NPR-C), while retaining the functionality of CNP.

The wild-type sequence of human CNP-22 (referred to herein as "hCNP22", "wtCNP22" or "CNP22") is set forth below:

(SEQ ID NO: 1)
(N-terminus) Gly_1-Leu_2-Ser_3-Lys_4-Gly_5-Cys_6-Phe_7-Gly_8-Leu_9-Lys_{10}-Leu_{11}-Asp_{12}-Arg_{13}-Ile_{14}-Gly_{15}-Ser_{16}-Met_{17}-Ser_{18}-Gly_{19}-Leu_{20}-Gly_{21}-Cys_{22}.

Positions 6 to 22 of CNP22 form a cyclic domain by means of a disulfide bond between Cys6 and Cys22. The 17-amino acid cyclic structure has been shown to be important for binding of CNP to NPR-B (Schiller, Biochem. Biophys. Res. Commun., 138: 880-886 (1986)). The amino acid sequence of positions 6 to 22 of CNP22 is referred to herein as "CNP17" (SEQ ID NO: 2).

CNP is susceptible to NEP cleavage at a number of sites: Cys6-Phe7, Gly8-Leu9, Lys10-Leu11, Arg13-Ile14, Ser16-Met17 and Gly19-Leu20. In one embodiment, the disclosure encompasses a CNP variant that is (1) modified to increase its overall size or molecular weight, e.g., to a range from about 2.6 kDa or 2.8 kDa to about 4 kDa, 4.2 kDa, 4.4 kDa, 4.6 kDa, 4.8 kDa, 5 kDa, 5.2 kDa, 5.4 kDa, 5.6 kDa, 5.8 kDa, 6 kDa, 6.2 kDa, 6.4 kDa, or to about 7 kDa, 7.2 kDa or about 8.2 kDa, and/or (2) modified at certain amino acid positions to reduce its susceptibility to NEP cleavage at 1, 2, 3, 4, 5 or all 6 of the sites listed above. The size or molecular weight of the CNP variant can be increased by various means, e.g., by conjugating additional amino acids and/or other kinds of chemical (e.g., natural or synthetic polymeric) groups to the peptide sequence at, e.g., the N-terminus, the C-terminus and/or side chain(s), and/or by using natural amino acids, unnatural amino acids, and/or peptidomimetics with bulkier side chains. The CNP variant is optionally further conjugated to other functional or structural moieties. Optionally in combination with any of the embodiments described herein, mutation(s) (e.g., substitution(s), addition(s), and/or deletion(s))

may be introduced to certain position(s) of CNP22 to reduce the CNP variants' affinity to NPR-C. Further modifications may be made without affecting NEP resistance or CNP activity, e.g., conservative substitutions, or other modifications known in the art.

In one embodiment, the CNP variant is represented by the general formula: (x)-Gly_1-Leu_2-Ser_3-Lys_4-Gly_5-Cys_6-Phe_7-Gly_8-Leu_9-Lys_{10}-Leu_{11}-Asp_{12}-Arg_{13}-Ile_{14}-Gly_{15}-Ser_{16}-Met_{17}-Ser_{18}-Gly_{19}-Leu_{20}-Gly_{21}-Cys_{22}-(z) (SEQ ID NO: 5), wherein:

the CNP variant comprises one or more modified amino acids, which may result in modified peptide bonds (e.g., through use of peptide bond isosteres), at a position corresponding to one or more of the following CNP residues: Gly1, Lys4, Gly5, Cys6, Phe7, Gly8, Leu9, Lys10, Leu11, Ile14, Gly15, Ser16, Met17, Gly19, Leu20 and Gly21; and

(x) and (z) independently may be absent or may be an amino acid sequence derived from a natriuretic polypeptide (e.g., NPPC, ANP, BNP) or a non-natriuretic polypeptide (e.g., human serum albumin (HSA), IgG, etc.).

In an embodiment, the CNP variant includes: (1) a modification at an amino acid position corresponding to one of positions 6, 7 or 8 (Cys6, Phe7 or Gly8) of CNP22, (2) optionally deletion, addition and/or substitution of any or all of the amino acids at positions 1-5 (Gly1, Leu2, Ser3, Lys4, and Gly5) and (3) optionally up to 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 further modifications (deletions, additions and/or substitutions) at positions corresponding to positions 6-22, of which 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 may be conservative substitutions or other substitutions described herein or known in the art.

It is understood that a reference to a particular amino acid position by number (e.g. position 7 of CNP22) refers to the corresponding amino acid position in any CNP variant, even if the number of the position in that CNP variant has changed due to preceding insertions or deletions. For example, a reference to "position 7" or "Phe7" would mean the corresponding position 2 for a CNP variant in which the first five amino acids had been deleted. Similarly, a reference to "position 7" would mean the corresponding position 8 for a CNP variant in which one amino acid had been added to the N-terminus.

In any of the embodiments described herein, the CNP variant may be cyclized through a covalent bond between positions corresponding to 6 and 22 of CNP22. It is contemplated that the covalent bond is formed using any methods known in the art. In another embodiment, the CNP variant may be cyclized through a covalent bond formed between an amino acid at or toward the N-terminus and an amino acid at or toward the C-terminus (referred to as "terminal" amino acids for this purpose) of the peptide. In one embodiment, the covalent bond is formed between the side chains of the two terminal amino acids or the amino acids at positions corresponding to 6 and 22 of CNP22. In another embodiment, the covalent bond is formed between the side chain of one terminal amino acid and the terminal group of the other terminal amino acid, or between the terminal groups of each terminal amino acid. For example, head-to-tail, side chain-to-side chain, side chain-to-head, or side chain-to-tail bonds are possible for the covalent bond formed between the terminal amino acids or between the amino acids at positions corresponding to 6 and 22 of CNP22.

In one embodiment, the disclosure provides a CNP variant having reduced affinity to NEP, and/or greater resistance to cleavage by NEP and/or increased in vivo serum half-life, while retaining functionality of CNP (e.g., stimulation of cGMP production). NEP preferably recognizes substrates smaller than about 3 kDa, due to the limited size of its active site cavity (Oefner, J. Mol. Biol., 296: 341-349 (2000)). In an embodiment, the CNP variants are modified to increase their overall molecular weight to a range from about 2.6 or 2.8 kDa to about 4, 4.6, 5, 5.2, 5.8, 6, 6.4 or 7 kDa, e.g., by adding about 0.6 to about 5 kDa of amino acids, hydrophilic or water-soluble polymers, hydrophobic acids (including fatty acids), and/or carbohydrates. In specific exemplary embodiments, the CNP variants have a molecular weight between about 2.6 kDa and about 7 kDa, or between about 2.8 kDa and 6 kDa, or between about 2.8 kDa and about 5.8 kDa. In certain embodiments, at least about 0.6, 0.8, 1, 1.2, 1.4, 1.6 or 1.8 kDa, or up to 2, 2.2, 2.4, 2.6, 2.8, 3, 3.2, 3.4, 3.6, 3.8, 4, 4.2, 4.4, 4.6, 4.8 or 5 kDa are added, to increase the total molecular weight of the CNP variant, e.g., to a range from about 2.6 or 2.8 kDa up to about 4 kDa, 4.2 kDa, 4.4 kDa, 4.6 kDa, 4.8 kDa, 5 kDa, 5.2 kDa, 5.4 kDa, 5.6 kDa, 5.8 kDa, 6 kDa, 6.2 kDa, 7.2 kDa, 8.2 kDa or higher. In some embodiments, such CNP variants comprise an amino acid sequence at least about 70%, 75%. 80%, 85%, 90%, or 95% identical or homologous to amino acids 6-22 of CNP22. In other embodiments, such CNP variants comprise a substitution, insertion or deletion of 1, 2, 3, 4, 5, 6 or 7 amino acids with another natural or unnatural amino acid or peptidomimetic. While both conservative and non-conservative substitutions or insertions are envisioned at any position, introduction of modifications may commence, e.g., by conservative substitutions in regions that have been identified in the art as involved in CNP activity or NPR-B binding, while non-conservative substitutions may be made in those regions that have been previously shown to be tolerant of modification.

In another embodiment, the CNP variants comprise a CNP having an intact cyclized portion between Cys6 and Cys22, and N-terminal and/or C-terminal tails that contain about 1-40, 1-20, 5-40, 5-35, 10-35, 15-35, 5-31, 10-31, or 15-31 amino acids and are fragments derived from a CNP polypeptide and/or a non-CNP polypeptide. In an embodiment, such CNP variants have a molecular weight in a range from about 2.8 kDa to about 4, 4.6, 5, 5.2, 5.8, 6, 6.4 or 7 kDa. Non-limiting examples of such CNP variants include wild-type CNP22 or CNP22 with one or more amino acid substitutions (e.g., a K4R substitution), having an N-terminal and/or C-terminal extension derived from natriuretic peptide precursor sequences (e.g., ANP, BNP or CNP) from human or other species, a natriuretic peptide precursor C (NPPC) variant with amino acid substitutions, additions and/or deletions (e.g., the CNP variants may be truncations of CNP-53 which result in peptides with a molecular weight between about 2.8 kDa and 5.8 kDa), or other non-CNP polypeptides such as, e.g., serum albumin or IgG protein (e.g., the CNP variants may be CNP chimeras containing fragments of serum albumin or IgG from human or other species).

In one embodiment, CNP variants having a total mass characterized by the ranges described generally herein, e.g., from about 2.6 kDa or 2.8 kDa to about 6 or 7 kDa, designed for increased resistance to NEP degradation, are represented by the general formula:

(x)-Gly_1-Leu_2-Ser_3-$(b)_4$-Gly_5-Cys_6-Phe_7-Gly_8-Leu_9-$(h)_{10}$-Leu_{11}-Asp_{12}-Arg_{13}-Ile_{14}-Gly_{15}-Ser_{16}-Met_{17}-Ser_{18}-Gly_{19}-Leu_{20}-Gly_{21}-Cys_{22}-(z) (SEQ ID NO: 6), wherein:

(x) is a synthetic or natural polymeric group, or a combination thereof, wherein a non-limiting example of a synthetic polymeric group is polyethylene glycol (PEG, also called polyethylene oxide (PEO)), and a non-limiting example of a natural polymeric group is an amino acid sequence containing from 1 to 35 amino acids and derived from NPPC or variants thereof with substitutions and/or deletions, ANP, BNP, or other non-CNP (poly)peptides such as, e.g., serum albumin, IgG, histidine-rich glycoproteins, fibronectin,

fibrinogen, zinc finger-containing polypeptides, osteocrin or fibroblast growth factor 2 (FGF2);

(z) may be absent or may be a synthetic or natural polymeric group, or a combination thereof, wherein a non-limiting example of a synthetic polymeric group is PEG, and a non-limiting example of a natural polymeric group is an amino acid sequence derived from a natriuretic polypeptide (e.g., NPPC, CNP, ANP or BNP) or non-natriuretic polypeptide (e.g., serum albumin or IgG); and

(b) and (h) independently may each be the wild type Lys at that position or may be replaced with a conservative amino acid substitution or any natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amine on a side chain, including but not limited to Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Glu or Ser. In one embodiment, (b) is Arg. In another embodiment, for improved NEP resistance, (b) is not Gly. In yet another embodiment, (h) is not Arg.

Non-limiting examples of amino acid sequences derived from NPPC or variants thereof include:

Arg,

Glu-Arg,

(SEQ ID NO: 7)
Gly-Ala-Asn-Lys-Lys,

(SEQ ID NO: 8)
Gly-Ala-Asn-Arg-Arg,

(SEQ ID NO: 9)
Gly-Ala-Asn-Pro-Arg,

(SEQ ID NO: 10)
Gly-Ala-Asn-Gln-Gln,

(SEQ ID NO: 11)
Gly-Ala-Asn-Ser-Ser,

(SEQ ID NO: 12)
Gly-Ala-Asn-Arg-Gln,

(SEQ ID NO: 13)
Gly-Ala-Asn-Arg-Met,

(SEQ ID NO: 14)
Gly-Ala-Asn-Arg-Thr,

(SEQ ID NO: 15)
Gly-Ala-Asn-Arg-Ser,

(SEQ ID NO: 16)
Ala-Ala-Trp-Ala-Arg-Leu-Leu-Gln-Glu-His-Pro-Asn-Ala,

(SEQ ID NO: 17)
Ala-Ala-Trp-Ala-Arg-Leu-Leu-Gln-Glu-His-Pro-Asn-Ala-Arg,

(SEQ ID NO: 18)
Asp-Leu-Arg-Val-Asp-Thr-Lys-Ser-Arg-Ala-Ala-Trp-Ala-Arg,

(SEQ ID NO: 19)
Gln-Glu-His-Pro-Asn-Ala-Arg-Lys-Tyr-Lys-Gly-Ala-Asn-Lys-Lys,

(SEQ ID NO: 20)
Gln-Glu-His-Pro-Asn-Ala-Arg-Lys-Tyr-Lys-Gly-Ala-Asn-Arg-Arg,

(SEQ ID NO: 21)
Asp-Leu-Arg-Val-Asp-Thr-Lys-Ser-Arg-Ala-Ala-Trp-Ala-Arg-Leu-Leu-Gln-Glu-His-Pro-Asn-Ala-Arg-Lys-Tyr-Lys-Gly-Ala-Asn-Lys-Lys,

-continued

and

(SEQ ID NO: 22)
Asp-Leu-Arg-Val-Asp-Thr-Lys-Ser-Arg-Ala-Ala-Trp-Ala-Arg-Leu-Leu-Gln-Glu-His-Pro-Asn-Ala-Arg-Lys-Tyr-Lys-Gly-Ala-Asn-Arg-Arg.

Non-limiting examples of amino acid sequences derived from non-CNP polypeptides such as, e.g., ANP, BNP, serum albumin and IgG include:

(SEQ ID NO: 23)
Ser-Leu-Arg-Arg-Ser-Ser;

(SEQ ID NO: 24)
Asn-Ser-Phe-Arg-Tyr;

(SEQ ID NO: 25)
Ser-Pro-Lys-Met-Val-Gln-Gly-Ser-Gly;

(SEQ ID NO: 26)
Met-Val-Gln-Gly-Ser-Gly;

(SEQ ID NO: 27)
Lys-Val-Leu-Arg-Arg-Tyr;

(SEQ ID NO: 28)
Lys-Val-Leu-Arg-Arg-His;

(SEQ ID NO: 29)
Gly-Gln-His-Lys-Asp-Asp-Asn-Pro-Asn-Leu-Pro-Arg;

(SEQ ID NO: 30)
Gly-Val-Pro-Gln-Val-Ser-Thr-Ser-Thr;

(SEQ ID NO: 31)
Gly-Glu-Arg-Ala-Phe-Lys-Ala-Trp-Ala-Val-Ala-Arg-Leu-Ser-Gln;
and

(SEQ ID NO: 32)
Gly-Gln-Pro-Arg-Glu-Pro-Gln-Val-Tyr-Thr-Leu-Pro-Pro-Ser.

In an embodiment, the N-terminus and/or C-terminus of CNP22 or a variant thereof independently may be conjugated to an amino acid extension containing 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39 or 40 amino acids. In one embodiment, the amino acid extension is derived from NPPC, CNP53, ANP or BNP. In a specific embodiment, the amino acid extension is Gln-Glu-His-Pro-Asn-Ala-Arg-Lys-Tyr-Lys-Gly-Ala-Asn-Lys-Lys (SEQ ID NO: 33). In a related embodiment, this 15-amino acid extension is added to the N-terminus to provide a CNP variant of the formula Gln-Glu-His-Pro-Asn-Ala-Arg-Lys-Tyr-Lys-Gly-Ala-Asn-Lys-Lys-Gly_1-Leu_2-Ser_3-$(b)_4$-Gly_5-Cys_6-Phe_7-Gly_8-Leu_9-$(h)_{10}$-Leu_{11}-Asp_{12}-Arg_{13}-Ile_{14}-Gly_{15}-Ser_{16}-Met_{17}-Ser_{18}-Gly_{19}-Leu_{20}-Gly_{21}-Cys_{22}-(z) (SEQ ID NO: 34).

In one embodiment, the CNP variants comprise wtCNP22 or a variant thereof (e.g., one having addition(s), deletion(s), and/or substitution(s) such as, e.g., a K4R substitution) (SEQ ID NO: 35) conjugated at the N-terminus and/or C-terminus to a hydrophilic polymer (e.g., PEG) to increase their overall molecular size to a range from about 2.6 kDa or 2.8 kDa to about 4, 5, 6 or 7 kDa. Such CNP variants are optionally further conjugated at the N-terminus and/or C-terminus to a polymeric group comprising, e.g., amino acids, carbohydrates, hydrophobic acids and/or phospholipids, a non-limiting example of which is an N-terminal amino acid extension containing 1 to 35, or 5 to 31, amino acids. In an embodiment, a hydrophilic polymeric (e.g., PEG) moiety of at least about

0.4, 0.6, 0.8, 1, 1.2. 1.4, 1.6 or 1.8 kDa, or up to about 2, 2.2, 2.4, 2.6, 2.8, 3, 3.2, 3.4, 3.6, 3.8, 4, 4.2, 4.4, 4.6, 4.8 or 5 kDa, is added to the N-terminus and/or C-terminus of wtCNP22 or a variant thereof.

As shown herein, conjugation of a hydrophilic or water-soluble PEG (or PEO) polymer of about 0.6 kDa or more to CNP22 or variants thereof generally increases resistance to NEP cleavage markedly. However, addition of PEG, even as small as 0.6 kDa, to wtCNP22 may reduce CNP functionality (e.g., stimulation of cGMP signaling), and addition of greater than about 2 or 3 kDa of PEG to CNP22 or variants thereof may reduce CNP functional activity in a size-dependent manner. But CNP functionality (at least comparable to that of wtCNP22) is retained when a PEG (or PEO) polymer of about 0.6 kDa to about 1.2 kDa, or potentially to about 2 kDa, is conjugated to a CNP variant having an N-terminal amino acid extension in which at least one relatively large amino acid that may potentially be positively charged under physiological conditions (e.g., arginine) immediately precedes the position corresponding to Gly1 of CNP22, such as, e.g., GANRR-CNP22(K4R) (SEQ ID NO: 36), GANPR-CNP22(K4R) (SEQ ID NO: 37), ER-CNP22 (SEQ ID NO: 38), ER-CNP22 (K4R) (SEQ ID NO: 39), R-CNP22 (SEQ ID NO: 40) and R-CNP22(K4R) (SEQ ID NO: 41).

Accordingly, in one embodiment, PEGylated CNP variants comprise at the N-terminus of CNP22 or a variant thereof (e.g., one having a K4R substitution) an amino acid extension containing at least 1, 2, 3, 4 or 5 amino acids, wherein the PEG polymer is conjugated to the N-terminus of the amino acid-extended CNP variant to result in a total mass characterized by the ranges described generally herein, e.g., from about 2.6 kDa or 2.8 kDa to about 6 or 7 kDa for increased resistance to NEP cleavage. In an embodiment, for enhanced CNP functionality, such pegylated, amino acid-extended CNP variants contain at least one relatively large natural or unnatural amino acid that may potentially be positively charged under physiological conditions, immediately preceding the position corresponding to Gly1 of CNP22. In a specific embodiment, the pegylated, amino acid-extended CNP variants contain at least one arginine residue immediately preceding the position corresponding to Gly1 of CNP22.

In addition to CNP variants conjugated at the N-terminus and/or C-terminus to a hydrophilic or water-soluble polymer such as, e.g., PEG (or PEO), the disclosure encompasses CNP variants conjugated to such a polymer at an internal site. For purposes of brevity here, PEG (or PEO) will be used as a representative example of a hydrophilic or water-soluble polymer. Various sites of PEGylation of a CNP variant are possible, including but not limited to: (1) PEGylation only at the N-terminus; (2) PEGylation only at the C-terminus; (3) PEGylation only at an internal site (e.g., Lys4); (4) PEGylation at both the N-terminus and the C-terminus; (5) PEGylation at the N-terminus and an internal site; and (6) PEGylation at the C-terminus and an internal site. For increased resistance to NEP degradation and retention of CNP functionality, in certain embodiments the total mass of PEGylatedCNP variants is characterized by the ranges described generally herein, e.g., in the range from about 2.6 kDa or 2.8 kDa to about 4, 5, 6 or 7 kDa. In a particular embodiment, CNP17, CNP22, CNP37 (defined below) or variants thereof (including those having amino acid additions, substitutions and/or deletions) are PEGylated only at the N-terminus. In another embodiment, the CNP variants are PEGylated only at an internal site (e.g., Lys4). In yet another embodiment, the CNP variants are PEGylated at the N-terminus and an internal site (e.g., Lys4). In still another embodiment, for better functionality the CNP variants are not PEGylated at a site (e.g., Lys10) within the cyclic domain (corresponding to Cys6 to Cys22 of CNP22). To prevent PEGylation at an internal site, Lys4 and/or Lys10 can be substituted with a natural or unnatural amino acid or peptidomimetic that does not contain a reactive primary amino group on a side chain, such as, e.g., Gly, Ser, Arg, Asn, Gln, Asp, Glu or citrulline (Cit). In a particular embodiment, Lys4 and/or Lys10 are replaced with Arg. In another embodiment, Lys10 is not replaced with Arg.

The disclosure contemplates use of hydrophilic or water soluble polymers (e.g., PEG molecules) that may vary in type (e.g., homopolymer or copolymer; random, alternating or block copolymer; linear or branched; monodispersed or polydispersed), linkage (e.g., hydrolysable or stable linkage such as, e.g., amide, imine, aminal, alkylene, or ester bond), conjugation site (e.g., at the N-terminus and/or C-terminus, preferably not at any of the residues in the cyclized region of CNP (corresponding to residues 6-22 of CNP22)), and length (e.g., from about 0.2, 0.4 or 0.6 kDa to about 2, 3, 4 or 5 kDa). The hydrophilic or water-soluble polymer can be conjugated to the CNP peptide by means of N-hydroxy succinimide (NHS)- or aldehyde-based chemistry or other chemistry, as is known in the art. Such CNP variants can be generated using, e.g., wtCNP-22 (2.2 kDa), CNP-17 retaining only the cyclized region (residues 6-22) of wtCNP22, CNP variants having an amino acid extension at the N-terminus and/or C-terminus of CNP22, or variants with amino acid substitutions, additions and/or deletions, for example, GANRR-CNP22(K4R) (SEQ ID NO: 36), GANPR-CNP22(K4R) (SEQ ID NO: 37), R-CNP22 (SEQ ID NO: 40), R-CNP22(K4R) (SEQ ID NO: 41), ER-CNP22 (SEQ ID NO: 38) and ER-CNP22(K4R) (SEQ ID NO: 39). In an embodiment, the PEG-CNP variants having a total mass characterized by the ranges described generally herein, e.g., from about 2.6 kDa or 2.8 kDa to about 6 or 7 kDa, contain a monodispersed, linear PEG (or PEO) group conjugated at the N-terminus and/or C-terminus via NHS- or aldehyde-based chemistry, or a two-arm or three-arm branched PEG group conjugated at the N-terminus and/or C-terminus via NHS-based chemistry. The disclosure further contemplates negatively charged PEG-CNP variants designed for reduced renal clearance, including but not limited to carboxylated, sulfated and phosphorylated compounds.

In a related embodiment, the disclosure contemplates PEG-CNP conjugates comprising NHS- or aldehyde-based PEG of the formula $(CH_2CH_2O)_n$, wherein n is an integer from 12 to 50, and the PEG polymer is up to about 2.5 kDa in molecular weight. In a specific embodiment, n is 12 or 24. In an embodiment, the terminal hydroxyl group of the PEG polymer is capped with a non-reactive group. In a particular embodiment, the capping group is an alkyl group, e.g., a lower alkyl group such as methyl.

In an additional embodiment, the PEG polymers or derivatives thereof have a polymer number-average molecular weight in the range from about 0.4 kDa to about 2.5 kDa or from about 0.6 kDa to about 1.5 kDa.

In a further embodiment, the wtCNP or CNP variant peptide is conjugated to a moiety including, e.g., bisphosphonates, carbohydrates, hydrophobic acids (including fatty acids) or amino acid sequences. Such amino acid sequences include for example polyAsp or polyGlu useful in bone/cartilage targeting, or can be derived from bone proteins with elucidated bone-targeting domains or derivatives thereof, such as for example fusion proteins or peptide sequences of osteopontin, osteocalcin, sialoprotein. etc. In embodiments described herein where CNP22 or a variant thereof is attached to a bone- or cartilage-targeting moiety, such a moiety is designed to promote getting the modified CNP peptide to

chondrocytes of bone growth plates, where the peptide can bind and activate NPR-B on the chondrocytes.

In another embodiment, the disclosure provides CNP variants with a peptide bond that is less susceptible to cleavage by peptidases including NEP. The disclosure encompasses a CNP variant comprising at least one modified residue at a site of endopeptidase cleavage. In one embodiment, the Cys6-Phe7 peptide bond (—C(—O)—NH—) at an NEP cleavage site in CNP can be replaced with anyone of the following peptide-bond isosteres:

—CH_2—NH—,

—C(=O)—N(R)—, where the amide group is alkylated with any of the following R groups: methyl, ethyl, n-propyl, isopropyl, cyclopropyl, n-butyl, isobutyl, sec-butyl or tert-butyl,

—C(=O)—NH—CH_2—,

—CH_2—S—,

—CH_2—S(O)$_n$—, where n is 1 or 2,

—CH=CH—,

—C(=O)—CH_2—,

—CH(CN)—NH—,

—CH(OH)—CH_2—,

—O—C(=O)—NH—, and

—NHC(=O)NH—.

In another embodiment, Phe7 is substituted with its enantiomer D-Phe. In yet another embodiment, D-enantiomers are introduced to one or more, up to all 22, positions within wtCNP-22. In a further embodiment, a beta amino acid such as 3-amino-2-phenylpropionic acid is substituted for Phe7, which increases the length of the backbone while reducing the length of the side chain. In yet another embodiment, the disclosure contemplates Cys analogs at the Cys6 position including but not limited to homocysteine, penicillamine, 2-mercaptopropionic acid, and 3-mercaptopropionic acid.

Even in the presence of a NEP-resistant bond between Cys6 and Phe7, other peptide bonds can be hydrolyzed by NEP, including Gly8-Leu9, Lys10-Leu11, Arg13-Ile14, Ser16-Met17 and Gly19-Leu20. Accordingly, the disclosure encompasses CNP analogs containing peptide bond isosteres at multiple locations in the backbone of the CNP analogs. In one embodiment, CNP analogs or variants comprise modifications at more than one peptidase cleavage site. In a further embodiment, such variant comprises a CNP with substitutions at amino acid residues important in binding to the NEP active site, thereby increasing resistance to NEP degradation. One or more NEP-binding residues, including but not limited to Gly8, Gly15, Ser18, Gly19 and/or Gly21, are replaced with larger-size natural or unnatural amino acid residues to reduce affinity to the NEP active site. In yet another embodiment, one or more hydrophobic residues essential in NEP recognition, including but not limited to Phe7, Leu9, Leu11, Ile14, Met17 and Leu20, are substituted with natural or unnatural amino acids and/or peptidomimetics that decrease NEP binding. In yet another embodiment, one to five of the first five amino acids of CNP can be deleted or substituted with any other natural amino acids or unnatural amino acids or peptidomimetics, or one or more natural or unnatural amino acids or peptidomimetics can be added to any one or to all of the first five positions of CNP.

In a further embodiment, the CNP variants have a total mass characterized by the ranges described generally herein, e.g., from about 2.6 kDa or 2.8 kDa to about 6 or 7 kDa for increased resistance to NEP, and are represented by the formula:

(x)-Gly_1-Leu_2-Ser_3-(a)$_4$-Gly_5-(b)$_6$-(c)$_7$-(d)$_8$-(e)$_9$-(f)$_{10}$-(g)$_{11}$-Asp_{12}-Arg_{13}-(h)$_{14}$-Gly_{15}-Ser_{16}-(i)$_{17}$-Ser_{18}-Gly_{19}-(j)$_{20}$-Gly_{21}-Cys_{22}-(z) (SEQ ID NO: 46), wherein:

(x) may be absent or may be selected from the group consisting of synthetic bone-targeting compounds such as, e.g., bisphosphonates; amino acid sequences useful in bone or cartilage targeting such as, e.g., polyAsp and polyGlu; amino acid sequences derived from bone proteins with elucidated bone-targeting domains, such as, e.g., fusion proteins or peptide sequences of osteopontin, osteocalcin, sialoprotein, etc.; polymeric or non-polymeric molecules that reduce renal clearance such as, e.g., charged PEG molecules; and extensions comprising, e.g., polymers (e.g., PEGs), carbohydrates, hydrophobic acids (including fatty acids), and/or amino acids, and wherein such amino acid extensions can contain, e.g., from 1 to 31, or 1 to 35, or 5 to 35, or 10 to 35, or 15 to 35 amino acid residues, and can be derived from NPPC, ANP, BNP, other non-CNP (poly)peptides such as, e.g., serum albumin or IgG, or variants of the aforementioned polypeptides having substitutions, additions and/or deletions, or combinations thereof;

(z) may be absent or may be selected from the group consisting of amino acid sequences useful in bone or cartilage targeting such as for example polyAsp and polyGlu, amino acid sequences from bone-targeting domains of bone proteins such as, e.g., osteopontin, osteocalcin and sialoprotein, and amino acid sequences derived from non-CNP (poly)peptides such as, e.g., ANP or BNP;

(a) may be the wild type Lys at that position or may be replaced with a conservative amino acid substitution or a natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amine on a side chain, including but not limited to Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu, wherein in one embodiment (a) is Arg;

(b) is selected from the group consisting of Cys and peptide-bond isosteres between Cys6 and Phe7 such as, e.g., Cys-CH_2—NH;

(c) is selected from the group consisting of L-Phe; D-Phe; 3-amino-2-phenylpropionic acid; N-alkylated derivatives of Phe, wherein the N-alkyl group is methyl, ethyl, n-propyl, isopropyl, cyclopropyl, n-butyl, isobutyl, sec-butyl or tert-butyl; and Phe analogs, wherein one or more ortho-, meta-, and/or para-positions of the benzene ring of the Phe analog are substituted with one or more substituents selected from the group consisting of halogen, hydroxyl, cyano, straight or branched C_{1-6} alkyl, straight or branched C_{1-6} alkoxy, straight or branched halo-C_{1-6} alkyl, C_{3-10} cycloalkyl, heterocyclyl, C_{6-14} aryl and heteroaryl (examples include, but are not limited to, tyrosine, 3-chlorophenylalanine, 2,3-chloro-phenylalanine, 3-chloro-5-fluoro-phenylalanine, 2-chloro-6-fluoro-3-methyl-phenylalanine), or wherein the benzene ring of the Phe analog can be replaced with another aryl group (non-limiting examples include 1- and 2-naphthylalanine) or with a heteroaryl group (non-limiting examples include pyridylalanine, thienylalanine and furylalanine);

(d) is selected from the group consisting of Gly, tert-butyl-Gly (tBu-Gly), Thr, Ser, Val and Asn;

(e) is selected from the group consisting of Leu, Ser, Thr and peptide-bond isosteres such as, e.g., N-Me-Leu;

(f) may be the wild type Lys at that position or may be replaced with a conservative amino acid substitution or a natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amine on a side chain, including but not limited to Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu, wherein in one embodiment (f) is not Arg;

(g) is selected from the group consisting of Leu and peptide-bond isosteres such as, e.g., N-Me-Leu;

(h) is selected from the group consisting of Ile, tBu-Gly, and peptide-bond isosteres such as, e.g., N-Me-Ile;

(i) is selected from the group consisting of Met, Val, Asn, beta-Cl-Ala, 2-aminobutyric acid (Abu) and 2-amino-isobutyric acid (Aib); and

(j) is selected from the group consisting of Leu, norleucine (Nle), homoleucine (Hleu), Val, tert-butyl-Ala (tBu-Ala), Ser, Thr, Arg, and peptide-bond isosteres such as, e.g., N-Me-Leu.

In one embodiment, the CNP variants comprise a modification at one or more of positions 6, 7, 8, 9, 10, 11, 13, 14, 16, 17, 19 and/or 20, and may optionally have modifications at any of the other positions disclosed herein.

In embodiments described herein where CNP22 or variants thereof can be attached to a hydrophobic acid, the CNP peptides can be attached to one or hydrophobic acids. Non-limiting examples of hydrophobic acids include straight-chain or branched, saturated or unsaturated C_5-C_{12} carboxylic acids (e.g., pentanoic acid, heptanoic acid, etc.) and natural fatty acids. The hydrophobic acids can be attached to the N-terminus, the C-terminus, and/or the side chain of one or more amino acid residues. In one embodiment, the hydrophobic acids are conjugated to the N-terminus. In an embodiment, conjugation of CNP22 or a variant thereof to a hydrophobic acid is designed, inter alia, to promote non-specific interaction between the modified CNP peptide and serum albumin, thereby increasing the size of the CNP peptide and protecting it from cleavage by proteases such as, e.g., NEP. The interaction between the hydrophobic acid-conjugated CNP peptide and albumin is designed to be not too strong, so that the modified CNP peptide can diffuse through cartilage, get to chondrocytes of bone growth plates, and bind and activate NPR-B.

In a further embodiment, the disclosure provides CNP variants that in vitro or in vivo stimulate the production of at least about 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140% or 150% of the cGMP level produced under the same concentration of wtCNP22 (e.g., 1 uM), comprise at least one modified amino acid at position $(b)_6$, $(c)_7$ and/or $(d)_8$, and are represented by the general formula:

(x)-Gly_1-Leu_2-Ser_3-$(a)_4$-Gly_5-$(b)_6$-$(c)_7$-$(d)_8$-$(e)_9$-$(f)_{10}$-$(g)_{11}$-Asp_{12}-Arg_{13}-$(h)_{14}$-Gly_{15}-Ser_{16}-$(i)_{17}$-Ser_{18}-Gly_{19}-$(j)_{20}$-Gly_{21}-Cys_{22}-(z) (SEQ ID NO: 47), wherein:

(x) may be absent or may be a peptide sequence containing one to five amino acids which is derived from a natriuretic polypeptide (e.g. NPPC, CNP, ANP or BNP) or a non-natriuretic polypeptide as described herein (e.g., HSA, IgG, a bone-targeting protein, etc.);

(z) may be absent or may be selected from the group consisting of synthetic bone-targeting compounds such as, e.g., bisphosphonates; amino acid sequences useful in bone/cartilage targeting such as, e.g., polyAsp and polyGlu; bone proteins with bone-targeting domains and derivatives thereof, such as fusion proteins or peptides sequences of osteopontin, osteocalcin, and sialoprotein; molecules that reduce renal clearance, such as, e.g., charged PEGs; and molecules that increase resistance of CNP to NEP-mediated degradation, as described herein;

(a) may be the wild type Lys at that position or may be replaced with a conservative amino acid substitution or a natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amine on a side chain, including but not limited to Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu, wherein in one embodiment (a) is Arg;

(b) may be Cys or descarboxy cysteine, or the $(b)_6$-$(c)_7$ peptide bond (—C(=O)—NH—) may be replaced with any one of the following peptide bond isosteres:

—CH_2—NH—,
—C(=O)—N(R)—, where the amide group is alkylated with any of the following R groups: methyl, ethyl, n-propyl, isopropyl, cyclopropyl, n-butyl, isobutyl, sec-butyl or tert-butyl,
—C(=O)—NH—CH_2—,
—CH_2—S—,
—CH_2—$S(O)_n$—, where n is 1 or 2,
—CH_2—CH_2—,
—CH=CH—,
—C(=O)—CH_2—,
—CH(CN)—NH—,
—CH(OH)—CH_2—,
—O—C(=O)—NH—, or
—NHC(=O)NH—;

(c) is selected from the group consisting of L-Phe; D-Phe; 3-amino-2-phenylpropionic acid; peptide bond isosteres of Phe such as N-alkylated derivatives of Phe wherein the N-alkyl group is selected from the group consisting of methyl, ethyl, n-propyl, isopropyl, cyclopropyl, n-butyl, isobutyl, sec-butyl and tert-butyl; and Phe analogs, wherein one or more ortho-, meta-, and/or para-positions of the benzene ring of the Phe analog are substituted with one or more substituents selected from the group consisting of halogen, hydroxyl, cyano, straight or branched C_{1-6} alkyl, straight or branched C_{1-6} alkoxy, straight or branched halo-C_{1-6} alkyl, C_{3-10} cycloalkyl, C_{6-14} aryl, heterocyclyl and heteroaryl (examples include, but are not limited to, tyrosine, 3-chlorophenylalanine, 2,3-chloro-phenylalanine, 3-chloro-5-fluoro-phenylalanine, 2-chloro-6-fluoro-3-methyl-phenylalanine), or wherein the benzene ring of the Phe analog can be replaced with another aryl group (non-limiting examples include 1- and 2-naphthylalanine) or with a heteroaryl group (non-limiting examples include pyridylalanine, thienylalanine and furylalanine);

(d) is selected from the group consisting of Gly, tert-butyl-Gly (tBu-Gly), Val, Ser, Thr and Asn;

(e) is selected from the group consisting of Leu, Ser, Thr, and peptide-bond isosteres such as, e.g., N-Me-Leu;

(f) is any natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amino group on a side chain, including but not limited to Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu, wherein in one embodiment (f) is not Arg;

(g) is selected from the group consisting of Leu and peptide-bond isosteres such as, e.g., N-Me-Leu;

(h) is selected from the group consisting of Ile, tBu-Gly and peptide-bond isosteres such as, e.g., N-Me-Ile;

(i) is selected from the group consisting of Met, Val, Asn, beta-Cl-Ala, 2-aminobutyric acid (Abu) and 2-amino-isobutyric acid (Aib); and

(j) is selected from the group consisting of Leu, norleucine (Nle), homoleucine (Hleu), Val, tert-butyl-Ala (tBu-Ala), Ser, Thr, Arg, and peptide-bond isosteres such as, e.g., N-Me-Leu.

In a further embodiment, the disclosure encompasses CNP variants that in vitro or in vivo stimulate the production of at least about 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140% or 150% of the cGMP level produced under the same concentration of wtCNP22 (e.g., 1 uM), have a total mass characterized by the ranges described generally herein, e.g., from about 2.6 kDa or 2.8 kDa to about 6 or 7 kDa for increased resistance to NEP degradation, and are represented by the general formula:

(x)-(y)-Cys_6-Phe_7-Gly_8-Leu_9-$(h)_{10}$-Leu_{11}-Asp_{12}-Arg_{13}-Ile_{14}-Gly_{15}-Ser_{16}-Met_{17}-Ser_{18}-Gly_{19}-Leu_{20}-Gly_{21}-Cys_{22}-(z) (SEQ ID NO: 48), wherein:

(x) is a synthetic or natural polymeric group, or a combination thereof, wherein a non-limiting example of a synthetic

polymeric group is polyethylene glycol (PEG), and a non-limiting example of a natural polymeric group is an amino acid sequence containing from 1 to 35 amino acids and derived from NPPC or variants thereof with substitutions and/or deletions, ANP, BNP, or other non-CNP (poly)peptides such as, e.g., serum albumin, IgG, histidine-rich glycoproteins, fibronectin, fibrinogen, zinc finger-containing polypeptides, osteocrin or FGF2;

(y) may be absent or may be one or more amino acids from Gly_1-Leu_2-Ser_3-Lys_4-Gly_5 (corresponding to positions 1 to 5 of CNP22) (SEQ ID NO: 1) and/or substitutions at one or more of those positions using natural or unnatural amino acids (e.g., K4R substitution);

(h) may be the wild-type Lys at that position or may be replaced with a conservative amino acid substitution or a natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amine on a side chain, including but not limited to Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu, wherein in one embodiment (h) is not Arg; and

(z) may be absent or may be a synthetic or natural polymeric group, or a combination thereof, wherein a non-limiting example of a synthetic polymeric group is PEG, and a non-limiting example of a natural polymeric group is an amino acid sequence derived from a natriuretic polypeptide (e.g., NPPC, CNP, ANP or BNP) or non-natriuretic polypeptide (e.g., serum albumin or IgG).

In an embodiment, (x), (y) and (z) together contain from about 10 to about 40, or from about 15 to about 35 amino acids. In another embodiment, (x) is an amino acid sequence comprising from 1 to 40 amino acids, or from 1 to 20 amino acids.

Further contemplated are CNP variants that in vitro or in vivo stimulate the production of at least about 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140% or 150% of the cGMP level produced under the same concentration of wtCNP22 (e.g., 1 uM), and comprise the sequence:

(y)-Cys_6-Phe_7-Gly_8-Leu_9-Lys_{10}-Leu_{11}-Asp_{12}-Arg_{13}-Ile_{14}-Gly_{15}-Ser_{16}-Met_{17}-Ser_{18}-Gly_{19}-Leu_{20}-Gly_{21}-Cys_{22} (SEQ ID NO: 138), wherein:

(y) comprises one or more amino acids selected from Gly_1-Leu_2-Ser_3-Lys_4-Gly_5 (SEQ ID NO: 1) and/or substitutions at one or more of those positions using natural or unnatural amino acids (e.g., K4R substitution), and further comprises a hydrophilic or water soluble polymer of molecular weight from about 0.6 kDa to about 5 kDa. In an embodiment, the hydrophilic or water-soluble polymer is conjugated to the N-terminus of such amino acid-extended CNP variant. In a particular embodiment, the hydrophilic or water-soluble polymer is PEG (or PEO).

In yet another embodiment, the disclosure provides CNP variants that in vitro or in vivo stimulate the production of at least about 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140% or 150% of the cGMP level produced under the same concentration of wtCNP22 (e.g. 1 uM), wherein the CNP variants comprise an N-terminal and/or C-terminal peptide extension containing from 1 to 15 amino acids, and are conjugated to a hydrophilic or water soluble polymer. In an embodiment, the peptide extension contains from 5 to 10 amino acids. In a specific embodiment, the peptide extension contains 5 amino acids. In another specific embodiment, the hydrophilic or water-soluble polymer is PEG (or PEO).

In a still further embodiment, the CNP variants of the disclosure in vitro or in vivo stimulate the production of at least about 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140% or 150% of the cGMP level produced under the same concentration of wtCNP22 (e.g. 1 uM), and comprise at least a 15 amino acid fragment derived from natriuretic peptide precursor C(NPPC), wherein the fragment is at least 70% homologous to a sequence from wild type NPPC containing the same number of amino acid residues.

In still another embodiment, the CNP variants have a total mass characterized by the ranges described generally herein, e.g., from about 2.6 kDa or 2.8 kDa to about 6 or 7 kDa for increased NEP resistance, and are represented by the formula:

(x)-$(b)_6$-$(c)_7$-$(d)_8$-$(e)_9$-$(f)_{10}$-$(g)_{11}$-Asp_{12}-Arg_{13}-$(h)_{14}$-Gly_{15}-Ser_{16}-$(i)_{17}$-Ser_{18}-Gly_{19}-$(j)_{20}$-Gly_{21}-Cys_{22}-(z) (SEQ ID NO: 49), wherein:

(x) may be absent (i.e., the N-terminus ends with an —NH_2 group) or may be selected from the group consisting of a sequence of 1, 2, 3, 4 or 5 amino acids from the peptide Gly_1-Leu_2-Ser_3-Lys_4-Gly_5 (SEQ ID NO: 1); amino acid sequences useful in bone/cartilage targeting such as for example polyAsp or polyGlu; bone-targeting domains from bone proteins such as for example osteopontin, osteocalcin or sialoprotein; molecules that reduce renal clearance such as hydrophilic or water-soluble polymers, including but not limited to charged PEG molecules; and moieties comprising PEG, carbohydrates, hydrophobic acids, amino acids, or combinations thereof, wherein such moieties can be amino acid extensions including but not limited to amino acid sequences derived from NPPC or non-CNP (poly)peptides such as, e.g., BNP, ANP, serum albumin or IgG;

(z) may be absent or may be selected from the group consisting of amino acid sequences useful in bone/cartilage targeting such as for example polyAsp or polyGlu; amino acid sequences derived from bone-targeting proteins, such as for example osteopontin, osteocalcin or sialoprotein; and amino acid sequences derived from NPPC or non-CNP (poly) peptides, as described herein;

(b) is selected from the group consisting of Cys and peptide bond isosteres between Cys6 and Phe7 such as, e.g., Cys-CH_2—NH;

(c) is selected from the group consisting of L-Phe; D-Phe; 3-amino-2-phenylpropionic acid; N-alkylated derivatives of Phe, wherein the N-alkyl group is methyl, ethyl, n-propyl, isopropyl, cyclopropyl, n-butyl, isobutyl, sec-butyl or tert-butyl; and Phe analogs, wherein one or more ortho-, meta-, and/or para-positions of the benzene ring of the Phe analog are substituted with one or more substituents selected from the group consisting of halogen, hydroxyl, cyano, straight or branched C_{1-6} alkyl, straight or branched C_{1-6} alkoxy, straight or branched halo-C_{1-6} alkyl, C_{3-10} cycloalkyl, C_{6-14} aryl, heterocyclyl and heteroaryl (examples include, but are not limited to, tyrosine, 3-chlorophenylalanine, 2,3-chloro-phenylalanine, 3-chloro-5-fluoro-phenylalanine, 2-chloro-6-fluoro-3-methyl-phenylalanine), or wherein the benzene ring of the Phe analog can be replaced with another aryl group (non-limiting examples include 1- and 2-naphthylalanine) or with a heteroaryl group (non-limiting examples include pyridylalanine, thienylalanine and furylalanine);

(d) is selected from the group consisting of Gly, tert-butyl-Gly (tBu-Gly), Val, Ser, Thr and Asn;

(e) is selected from the group consisting of Leu, Ser, Thr, and peptide-bond isosteres such as, e.g., N-Me-Leu;

(f) may be the wild type Lys at that position or may be replaced with a conservative amino acid substitution or a natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amine on a side chain, including but not limited to Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu, wherein in one embodiment (f) is not Arg;

(g) is selected from the group consisting of Leu and peptide-bond isosteres such as, e.g., N-Me-Leu;

(h) is selected from the group consisting of Ile, tBu-Gly and peptide-bond isosteres such as, e.g., N-Me-Ile;

(i) is selected from the group consisting of Met, Val, Asn, beta-Cl-Ala, 2-aminobutyric acid (Abu) and 2-amino-isobutyric acid (Aib); and

(j) is selected from the group consisting of Leu, norleucine (Nle), homoleucine (Hleu), Val, tert-butyl-Ala (tBu-Ala), Ser, Thr, Arg, and peptide-bond isosteres such as, e.g., N-Me-Leu.

In a further embodiment, the CNP variants have a total mass characterized by the ranges described generally herein, e.g., from about 2.6 kDa or 2.8 kDa to about 6 or 7 kDa for increased NEP resistance, and are represented by the formula: (x)-Gly_1-Leu_2-Ser_3-(a)$_4$-Gly_5-(b)$_6$-(c)$_7$-(d)$_8$-(e)$_9$-(f)$_{10}$-(g)$_{11}$-Asp_{12}-Arg_{13}-(h)$_{14}$-(i)$_{15}$-Ser_{16}-(j)$_{17}$-Ser_{18}-Gly_{19}-(k)$_{20}$-Gly_{21}-Cys_{22}-(z) (SEQ ID NO: 50), wherein:

(x) and (z) independently may be absent or may be selected from the group consisting of synthetic bone-targeting compounds such as, e.g., bisphosphonates; amino acid sequences useful in bone/cartilage targeting such as for example polyAsp or polyGlu; amino acid sequences derived from bone-targeting domains of bone proteins and derivatives thereof, such as for example fusion proteins or peptide sequences of osteopontin, osteocalcin, sialoprotein, etc.; moieties that reduce renal clearance, including but not limited to hydrophilic or water-soluble polymers such as, e.g., charged PEG molecules; and moieties comprising, e.g., hydrophilic polymers (e.g., PEG), carbohydrates, hydrophobic acids, and/or amino acids;

(a) may be the wild type Lys at that position or may be replaced with a conservative amino acid substitution or a natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amine on a side chain, including but not limited to Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu, wherein in one embodiment (a) is Arg;

(b) is selected from the group consisting of Cys and peptide bond isosteres between Cys6 and Phe7 such as, e.g., Cys-CH_2—NH;

(c) is selected from the group consisting of L-Phe; D-Phe; 3-amino-2-phenylpropionic acid; N-alkylated derivatives of Phe, wherein the N-alkyl group is methyl, ethyl, n-propyl, isopropyl, cyclopropyl, n-butyl, isobutyl, sec-butyl or tert-butyl; and Phe analogs, wherein one or more ortho-, meta-, and/or para-positions of the benzene ring of the Phe analog are substituted with one or more substituents selected from the group consisting of halogen, hydroxyl, cyano, straight or branched C_{1-6} alkyl, straight or branched C_{1-6} alkoxy, straight or branched halo-C_{1-6} alkyl, C_{3-10} cycloalkyl, C_{6-14} aryl, heterocyclyl and heteroaryl (examples include, but are not limited to, tyrosine, 3-chlorophenylalanine, 2,3-chloro-phenylalanine, 3-chloro-5-fluoro-phenylalanine, 2-chloro-6-fluoro-3-methyl-phenylalanine), or wherein the benzene ring of the Phe analog can be replaced with another aryl group (non-limiting examples include 1- and 2-naphthylalanine) or with a heteroaryl group (non-limiting examples include pyridylalanine, thienylalanine and furylalanine);

(d) is selected from the group consisting of Gly, tert-butyl-Gly, Thr, Ser, Val and Asn;

(e) is selected from the group consisting of Leu, Ser, Thr, and peptide bond isosteres such as, e.g., N-Me-Leu;

(f) may be the wild type Lys at that position or may be replaced with a conservative amino acid substitution or a natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amine on a side chain, including but not limited to Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu, wherein in one embodiment (f) is not Arg;

(g) is selected from the group consisting of Leu, Asn, and peptide bond isosteres such as, e.g., N-Me-Leu;

(h) is selected from the group consisting of Ile, tert-butyl-Gly (tBu-Gly), Asn, and peptide bond isosteres such as, e.g., N-Me-Ile;

(i) is selected from the group consisting of Gly, Arg, Ser and Asn;

(j) is selected from the group consisting of Met, Val, Asn, beta-Cl-Ala, 2-aminobutyric acid (Abu) and 2-amino-isobutyric acid (Aib); and

(k) is selected from the group consisting of Leu, norleucine (Nle), homoleucine (Hleu), Val, tert-butyl-Ala (tBu-Ala), Arg, Thr, Ser, and peptide bond isosteres such as, e.g., N-Me-Leu.

In a further embodiment, the CNP variants can have amino acid substitution(s) at one or more of any of positions 1 to 22 of CNP22. In one embodiment, Gly1 is substituted with Arg or Glu. In another embodiment, Lys4 is replaced with Arg. In still another embodiment, Gly5 is substituted with Arg, Gln or Ser. In yet another embodiment, Gly15 is substituted with Ser, Asn, Arg or Cit. In a further embodiment, Gly19 is substituted with Ser, Arg or Asn. In yet another embodiment, Gly21 is substituted with Ser, Thr, or Arg.

In one embodiment, the CNP variant is selected from the group consisting of GLSKGC(CH_2NH)FGLKLDRIGSMSGLGC (formed using descarboxy-Cys) (SEQ ID NO: 56), GLSKGC-(N-Me-Phe)-GLKLDRIGSMSGLGC (SEQ ID NO: 57), GLSKGC-(D-Phe)-GLKLDRIGSMSGLGC(SEQ ID NO:136), GLSKGCF-(tBuG)-LKLDRIGSMSGLGC (SEQ ID NO: 58), GLSKGC-(3-Cl-Phe)-GLKLDRIGSMSGLGC (SEQ ID NO:137), and GLSKGC-[NHCH_2CH(Ph)CO]-GLKLDRIGSMSGLGC (formed using 3-amino-2-phenylpropionic acid) (SEQ ID NO: 59). In a further embodiment, a disulfide bond exists between Cys6, descarboxy-Cys or another sulfhydryl-containing cysteine analog at the Cys6 position, and Cys22 of any CNP variant described herein.

In another embodiment, the CNP variants contain an amino acid extension at the N-terminus and/or C-terminus of CNP22 or CNP17, including but not limited to:

(SEQ ID NO: 4)
DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMS GLGC (CNP-53);

(SEQ ID NO: 60)
QEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-37, Analog BL);

(SEQ ID NO: 61)
AAWARLLQEHPNAGLSKGCFGLKLDRIGSMSGLGC (Analog CA);

(SEQ ID NO: 62)
AAWARLLQEHPNARGLSKGCFGLKLDRIGSMSGLGC (Analog CB);

(SEQ ID NO: 63)
DLRVDTKSRAAWARGLSKGCFGLKLDRIGSMSGLGC (Analog CC);

(SEQ ID NO: 40)
RGLSKGCFGLKLDRIGSMSGLGC;

(SEQ ID NO: 38)
ERGLSKGCFGLKLDRIGSMSGLGC;

(SEQ ID NO: 64)
GANQQGLSKGCFGLKLDRIGSMSGLGC;

(SEQ ID NO: 65)
GANRRGLSKGCFGLKLDRIGSMSGLGC;

-continued

(SEQ ID NO: 66)
GANPRGLSKGCFGLKLDRIGSMSGLGC;

(SEQ ID NO: 67)
GANSSGLSKGCFGLKLDRIGSMSGLGC;

(SEQ ID NO: 144)
GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC
(sometimes designated "CNP27-HSA" or "HSA-CNP27"
in the Examples and figures);

(SEQ ID NO: 68)
SPKMVQGSG-CNP17-KVLRRH (Analog CD) (CNP17 having
N-terminal and C-terminal tails derived from BNP).

In a further embodiment, the CNP variants have a K4R substitution at position 4 of CNP22. Non-limiting examples of CNP(K4R) variants include:

((SEQ ID NO: 36)
GANRRGLSRGCFGLKLDRIGSMSGLGC (Analog AY);

(SEQ ID NO: 37)
GANPRGLSRGCFGLKLDRIGSMSGLGC (Analog CI);

(SEQ ID NO: 41)
RGLSRGCFGLKLDRIGSMSGLGC (Analog AZ);

(SEQ ID NO: 39)
ERGLSRGCFGLKLDRIGSMSGLGC (Analog BA);

(SEQ ID NO: 69)
GANQQGLSRGCFGLKLDRIGSMSGLGC (Analog CH);
and

(SEQ ID NO: 70)
GANSSGLSRGCFGLKLDRIGSMSGLGC (Analog CG).

In one embodiment, CNP variants having a PEG (or PEO) moiety and an amino acid extension at the N-terminus contain arginine at the position immediately preceding the position corresponding to Gly1 of CNP22. Such PEGylated CNP variants are designed for increased resistance to NEP degradation, reduced binding to serum albumin, and enhanced CNP functional activity (e.g., activation of cGMP signaling). Non-limiting examples of PEGylated CNP variants include PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36), PEO12-GANRR-CNP22(K4R) (SEQ ID NO: 36), PEO24-GANRR-CNP22(SEQ ID NO: 65), PEO12-GANRR-CNP22(SEQ ID NO: 65), PEO24-GANPR-CNP22(K4R) (SEQ ID NO: 37), PEO12-GANPR-CNP22(K4R) (SEQ ID NO: 37), PEO24-GANPR-CNP22(SEQ ID NO: 37), PEO12-GANPR-CNP22 (SEQ ID NO: 66), PEO24-GANQQ-CNP22(SEQ ID NO: 64), PEO12-GANQQ-CNP22(SEQ ID NO: 64), PEO24-ER-CNP22(K4R) (SEQ ID NO: 39), PEO12-ER-CNP22(K4R) (SEQ ID NO: 39), PEO24-ER-CNP22(SEQ ID NO: 38), PEO12-ER-CNP22(SEQ ID NO: 38), PEO24-R-CNP22 (K4R) (SEQ ID NO: 41), PEO12-R-CNP22(K4R) (SEQ ID NO: 41), PEO24-R-CNP22(SEQ ID NO: 40), and PEO12-R-CNP22(SEQ ID NO: 40), wherein PEO24 is a monodispersed 1.2 kDa PEG polymer and PEO12 is a monodispersed 0.6 kDa PEG polymer. In an embodiment, the PEG (or PEO) polymer is attached to the N-terminus of the CNP variants.

Additional CNP variants include, but are not limited to, derivatives of CNP37 having mutation(s) at the furin cleavage site (underlined), designed to improve in vivo resistance to the furin protease, and/or having glycine (underlined) preceding glutamine, designed to prevent pyroglutamine formation, including but not limited to:

(SEQ ID NO: 71)
GQEHPNARKYKGANPKGLSKGCFGLKLDRIGSMSGLGC (An. CS);

(SEQ ID NO: 72)
GQEHPNARKYKGANQKGLSKGCFGLKLDRIGSMSGLGC (An. CT);

(SEQ ID NO: 73)
GQEHPNARKYKGANQQGLSKGCFGLKLDRIGSMSGLGC (An. CU);

(SEQ ID NO: 74)
GQEHPNARKYKGANKPGLSKGCFGLKLDRIGSMSGLGC (An. CW);

(SEQ ID NO: 75)
GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC
(Gly-CNP37, An. DB);

(SEQ ID NO: 145)
PGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC
(Pro-Gly-CNP37).

In another embodiment, the CNP variants are chimera comprising CNP22 and an N-terminal peptide fragment, including but not limited to:

(SEQ ID NO: 76)
GHHSHEQHPHGANQQGLSKGCFGLKLDRIGSMSGLGC (Analog CQ)
(histidine-rich glycoprotein (HRGP) fragment-
CNP22 chimera);

(SEQ ID NO: 77)
GAHHPHEHDTHGANQQGLSKGCFGLKLDRIGSMSGLGC (Analog CR)
(HRGP fragment-CNP22 chimera);

(SEQ ID NO: 78)
GHHSHEQHPHGANPRGLSKGCFGLKLDRIGSMSGLGC (Analog CX)
(HRGP fragment-CNP22 chimera);

(SEQ ID NO: 79)
GQPREPQVYTLPPSGLSKGCFGLKLDRIGSMSGLGC (Analog CF)
($IgG_1(F_c)$ fragment-CNP22 chimera);

(SEQ ID NO: 80)
GQHKDDNPNLPRGANPRGLSKGCFGLKLDRIGSMSGLGC (Analog
CY) (human serum albumin (HSA) fragment-CNP22
chimera);

(SEQ ID NO: 81)
GERAFKAWAVARLSQGLSKGCFGLKLDRIGSMSGLGC (Analog CE)
(HSA fragment-CNP22 chimera);

(SEQ ID NO: 82)
FGIPMDRIGRNPRGLSKGCFGLKLDRIGSMSGLGC (Analog CZ)
(osteocrin "NPR C inhibitor" fragment-CNP22
chimera);
and

(SEQ ID NO: 83)
GKRTGQYKLGSKTGPGPKGLSKGCFGLKLDRIGSMSGLGC
(Analog DA) (FGF2 "heparin-binding domain"
fragment-CNP22 chimera).

In a further embodiment, the CNP variants are chimera comprising an N-terminal peptide fragment and CNP22 in which arginine is substituted for Lys4 of CNP22 ("CNP22 (K4R)"), including but not limited to:

(SEQ ID NO: 84)
GQPREPQVYTGANQQGLSRGCFGLKLDRIGSMSGLGC (Analog CK)
($IgG_1(F_c)$ fragment-CNP22(K4R) chimera);

(SEQ ID NO: 85
GVPQVSTSTGANQQGLSRGCFGLKLDRIGSMSGLGC (Analog CL)
(HSA fragment-CNP22(K4R) chimera)

-continued

(SEQ ID NO: 86)
GQPSSSSQSTGANQQGLSRGCPGLKLDRIGSMSGLGC
(Analog CM) (fibronectin fragment-CNP22(K4R)
chimera);

(SEQ ID NO: 87)
GQTHSSGTQSGANQQGLSRGCPGLKLDRIGSMSGLGC
(Analog CN) (fibrinogen fragment-CNP22(K4R)
chimera);

(SEQ ID NO: 88)
GSTGQWHSESGANQQGLSRGCPGLKLDRIGSMSGLGC
(Analog CO) (fibrinogen fragment-CNP22(K4R)
chimera);
and

(SEQ ID NO: 89)
GSSSSSSSSSGANQQGLSRGCPGLKLDRIGSMSGLGC
(Analog CP) (zinc finger fragment-CNP22(K4R)
chimera).

In yet another embodiment, the CNP variants are chimera, or fusion proteins, comprising a CNP peptide or variant, and a cleavable peptide or protein, or peptide tag. Exemplary cleavable proteins or peptides include, but are not limited to, histidine (e.g., hexa-His) tags; TAF12: human transcription factor TAF12; KSI: ketosteroid isomerase; MBP: maltose-binding protein; β-Gal: β-galactosidase; GST: glutathione-S-transferase; Trx: thioredoxin; CBD: chitin binding domain; BMPM: BMP-2 mutation, SUMO, CAT, TrpE, staphylococcal protein A, streptococcal proteins, starch-binding protein, cellulose-binding domain of endoglucanase A, cellulose-binding domain of exoglucanase Cex, biotin-binding domain, recA, Flag, c-Myc, poly(His), poly(Arg), poly(Asp), poly (Gln), poly(Phe), poly(Cys), green fluorescent protein, red fluorescent protein, yellow fluorescent protein, cyan fluorescent protein, biotin, avidin, streptavidin, antibody epitopes, and fragments thereof.

In yet another embodiment, the CNP variant may be a monomer or a dimer. In a related embodiment the monomers of dimeric CNP variants can be attached N-terminus to N-terminus via a linker or no linker, N-terminus to C-terminus via a linker or no linker, or C-terminus to C-terminus via a linker or no linker.

Chimera comprising an IgG fragment and CNP22 or a variant thereof are designed for, inter alia, increased resistance to NEP degradation and reduced binding to serum albumin. CNP chimera comprising a surface fragment of HSA are designed for, inter alia, reduced immunogenicity and reduced binding to serum albumin. HRGP-CNP22 and HRGP-CNP22(K4R) chimera containing a cationic, histidine-rich, non-lysine, non-arginine sequence at the N-terminus are designed for, inter alia, increased stability to proteases. Chimera containing an osteocrin fragment are designed to release, upon protease (e.g., furin) cleavage, the osteocrin fragment at bone growth plates, where the fragment would inhibit the clearance receptor NPR-C. With respect to chimera comprising an FGF2 heparin-binding fragment, heparin binding to the fragment is designed to protect the chimera from degradation, thereby providing a longer serum half-life. Chimera containing a fibronectin, fibrinogen, or zinc-finger fragment are designed for reduced binding to serum albumin, among other advantageous features.

Not intending to be bound by theory, a CNP variant of molecular weight from about 2.6 kDa or 2.8 kDa to about 6 or 7 kDa which has increased resistance to NEP degradation and has similar or improved functionality (e.g., binding to NPR-B and stimulation of cGMP signaling) as compared to wtCNP22, may be more effective if it does not bind tightly to plasma proteins such as serum albumin. A CNP variant that does not bind tightly to plasma proteins (e.g., serum albumin) may be more effective in diffusing through cartilage, getting to chondrocytes of bone growth plates, and binding to and activating NPR-B for cGMP signaling. In one embodiment, CNP variants designed for reduced binding to plasma proteins (e.g., serum albumin) are chimeras comprising CNP22 or a variant thereof and a peptide fragment from IgG. In another embodiment, CNP variants designed for reduced binding to plasma proteins are chimeras comprising CNP22 or CNP22(K4R) and a fragment from a polypeptide (e.g., IgG, HSA, fibronectin, fibrinogen, a zinc finger-containing polypeptide, etc.). In yet another embodiment, CNP variants designed for reduced binding to plasma proteins comprise CNP22 or a variant thereof conjugated to a hydrophilic or water-soluble polymer. In one embodiment, the hydrophilic or water-soluble polymer is PEG (or PEO). In another emhodiment, the hydrophilic or water-soluhle polymer (e.g., PEG) is functionalized with one or more functional groups that impart a negative charge to the polymer under physiological conditions, such as, e.g, carboxyl, sulfate or phosphate groups, or a combination thereof.

In any of the embodiments disclosed herein, the CNP variants may have substantially the same or better biological activity than wild-type CNP22. For example, the CNP variants may retain at least 50%, 60%, 70%, 80%, 90%, 95% or more of the activity of wild-type CNP22, or may have greater activity than CNP22, e.g., with respect to interaction with NPR-B (GC-B) to stimulate the generation of cGMP. Alternatively, or in addition, the CNP variants may retain at least 50%, 60%, 70%, 80%, 90%, 95% or more of the activity of wild-type CNP22, or may have greater activity than CNP22, with respect to regulating endochondral bone growth and chondrocyte activity, including but not limited to chondrocyte proliferation, chondrocyte differentiation, inhibition of the mitogen activated protein (MAP) kinase/MEK (Raf-1) kinase signaling pathway, and promoting endochondral ossification. In any of the embodiments described herein, the CNP variants may comprise an amino acid sequence that is at least 40%, 50%, 60%, 70%, 80%, 90%, 95% or more identical or homologous to amino acids 6-22 or 1-22 of wild-type CNP22.

In a further embodiment, the disclosure provides variants of CNP22 having less affinity to the NPR-C clearance receptor while retaining the ability to bind and activate NPR-B. The present disclosure encompasses variants that were, or can be, generated from a homology-based structural model of the NPR-B/CNP complex as described in the Detailed Description. In another embodiment, the CNP variants have substitution(s) at one or more Gly sites at positions 1, 5, 8, 15, 19 and 21, to reduce conformational flexibility, which may increase their specificity for binding to NPR-B over NPR-C. Variants of CNP having potentially reduced affinity to the NPR-C include but are not limited to those having one or more of the following substitutions: G1R, G1E, G5R, G5Q, G5S, F7Y, G8T, G8S, G8V, G8N, L9S, L9T, K10Cit, K10Q, K10S, I14N, G15R, G15S, G15N, G15Cit, S16Q, M17V, M17N, G19S, G19R, G19N, L20V, L20R, L20T, L20S, G21S, G21T and G21R.

In yet another embodiment, the CNP variants have a modification and/or substitution at one or more of positions 5, 7, 8, 9, 10, 14, 15, 16, 17, 19, 20 and 21, and may optionally have modifications and/or substitutions at any of the other positions disclosed herein. In a further embodiment, the CNP variants can optionally have conjugation(s) or extension(s), e.g., at the N- and/or C-terminus to facilitate bone/cartilage targeting, reduce renal clearance, and/or increase resistance to NEP degradation. Such conjugation(s) or extension(s) can comprise molecules or sequences formed or derived from,

e.g., polyAsp, polyGlu, bone- or cartilage-targeting peptides, osteopontin, osteocalcin, sialoprotein, PEGs, carbohydrates, hydrophobic acids, NPPC or non-CNP (poly)peptides, or combinations thereof.

In still another embodiment, the CNP variants are prepared by standard solid-phase peptide synthesis methods with natural or unnatural amino acid(s) or peptidomimetic(s) being substituted and/or added where appropriate. In another embodiment, the CNP variants are produced by recombinant synthesis processes, e.g., via fusion proteins containing a tag or carrier protein, wherein use of the tag or carrier protein facilitates, e.g., detection, isolation and/or purification of the fusion protein, and selective chemical or proteolytic cleavage of the tag or carrier protein from the fusion protein provides the target CNP variant. In a further embodiment, PEGylation of the CNP variants occurs following, or part of, chemical or biological synthesis with the conjugation reaction being performed by NHS- or aldehyde-based chemistry or other chemistry known in the art. In another embodiment, the CNP variants comprise a disulfide bond. In a related embodiment, the disulfide bond forms a cyclic peptide. In a particular embodiment, the disulfide bond is formed between cysteine residues at positions corresponding to positions 6 and 22 of CNP22.

It is further contemplated that the CNP variants can be conjugated to a hydrophobic polymeric or non-polymeric moiety, such as, e.g., heptanoic acid, pentanoic acid, or fatty acids. The hydrophobic moiety can be conjugated to the side chain of an amino acid residue, including but not limited to a lysine, a serine, a cysteine or a threonine, or can be attached to the N-terminus and/or C-terminus of the CNP variant.

In an embodiment, the CNP variants as described herein have a pI in the range from about 8 to about 10.5 or from about 8.5 to about 10.

In a further embodiment, the disclosure provides a pharmaceutical composition comprising a CNP variant, optionally another biologically active agent, and optionally a pharmaceutically acceptable excipient, carrier or diluent. In some embodiments, the compositions are sterile pharmaceutical compositions suitable for parenteral injection. In some embodiments, the compositions comprise substantially pure CNP variant, e.g. at least about 90% or 95% pure. In some embodiments, the compositions contain less than about 5%, 4%, 3%, 2%, 1% or 0.5% contaminants, such as other human proteins, porcine proteins, or CNP53 or fragments thereof (other than the desired CNP variant). In certain embodiments, the sterile composition is administered to a subject for treating or preventing any of the CNP-responsive conditions or disorders disclosed herein.

CNP variants of the disclosure advantageously retain CNP activity and exhibit increased serum half-life. Retention of CNP activity can be shown, for example, as retention of desired in vivo biological effect, or retention of at least about 50%, 60%, 70%, 80%, 90%, 95% or at least about 100% of the cGMP stimulating activity of CNP22, under the same concentration (e.g., 1 uM of CNP peptide or greater than the ED80). In some embodiments, CNP variants exhibit at least about 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, 15-fold, 20-fold, 25-fold, 30-fold, 35-fold or 40-fold increase in serum half-life compared to CNP22.

In a related embodiment, the CNP variants described herein have increased NEP resistance and exhibit increased half-life compared to wild-type CNP22. In one embodiment, the half-life of the CNP variants is increased by about 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or about 100% compared to wild-type CNP22.

In certain embodiments, the CNP variants described herein increase cGMP production in vitro, increase cGMP production in vivo, increase in vivo the level of one or more biomarkers associated with cartilage or bone formation or growth, increase resistance to NEP cleavage in vitro, increase plasma or serum half-life in vivo, increase bioavailability in vivo, or increase the length of particular bones in vivo, or effect combinations of such increases, by about 1.5-fold, about 2-fold, about 2.5-fold, about 3-fold, about 3.5-fold, about 4-fold, about 4.5-fold, or about 5-fold or more compared to wild-type CNP22.

In yet another embodiment, the disclosure provides methods of treating conditions or disorders responsive to CNP, comprising administering a therapeutically effective amount of a CNP variant or a composition comprising the same to a subject in need thereof. In one embodiment, disorders responsive to CNP are disorders of bone growth, including but not limited to skeletal dysplasias and inherited skeletal malformations such as disorders associated with fibroblast growth factor receptor 3 (FGFR-3) mutations. In a specific embodiment, the disorder associated with FGFR-3 mutation(s) is achondroplasia. In another embodiment, the disorders responsive to CNP are disorders associated with vascular smooth muscle cells and tissues. In a further embodiment, the CNP variants are useful for increasing the size of the growth plate of a bone (e.g., a limb bone). In another embodiment, the CNP variants are useful for elongating a bone or increasing long bone growth. In still another embodiment, the CNP variants are useful for enhancing matrix production, proliferation and differentiation of chondrocytes.

In certain embodiments, the CNP variants described herein are administered at a dose in the range from about 5 or 10 nmol/kg to about 300 nmol/kg, or from about 20 nmol/kg to about 200 nmol/kg. In some embodiments, the CNP variants are administered at a dose of about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 125, 150, 175, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 350, 400, 450, 500, 750, 1000, 1250, 1500, 1750 or 2000 nmol/kg or other dose deemed appropriate by the treating physician. In other embodiments, the CNP variants are administered at a dose of about 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750 or 800 ug/kg, or about 1 mg/kg, 1.25 mg/kg, 1.5 mg/kg, 2 mg/kg or other dose deemed appropriate by the treating physician. The doses of CNP variants described herein can be administered according to the dosing frequency/frequency of administration described herein, including without limitation daily, 2 or 3 times per week, weekly, every 2 weeks, every 3 weeks, monthly, etc.

In another embodiment, the CNP variants are administered in a single treatment or in multiple doses. The multiple doses may be administered daily, or in multiple doses over the course of treatment. In certain embodiments, it is contemplated that the CNP variant is administered, in a single dose or in multiple doses, daily, every other day, every 3 days, 2 times per week, 3 times per week, weekly, bi-weekly, every 3 weeks, monthly, every 6 weeks, every 2 months, every 3 months or as deemed appropriate by a treating physician.

In certain embodiments, administration of the CNP variant is adjusted to allow for periods of growth (e.g., chondrogenesis), followed by a recovery period (e.g., osteogenesis). For example, the CNP variant may be administered subcutaneously, intravenously, or by another mode daily or multiple times per week for a period of time, followed by a period of no treatment, then the cycle is repeated. In some embodiments, the initial period of treatment (e.g., administration of the CNP

variant daily or multiple times per week) is for 3 days, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks or 12 weeks. In a related embodiment, the period of no treatment lasts for 3 days, 1 week, 2 weeks, 3 weeks or 4 weeks. In certain embodiments, the dosing regimen of the CNP variant is daily for 3 days followed by 3 days off; or daily or multiple times per week for 1 week followed by 3 days or 1 week off; or daily or multiple times per week for 2 weeks followed by 1 or 2 weeks off; or daily or multiple times per week for 3 weeks followed by 1, 2 or 3 weeks off; or daily or multiple times per week for 4, 5, 6, 7, 8, 9, 10, 11 or 12 weeks followed by 1, 2, 3 or 4 weeks off.

In additional embodiments, the disclosure provides a method of treating a CNP-responsive condition or disorder, comprising administering a CNP peptide or variant to a subject, and monitoring the level of at least one bone- or cartilage-associated biomarker in the subject (e.g., in a biological sample from the subject), wherein an increase or decrease in the level of the bone- or cartilage-associated biomarker indicates a therapeutic effect of the CNP peptide or variant on the subject. In some embodiments, when the level of a biomarker increases in association with bone or cartilage formation or growth, an increase in the level of that biomarker indicates a therapeutic effect of the CNP peptide or variant on the subject. In other embodiments, when the level of a biomarker decreases in association with bone or cartilage formation or growth, a decrease in the level of that biomarker indicates a therapeutic effect of the CNP peptide or variant on the subject.

In further embodiments, the therapeutic method further comprises adjusting the amount (or dose) or frequency of administration of the CNP peptide or variant, wherein:

(i) the amount (or dose) or frequency of administration of the CNP peptide or variant is increased if the level of the at least one bone- or cartilage-associated biomarker is below a target level, where the level of the biomarker increases in association with bone or cartilage formation or growth; or

(ii) the amount (or dose) or frequency of administration of the CNP peptide or variant is decreased if the level of the at least one bone- or cartilage-associated biomarker is above a target level, where the level of the biomarker increases in association with bone or cartilage formation or growth; or

(iii) the amount (or dose) or frequency of administration of the CNP peptide or variant is increased if the level of the at least one bone- or cartilage-associated biomarker is above a target level, where the level of the biomarker decreases in association with bone or cartilage formation or growth; or

(iv) the amount (or dose) or frequency of administration of the CNP peptide or variant is decreased if the level of the at least one bone- or cartilage-associated biomarker is below a target level, where the level of the biomarker decreases in association with bone or cartilage formation or growth.

It is contemplated that the target level of a biomarker refers to the level or range of levels of the biomarker that is associated with therapeutic effect in the subject and/or beneficial effect in alleviating or ameliorating symptoms of the disorder or condition. In certain embodiments, a level of a biomarker above or below a target level may be deleterious to the subject.

In other embodiments, the disclosure contemplates a method for assessing the effect of administration of a CNP peptide or variant on bone or cartilage formation or growth. In one embodiment, the method provides for assaying or measuring the level of at least one bone- or cartilage-associated biomarker in a subject that has been administered a CNP peptide or variant in order to assess the effect of the CNP peptide or variant on bone and cartilage formation and growth in vivo. In a related embodiment, an increase in the level of the at least one bone- or cartilage-associated biomarker may indicate that administration of a CNP peptide or variant has a positive effect on bone or cartilage formation or growth and is a useful treatment for skeletal dysplasias and other bone- or cartilage-related diseases or disorders associated with decreased CNP activity. Exemplary bone- or cartilage-associated biomarkers include, but are not limited to, CNP (e.g, endogenous level of CNP-22 or CNP-53), cGMP, osteocalcin, proliferating cell nuclear antigen (PCNA), propeptides of type I procollagen (PINP) and fragments thereof, collagen type I and fragments thereof, propeptides of collagen type II and fragments thereof, collagen type II and fragments thereof, aggrecan chondroitin sulfate, and alkaline phosphatase.

In further embodiments, the disclosure contemplates a method for assessing the effect of a CNP peptide or variant on the level of at least one bone- or cartilage-associated biomarker in a subject, comprising assaying or measuring the level of the bone- or cartilage-associated biomarker in a biological sample from a subject that has been administered a CNP peptide or variant. In some embodiments, the method further comprises administering the CNP peptide or variant to the subject before assaying or measuring the level of the bone- or cartilage-associated biomarker.

In certain embodiments, the at least one bone- or cartilage-associated biomarker is selected from the group consisting of CNP (e.g, endogenous level of CNP-22 or CNP-53), cGMP, propeptides of collagen type II and fragments thereof, collagen type II and fragments thereof, osteocalcin, proliferating cell nuclear antigen (PCNA), propeptides of type I procollagen (PINP) and fragments thereof, collagen type I and fragments thereof, aggrecan chondroitin sulfate, and alkaline phosphatase.

In some embodiments of methods (e.g., therapeutic, diagnostic and assay methods) relating to bone- or cartilage-associated biomarkers, the CNP peptide or variant is CNP-22, CNP-53, or any of the CNP peptides and variants described herein. In certain embodiments of such methods, the CNP peptide or variant is not CNP-22 or CNP-53.

In other embodiments, the disclosure provides a method for recombinant production of a CNP variant, comprising culturing in a medium a host cell comprising a polynucleotide encoding a CNP variant peptide linked to a polynucleotide encoding a cleavable peptide or protein, under conditions that result in expression of a fusion polypeptide encoded by the polynucleotides. In a related embodiment, the host cell is transformed with an expression vector comprising a polynucleotide encoding a CNP variant peptide linked to a polynucleotide encoding a cleavable peptide or protein.

In one embodiment, the vector is a plasmid. In still another embodiment, the plasmid is selected from the group consisting of pET-21a, pJexpress, pET-31b, pET-15b, pET-32a, pET-41a, pMAL, pQE-30, pET-SUMO, pET-22b, and pTYB11.

In certain embodiments, the cleavable peptide or protein comprises a polypeptide that is selected from the group consisting of a histidine tag, human transcription factor TAF12, ketosteroid isomerase, maltose-binding protein, β-galactosidase, glutathione-S-transferase, thioredoxin, chitin binding domain, and BMP-2 mutation, or fragments thereof.

In a related embodiment, the cleavable peptide or protein is cleaved by a cleaving agent. In some embodiments, the cleaving agent is selected from the group consisting of formic acid, cyanogen bromide (CNBr), hydroxylamine, protein self cleavage, Factor Xa, enterokinase, ProTEV, and SUMO protease. Additional exemplary cleaving agents include, but are not limited to, palladium, clostripain, thrombin, chymotrypsin, trypsin, trypsin-like proteases, carboxypeptidase,

enteropeptidase, Kex 2 protease, Omp T protease, subtilisin, V8 protease, HIV protease, rhinovirus protease, furilisin protease, IgA proteases, human Pace protease, collagenase, Nia protease, poliovirus 2Apro protease, poliovirus 3C protease, genenase, furin, elastase, Proteinase K, pepsin, rennin (chymosin), microbial aspartic protcascs, papain, calpain, chymopapain, ficin (ficain), bromelain (bromelase), cathespisin B, caspases, thermolysin, Endoprotease Arg-C, Endoprotease Glu-C, Endoprotease Lys-C, kallikrein, and plasmin.

In certain embodiments, the fusion polypeptide is expressed as a soluble protein or as an inclusion body. In a related embodiment, the disclosure contemplates isolating the expressed fusion polypeptide from the host cell or culture medium. In a further embodiment, the isolated fusion polypeptide is contacted with a cleaving agent as described herein.

In one embodiment, the disclosure provides a bacterial host cell comprising an expression vector, said vector comprising a polynucleotide encoding a CNP variant peptide linked to a polynucleotide encoding a cleavable peptide or protein. In some embodiments, the cleavable peptide or protein is selected from the group consisting of a histidine tag, human transcription factor TAF12, ketosteroid isomerase, maltose-binding protein, β-galactosidase, glutathione-S-transferase, thioredoxin, chitin binding domain, and BMP-2 mutation, or fragments thereof.

In another embodiment, the host cell is a bacteria, such as *E. coli*. In a related embodiment, the *E. coli* cell is selected from the group consisting of BL21(DE3), BL21(DE3)pLysS, BL21(DE3)pGro7, ArcticExpress(DE3), C41 [also called C41(DE3)], C43 [also called C43(DE3)], Origami B(DE3), Origami B(DE3)pLysS, KRX, and Tuncr(DE3). In still a further embodiment, the host cell comprises a vector as described above. In some embodiments, the host cell is transformed with the vector prior to cell culture.

In certain embodiments, it is contemplated that the host cell is cultured in a medium under conditions suitable for expression of a fusion polypeptide encoded by the polynucleotides. In one embodiment, the fusion polypeptide is expressed as a soluble protein or as inclusion body. In a related embodiment, the expressed fusion polypeptide is isolated from the host cell or culture medium. In still another embodiment, the isolated fusion polypeptide is contacted with a cleaving agent as described herein.

BRIEF DESCRIPTION OF THE FIGURES

FIG. **1** shows expression of CNP fusion proteins in *E. coli* (FIG. **1**A: Coomassie blue stain, FIG. **1**B: Western blot). M: protein marker; T: total cell lysates; S: soluble supernatants; P: 2 ug CNP22; KSI: KSI-CNP(M/N) fusion protein expression (insoluble); N: Un-induced KSI-CNP fusion protein total lysates; KSI': KSI-P-CNP fusion protein expression (insoluble); Trx: Trx-P-CNP fusion protein expression (soluble); MBP: MBP-P-CNP fusion protein expression (soluble); TAF: TAF-P-CNP fusion protein expression (insoluble) (BL21); TAF': TAF-P-CNP fusion protein expression (insoluble) BL21(DE3), where CNP is Gly-CNP37.

FIG. **2** shows formic acid cleavage of TAF-CNP inclusion bodies. M: protein marker; 1: Gly-CNP37 positive control; 2: uncleaved TAF-CNP inclusion bodies; 3: 2% formic acid cleaved TAF-CNP inclusion bodies, where CNP is Gly-CNP37.

FIGS. **3**A-E depict expression of CNP fusion proteins in *E. coli*. M: protein marker; Tu: total un-induced cell lysates; Su: un-induced soluble supernatants; T: total induced cell lysates; S: soluble supernatants; C1: CNP22; C: Gly-wtCNP37 ("CNP38"); P: insoluble pellets. A: KSI: KSI-CNP38(M/N) fusion protein expression (Insoluble); KSI': KSI-Pro-CNP38 (Pro-Gly-wtCNP37 is designated "Pro-CNP38") fusion protein expression (Insoluble); Trx: Trx-Pro-CNP38 fusion expression (Soluble); MBP: MBP-Pro-CNP38 fusion protein expression (Soluble); TAF: TAF-Pro-CNP38 fusion protein expression (Insoluble) from BL21 cell; TAF': TAF-Pro-CNP38 fusion protein expression (Insoluble) from BL21 (DE3) cell. B: TAF-Pro-CNP37 and BMP-Pro-CNP37 fusion protein expression. C: BMP-Pro-CNP38 fusion protein and BMP protein expression. D: TAF-Pro-HSA-CNP ("Pro-GH-KSEVAHRFK-wtCNP27 (SEQ ID NO: 188) is designated "Pro-HSA-CNP") fusion protein expression. E: TAF-Pro-CNP38 fusion protein and TAF protein expression.

FIGS. **4**A-C depict formic acid cleavage of TAF-Pro-CNP38 inclusion bodies. A: 50% formic acid cleavage of TAF-Pro-CNP38 inclusion bodies. M: protein marker; U: un-cleaved TAF-Pro-CNP38 inclusion bodies; 25° C., 37° C., 42° C., 55° C.: TAF-Pro-CNP38 inclusion bodies were cleaved in 50% formic acid at 25° C., 37° C., 42° C. or 55° C. for 24 hours. 37° C.-S and 55° C.-S: soluble supernatants from the cleavage reactions at 37° C. and 55° C. neutralized with 10N NaOH and centrifuged at 14,000 rpm for 15 minutes. B: 10% and 2% formic acid cleavage of TAF-Pro-CNP38 inclusion bodies. M: protein marker; U: un-cleaved TAF-Pro-CNP38 inclusion bodies; C: formic acid cleaved TAF-Pro-CNP38; S: soluble supernatant after centrifuged at 14,000 rpm for 5 minutes without neutralization; P: insoluble pellet after centrifuged at 14,000 rpm for 5 minutes without neutralization. C: LC/MS analysis of 2% and 10% formic acid cleaved products from TAF-Pro-CNP38 inclusion bodies.

FIGS. **5**A-C depict formic acid cleavage of TAF-Pro-CNP38 inclusion bodies at different temperature and time of formic acid cleavage. M: protein marker; C: Gly-wtCNP37 ("CNP38") positive control; U: un-cleaved TAF-Pro-CNP38 inclusion bodies. A: 2% formic acid cleaved TAF-Pro-CNP38 at 42° C., 55° C. or 70° C. for 6, 24 or 48 hours. B: 2% formic acid cleaved TAF-Pro-CNP38 at 55° C., 60° C., 65° C. or 70° C. for 17 or 24 hours. C: LC/MS analysis of 2% formic acid cleaved products from TAF-Pro-CNP38 inclusion bodies.

FIG. **6** is an SDS-PAGE (Coomassie blue stain) for the expression of a TAF-CNP34 fusion protein. M: protein marker; C: control [Gly-CNP37 ("CNP38")]; T: total cell lysates; S: supernatant; TI: total cell lysates induced; SI: supernatant induced.

FIG. **7** is an SDS-PAGE (Coomassie blue stain) for the expression of fusion proteins TAF-NL-(C/A & 6D/6E)-Pro-CNP38, TAF(C/A & 10D/10E)-Pro-CNP38, and TAF-Pro-CNP53, where "Pro-CNP38" denotes Pro-Gly-CNP37. M: protein marker; C: control [Gly-CNP37 ("CNP38")]; T: total cell lysates; TI: total cell lysates induced; S: supernatant; SI: supernatant induced.

FIG. **8** is an SDS-PAGE (Coomassie blue stain) of the products of formic acid cleavage of the fusion proteins TAF-CNP34 and TAF-Pro-CNP53. M: protein marker; P: positive control [Gly-CNP37 ("CNP38")]; U: uncleaved; C: cleaved; CS: cleaved supernatant; CP: cleaved pellet.

FIG. **9** is an LC/MS chromatogram showing the peak for CNP-34 after formic acid cleavage of TAF-CNP34.

FIG. **10** is an LC/MS chromatogram showing the peak for Pro-CNP53 after formic acid cleavage of TAF-Pro-CNP53.

FIG. **11** is an SDS-PAGE (Coomassie blue stain) for the expression of fusion proteins TAF(C/A & 4D/4E)-Pro-CNP38 and TAF(4D/4E)-Pro-CNP38, where "Pro-CNP38" denotes Pro-Gly-CNP37. M: protein marker; C: control [Gly-

CNP37 ("CNP38")]; T: total cell lysates; TI: total cell lysates induced; S: supernatant; SI: supernatant induced.

FIG. **12** is an SDS-PAGE (Coomassie blue stain) of the products of formic acid cleavage of the fusion proteins TAF (4D/4E)-Pro-CNP38 and TAF(C/A & 4D/4E)-Pro-CNP38, where "Pro-CNP38" denotes Pro-Gly-CNP37. M: protein marker; P: positive control [Gly-CNP37 ("CNP38")]; U: uncleaved; C: cleaved; CS: cleaved supernatant; CP: cleaved pellet.

FIG. **13** is an SDS-PAGE (Coomassie blue stain) of the products of formic acid cleavage of the fusion proteins TAF-NL-(C/A & 6D/6E)-Pro-CNP38 and TAF(C/A & 10D/10E)-Pro-CNP38, where "Pro-CNP38" denotes Pro-Gly-CNP37. M: protein marker; P: positive control [Gly-CNP37 ("CNP38")]; U: uncleaved; C: cleaved; CS: cleaved supernatant; CP: cleaved pellet.

FIG. **14** is a Western blot, using an anti-CNP antibody, of a TAF-Pro-CNP38 fusion protein produced in a 10 L fermentation of BL21(DE3) cells, where the cells were induced at OD_{600}=64 and hour 17 and "Pro-CNP38" denotes Pro-Gly-CNP37.

FIG. **15** is an SDS-PAGE (Coomassie blue stain) of eluate fractions from SP-Sepharose cation-exchange column chromatography of a cruder Pro-Gly-CNP37 ("Pro-CNP38") product. A: TAF-Pro-CNP38 inclusion body (IB) in water; B: IB in formate; C: IB in formate, neutralized; D: neutralized pellet; E: neutralized supernatant; F: TMAE Hi-CAP load; G: TMAE Hi-CAP flow through/SP-Sepharose load; H: SP-Sepharose flow through; SP-Sepharose eluate fractions 1-47: 10 uL/lane.

FIG. **16** shows the degree of resistance of N-terminal PEGylated CNP22 conjugates to neutral endopeptidase (NEP) in vitro.

FIG. **17** depicts the degree of NEP resistance of CNP variants having an amino acid extension at the N-terminus ["CNP27" is GANRR-CNP22(K4R) (SEQ ID NO: 36)].

FIG. **18** illustrates the degree of NEP resistance of N-terminal PEGylated CNP17 and GANRR-CNP22(K4R) ("CNP27") (SEQ ID NO: 36).

FIG. **19** illustrates the degree of NEP resistance of wtCNP22 and CNP variants Gly-CNP37, GHKSEVAHRFK-wtCNP27 ("CNP27-HSA" in the figures) (SEQ ID NO: 144) and PEO12-GANRR-CNP22(K4R) ("CNP27-PEO12" in the figures) (SEQ ID NO: 36).

FIG. **20** shows the ability of CNP variants having an N-terminal amino acid extension to stimulate cGMP production in NIH3T3 cells in vitro. The results are relative to the level of cGMP produced in the presence of 1 uM CNP22. "CNP27" is GANRR-CNP22(K4R) (SEQ ID NO: 36)

FIG. **21** displays the ability of N-terminal PEGylated CNP17 and GANRR-CNP22(K4R) ("CNP27") (SEQ ID NO: 36) to stimulate cGMP production in NIH3T3 cells.

FIG. **22** illustrates the effects of N-terminal PEGylation of CNP22 on cGMP production.

FIG. **23** illustrates the cGMP production induced by wtCNP22 and CNP variants Gly-CNP37, GHKSEVAHRFK-wtCNP27 ("CNP27-HSA") (SEQ ID NO: 144), wtCNP29 and PEO12-GANRR-CNP22(K4R) ("CNP27-PEO12") (SEQ ID NO: 36) in NIH3T3 cells.

FIGS. **24**A and B show that CNP-22 and Pro-Gly-CNP37 ("Pro-CNP38") stimulated cGMP production through NPR-B with similar dose-response curves, and to a much greater extent than through NPR-A, and exhibited a similar profile for NPR-B vs. NPR-C selectivity in in vitro signaling competition assays.

FIG. **25** demonstrates that exposure of rat chondrosarcoma cells to CNP22 1 hour once daily or 2 hours once daily has substantially similar effectiveness in reversing FGF2-induced arrest of chondrocyte growth as continuous exposure to CNP22.

FIGS. **26**A and B show results from a dose response study of CNP22 effects on FGF2-arrested rat chondrosarcoma (RCS) cells.

FIGS. **27**A-D show that addition of CNP22 to RCS cells arrested by FGF2 increases matrix synthesis and partly inhibits FGF2, as assessed by ^{35}S-sulfate and ^{3}H-Pro incorporation into or decrease from matrix. Panels A and C, synthesis; B and D, degradation; A and B, ^{35}S measurement; C and D, ^{3}H measurement. Statistically significant differences are highlighted (ANOVA; *$p<0.05$, **$p<0.01$).

FIGS. **28**A-C show the levels of aggrecan and fibronectin production (mRNA, panels A and C, and protein, panel B) in RCS cells cultured with FGF2 and CNP22.

FIG. **29** shows the efficacy of CNP37 and PEO24-GANRR-CNP22(K4R) ("CNP27-PEO24" in the figures) (SEQ ID NO: 36) in stimulating longitudinal growth of wild-type femur in an ex vivo mouse organ model.

FIG. **30** shows longitudinal bone growth of 2-3 day-old wild-type mouse tibias treated with CNP22, CNP37 or PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) every two days. Results are normalized to measurements prior to treatment (day 0). Data is represented as means±SEM (n=8).

FIG. **31** shows longitudinal bone growth of 2-3 day old achondroplastic FGFR3^{ach} mouse tibias treated with CNP22, CNP37 or PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) every two days. Results are normalized to measurements prior to treatment (day 0). Data is represented as means±SEM (n=7-8).

FIG. **32** shows longitudinal bone growth of 2-3 day-old wild-type mouse femurs treated with CNP22, CNP37 or PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) every two days. Results are normalized to measurements prior to treatment (day 0). Data is represented as means±SEM (n=8).

FIG. **33** shows longitudinal bone growth of 2-3 day-old FGFR3^{ach} mouse femurs treated with CNP22, CNP37 or PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) every two days. Results are normalized to measurements prior to treatment (day 0). Data is represented as means±SEM (n=3-7).

FIGS. **34**A-I depict CNP37 biodistribution in FGFR3^{ach} mouse femurs treated ex vivo every two days. Panels A-C illustrate distribution in distal femurs, panels D-F illustrate distribution in articular chondrocytes and panels G-I illustrate distribution in hypertrophic chondrocytes.

FIGS. **35**A-C depict the cellularity of proliferating columns in growth plates after treatment of wild-type and FGFR3^{ach} mouse femurs with CNP22 or CNP37 every two days for 8 days. (A) no treatment, (B) cell numbers per column after treatment, (C) morphological studies after treatment. The panels in FIGS. **35**C(i) to C(vi). correspond to the sample order set out in FIG. **35**B. Data is represented as means±SEM (n=4-8).

FIGS. **36**A-C depict chondrocyte hypertrophy after ex vivo treatment of wild-type and FGFR3^{ach} mouse femurs with CNP22 or CNP37 every two days for 8 days. (A) no treatment, (B) cell size after treatment, (C) morphological studies after treatment. The panels in FIGS. **36**C(i) to C(vi) correspond to the sample order set out in FIG. **36**B. Data is represented as means±SEM (n=4-9).

FIGS. **37**A-I depict the biodistribution of CNP37 in FGFR3^{ach} mouse tibias treated in vivo. Panels A-C show distribution in distal femurs, panels D-F illustrate distribution in articular chondrocytes and panels G-I illustrate distribution in hypertrophic chondrocytes.

FIGS. **38**A-C illustrate the in vivo effects of CNP37 on FGFR3^{ach} mouse tibia growth plate: (A) total growth plate thickness, (B) proliferating zone thickness, and (C) hypertrophic zone thickness. Data is represented as means±SEM (n=7-15).

FIG. **39** shows cGMP levels in conditioned media from wild-type mouse femurs treated ex vivo with CNP22, CNP37 or PEO24-GANRR-CNP22(K4R) ("CNP27-PEO24") (SEQ ID NO: 36) ($p<0.01$).

FIG. **40** shows cGMP levels in conditioned media from FGFR3^{ach} mouse femurs treated ex vivo with CNP22, CNP37 or PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) ($p<0.01$).

FIG. **41** depicts cGMP levels in conditioned media from wild-type mouse tibias treated ex vivo with CNP22, CNP37 or PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) ($p<0.01$).

FIG. **42** shows cGMP levels in conditioned media from FGFR3^{ach} mouse tibias treated ex vivo with CNP22, CNP37 or PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) ($p<0.01$).

FIG. **43** demonstrates that ex vivo exposure of wild-type and FGFR3^{ach} mouse femurs to CNP22, CNP37 or PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) increased the levels of cleaved collagen type II in the conditioned media ($p<0.05$).

FIG. **44** depicts the hypertrophic region of femoral bones isolated from wild-type mice and FGFR3^{Y367C} mice (a mouse model of severe achondroplasia) and treated ex vivo with vehicle or 1 uM Pro-Gly-CNP37 ("ProCNP38") for 6 days, demonstrating that Pro-Gly-CNP37 treatment resulted in increase in bone growth and expansion in the growth plate.

FIG. **45** shows that CNP37 and PEO24-GANRR-CNP22 (K4R) (SEQ ID NO: 36) intravenously (i.v.) administered to rats have a much longer half-life and a much greater bioavailability in the plasma than CNP22.

FIG. **46** illustrates that PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) subcutaneously (s.c.) administered to rats also has a much longer half-life and a much greater bioavailability in the plasma than CNP22.

FIG. **47** demonstrates that i.v. administered CNP37 and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) stimulate a much greater level of cGMP production in rats than CNP22.

FIG. **48** shows that s.c. administered PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) and, to a lesser extent, CNP37 are substantially more effective in stimulating cGMP production in rats than CNP22.

FIG. **49** shows body weight measurements of wild-type mice treated with Gly-CNP37 or PEO12-GANRR-CNP22 (K4R) (SEQ ID NO: 36).

FIG. **50** shows tail length measurements of wild-type mice treated with Gly-CNP37 or PEO12-GANRR-CNP22(K4R) (SEQ ID NO: 36).

FIG. **51** illustrates the effect of treatment of FGFR3^{ach} mice with CNP22, CNP37 or PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) on body length ($p=0.02$, 1-tailed t-test, unequal variance).

FIG. **52** shows the effect of CNP22, CNP37 and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) on tail length in FGFR3^{ach} mice.

FIGS. **53**A and B show the effect of CNP22, CNP37 and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) on the length of distal long bones (A, ulna; B, tibia) in FGFR3^{ach} mice ($p<0.01$, one-tailed t-test, unequal variance).

FIGS. **54**A and B show the effect of CNP22, CNP37 and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) on the length of proximal bones (A, humerus; B, femur) in FGFR3^{ach} mice ($p<0.01$, one-tailed t-test, unequal variance).

FIG. **55** illustrates that CNP37 administration corrects rhizomelia (disproportion of the length of the proximal limbs) as assessed by the femur:tibia ratio observed in FGFR3^{ach} mice ($p<0.01$, one-tailed t-test, unequal variance).

FIG. **56** shows the effect of CNP22, CNP37 and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) on head length in FGFR3^{ach} mice ($p<0.01$ one-tailed t-test, unequal variance).

FIG. **57** shows that treatment of FGFR3^{ach} mice with CNP37 increases the size of the external auditory meatus (EAM) ($P=0.03$, one-tailed t-test, unequal variance).

FIG. **58** shows the effect of CNP22, CNP37 and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) on spinal length in achondroplasic mice, expressed as extension of vertebral bodies (e.g., lumbar vertebra 5).

FIG. **59** shows that treatment of FGFR3^{ach} mice with CNP37 or PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) results in increased cGMP plasma levels 15-min post-dose.

FIG. **60** illustrates the serum levels of cleaved collagen type II in FGFR3^{ach} mice treated 5 weeks with CNP22, CNP37 or PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36).

FIG. **61** shows the serum levels of osteocalcin in FGFR3^{ach} mice treated 5 weeks with CNP22, CNP37 or PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36).

FIG. **62** shows cGMP plasma levels 15-min post-dose from wild-type mice treated with Gly-CNP37 or PEO12-GANRR-CNP22(K4R) (SEQ ID NO: 36) ($p<0.05$).

FIG. **63** shows the serum levels of cleaved collagen type II in wild-type mice treated 5 weeks with Gly-CNP37 or PEO12-GANRR-CNP22(K4R) (SEQ ID NO: 36).

FIGS. **64-66** display the levels of cGMP, cleaved collagen type II and alkaline phosphatase after administration of vehicle or 20 nmol/kg or 70 nmol/kg Pro-Gly-CNP37 ("Pro-CNP38") to wild-type mice.

FIGS. **67** and **68** depict cleaved collagen type II and total alkaline phosphatase levels after administration of vehicle or Pro-Gly-CNP37 ("Pro-CNP38") under different dosing regimens.

FIG. **69** illustrates the relative increase in body length of Pro-Gly-CNP37 ("Pro-CNP38") and vehicle treated animals in two separate studies (S1 and S2) at Day 37 vs Day 1.

FIGS. **70**A and B show changes in bone mineral density (A) and bone mineral content (B) after administration of Pro-Gly-CNP37 ("Pro-CNP38").

FIG. **71** shows plasma cGMP levels 15 min after the last subcutaneous dose of CNP variant, Day 36, where Gly-wtCNP37 is "CNP38", Pro-Gly-wtCNP37 is "Pro-CNP38", and GHKSEVAHRFK-wtCNP27 (SEQ ID NO: 144) is "HSA-CNP27" in FIGS. **71-73**.

FIG. **72** shows serum levels of cleaved collagen type II from mice treated with Gly-CNP37, Pro-Gly-CNP37 or GHKSEVAHRFK-CNP27 (SEQ ID NO: 144).

FIG. **73** shows serum levels of alkaline phosphatase from mice treated with Gly-CNP37, Pro-Gly-CNP37 or GHKSEVAHRFK-CNP27 (SEQ ID NO: 144).

FIGS. **74**A and B depict desensitization of the cGMP response after acute (A) or chronic (B) treatment with 1 uM Gly-CNP37.

FIG. **75**A demonstrates that daily treatment of wild-type mice with 200 nmol/kg of Pro-Gly-CNP37 ("Pro-CNP38") for 8 days did not desensitize the cGMP response. FIG. **75**B shows that treatment of the mice with 200 nmol/kg of Pro-Gly-CNP37 once a day for two consecutive days potentiated the cGMP response.

FIGS. **76**A-D show that treatment of wild-type mice with 200 nmol/kg of Gly-CNP37 stimulated cGMP secretion in distal femurs (cartilage and bone) (A), femoral cortices (bone) (B), ear pinna (cartilage) (C), and kidney (D). FIGS.

76E-H show that liver (E), heart (F), lung (G) and brain (H) tissues did not exhibit appreciable cGMP secretion in response to Gly-CNP37 relative to vehicle control at the studied time points.

FIGS. **77-82** show results from an on-going study in normal juvenile cynomolgus monkeys subcutaneously injected daily with vehicle or 10 or 36 ug/kg of Pro-Gly-CNP37. Both doses of Pro-Gly-CNP37 have increased growth plate width (FIG. **77**), increased right and left tibia lengths (FIGS. **78**A and B), increased leg length (FIG. **79**), increased arm length (FIG. **80**), increased body length (FIG. **81**), and increased the serum level of alkaline phosphatase (FIG. **82**).

FIG. **83** depicts the observed plot of degradation rate constant (K_{obs}) vs. pH at pH 3-8 and 5° C., 25° C. and 40° C. for Gly-CNP37 formulations.

DETAILED DESCRIPTION OF THE DISCLOSURE

The present disclosure relates to novel variants of CNP having reduced affinity to NEP and/or NPR-C, and reduced susceptibility to cleavage by NEP and/or clearance by NPR-C, pharmaceutical compositions comprising such CNP variants, and methods of using such

CNP variants to treat disorders responsive to CNP, including but not limited to bone-related disorders such as achondroplasia and disorders associated with vascular smooth muscle cells and tissues.

A. Definitions

Unless otherwise stated, the following terms used in this application, including the specification and claims, have the definitions given below.

As used in the specification and the appended claims, the indefinite articles "a" and "an" and the definite article "the" include plural as well as singular referents unless the context clearly dictates otherwise.

The term "about" or "approximately" means an acceptable error for a particular value as determined by one of ordinary skill in the art, which depends in part on how the value is measured or determined. In certain embodiments, the term "about" or "approximately" means within 1, 2, 3, or 4 standard deviations. In certain embodiments, the term "about" or "approximately" means within 30%, 25%, 20%, 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, or 0.05% of a given value or range. Whenever the term "about" or "approximately" precedes the first numerical value in a series of two or more numerical values, it is understood that the term "about" or "approximately" applies to each one of the numerical values in that series.

The terms "ambient temperature" and "room temperature" are used interchangeably herein and refer to the temperature of the surrounding environment (e.g., the room in which a reaction is conducted or a composition is stored). In certain embodiments, ambient temperature or room temperature is a range from about 15° C. to about 28° C., or from about 15° C. to about 25° C., or from about 20° C. to about 28° C., or from about 20° C. to about 25° C., or from about 22° C. to about 28° C., or from about 22° C. to about 25° C. In other embodiments, ambient temperature or room temperature is about 15° C., 16° C., 17° C., 18° C., 19° C., 20° C., 21° C., 22° C., 23° C., 24° C., 25° C., 26° C., 27° C. or 28° C.

Definition of standard chemistry terms may be found in reference works, including Carey and Sundberg, Advanced Organic Chemistry, 3rd Edition, Vols. A and B (Plenum Press, New York 1992). The practice of the present disclosure may employ, unless otherwise indicated, certain conventional methods of synthetic organic chemistry, mass spectrometry, preparative and analytical chromatography, protein chemistry, biochemistry, recombinant DNA technology and pharmacology, within the skill of the art. See, e.g., T. E. Creighton, Proteins: Structures and Molecular Properties (W.H. Freeman and Company, 1993); A. L. Lehninger, Biochemistry (Worth Publishers, Inc., 4th Edition, 2004); Sambrook, et al., Molecular Cloning: A Laboratory Manual (2nd Edition, 1989); Methods In Enzymology (S. Colowick and N. Kaplan eds., Academic Press, Inc.); Remington's Pharmaceutical Sciences, 18th Edition (Easton, Pa.: Mack Publishing Company, 1990).

All publications, patents and patent applications cited herein, whether supra or infra, are hereby incorporated by reference in their entirety.

The following amino acid abbreviations are used throughout the text:

Alanine: Ala (A)	Arginine: Arg (R)
Asparagine: Asn (N)	Aspartic acid: Asp (D)
Cysteine: Cys (C)	Glutamine: Gln (Q)
Glutamic acid: Glu (E)	Glycine: Gly (G)
Histidine: His (H)	Isoleucine: Ile (I)
Leucine: Leu (L)	Lysine: Lys (K)
Methionine: Met (M)	Phenylalanine: Phe (F)
Proline: Pro (P)	Serine: Ser (S)
Threonine: Thr (T)	Tryptophan: Trp (W)
Tyrosine: Tyr (Y)	Valine: Val (V)

"Polypeptide" and "protein" refer to a polymer composed of amino acid residues, related naturally occurring structural variants, and synthetic non-naturally occurring analogs thereof, linked via peptide bonds or peptide bond isosteres. Synthetic polypeptides can be synthesized, for example, using an automated polypeptide synthesizer. The terms "polypeptide" and "protein" are not limited to a minimum length of the product. The term "protein" typically refers to large polypeptides. The term "peptide" typically refers to short polypeptides. Thus, peptides, oligopeptides, dimers, multimers, and the like, are included within the definition. Both full-length proteins and fragments thereof are encompassed by the definition. The terms "polypeptide" and "protein" also include postexpression modifications of the polypeptide or protein, for example, glycosylation, acetylation, phosphorylation and the like. Furthermore, for purposes of the present disclosure, a "polypeptide" can include "modifications," such as deletions, additions, substitutions (which may be conservative in nature or may include substitutions with any of the 20 amino acids that are commonly present in human proteins, or any other naturally or non-naturally-occurring or atypical amino acids), and chemical modifications (e.g., addition of or substitution with peptidomimetics), to the native sequence. These modifications may be deliberate, as through site-directed mutagenesis, or through chemical modification of amino acids to remove or attach chemical moieties, or may be accidental, such as through mutations arising with hosts that produce the proteins or through errors due to PCR amplification.

Conventional notation is used herein to portray polypeptide sequences: the left-hand end of a polypeptide sequence is the amino-terminus; the right-hand end of a polypeptide sequence is the carboxyl-terminus.

"Conservative substitution" refers to substitution of an amino acid in a polypeptide with a functionally, structurally or chemically similar natural or unnatural amino acid. In one

embodiment, the following groups each contain natural amino acids that are conservative substitutions for one another:

(1), Alanine (A) Serine (S), Threonine (T);

(2) Aspartic acid (D), Glutamic acid (E);

(3) Asparagine (N), Glutamine (Q);

(4) Arginine (R), Lysine (K);

(5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); and

(6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W).

In another embodiment, the following groups each contain natural amino acids that are conservative substitutions for one another:

(1) Glycine (G), Alanine (A);

(2) Aspartic acid (D), Glutamic acid (E);

(3) Asparagine (N), Glutamine (Q);

(4) Arginine (R), Lysine (K);

(5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V), Alanine (A);

(6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W); and

(7) Serine (S), Threonine (T), Cysteine (C).

In a further embodiment, amino acids may be grouped as set out below.

(1) hydrophobic: Met, Ala, Val, Leu, Ile, Phe, Trp;

(2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;

(3) acidic: Asp, Glu;

(4) basic: His, Lys, Arg;

(5) residues that influence backbone orientation: Gly, Pro; and

(6) aromatic: Trp, Tyr, Phe, His.

In one embodiment, the peptides or polypeptides described herein are generated via recombinant means, using a polynucleotide encoding a CNP variant. The disclosure thus encompasses polynucleotides encoding any of the CNP variants described herein, host cells or vectors comprising such polynucleotides, optionally linked to expression control sequences, and methods of using such polynucleotides, vectors or host cells to produce CNP variants of the disclosure. CNP variants expressed by such polynucleotides may be produced by methods including growing host cells in culture medium under conditions suitable for expression of the polynucleotide encoding a CNP variant, and isolating the expression product from the host cells or culture medium. Actual expression products may vary slightly from the encoded protein product depending on any post-translational processing.

"Polynucleotide" refers to a polymer composed of nucleotide units. Polynucleotides include naturally occurring nucleic acids, such as deoxyribonucleic acid ("DNA") and ribonucleic acid ("RNA") as well as nucleic acid analogs. The term "nucleic acid" typically refers to large polynucleotides. The term "oligonucleotide" typically refers to short polynucleotides, generally no greater than about 50 nucleotides. It will be understood that when a nucleotide sequence is represented by a DNA sequence (i.e., A, T, G, C), the nucleotide sequence also encompasses an RNA sequence (i.e., A, U, G, C) in which "U" replaces "T." "cDNA" refers to a DNA that is complementary or identical to an mRNA, in either single stranded or double stranded form.

"Expression control sequence" refers to a nucleotide sequence that regulates the expression of a nucleotide sequence operatively linked thereto. "Operatively linked" refers to a functional relationship between two parts in which the activity of one part (e.g., the ability to regulate transcription) results in an action on the other part (e.g., transcription of the sequence). Expression control sequences can include, for example and without limitation, sequences of promoters (e.g., inducible or constitutive), enhancers, transcription terminators, a start codon (i.e., ATG), splicing signals for introns, and stop codons.

"Recombinant polynucleotide" refers to a polynucleotide having sequences that are not naturally joined together. An amplified or assembled recombinant polynucleotide may be included in a suitable vector, and the vector can be used to transform a suitable host cell. A host cell that comprises the recombinant polynucleotide is referred to as a "recombinant host cell." The gene is then expressed in the recombinant host cell to produce, e.g., a "recombinant polypeptide." A recombinant polynucleotide may serve a non-coding function (e.g., promoter, origin of replication, ribosome-binding site, etc.) as well.

"Chimera" as used herein refers to a polynucleotide or polypeptide comprising at least two heterologous polynucleotide or polypeptide sequences (i.e. derived from different sources or not associated with each other as a naturally-occurring sequence) which are directly or indirectly attached or linked together using techniques commonly known in the art, e.g., recombinant expression or chemical crosslinking. In one embodiment, the heterologous sequence can comprise a protein or peptide directly or indirectly linked to a CNP peptide or variant, including proteins or peptides which are cleavable from the CNP peptide or variant. In a related embodiment, CNP variants are chimera as described herein.

In certain embodiments, chimeras include CNP fusion proteins comprising a cleavable carrier protein or peptide tag. The term "cleavable carrier protein" or "cleavable peptide tag" refers to a peptide or polypeptide sequence that may be fused, directly or indirectly via a linker, to a heterologous polypeptide sequence, and is removable from the heterologous sequence using an agent that cleaves or separates the cleavable peptide or polypeptide from the heterologous polypeptide or protein. In some embodiments, the cleavable carrier protein or peptide tag improves generation, purification and/or detection of the fusion protein or the heterologous polypeptide. Exemplary cleavable carrier proteins and peptide tags include, but are not limited to, human transcription factor TAF12 (TAF12), ketosteroid isomerase (KSI), maltose-binding protein (MBP), β-galactosidase (β-Gal), glutathione-S-transferase (GST), thioredoxin (Trx), chitin-binding domain (CBD), BMP-2 mutation (BMPM), SUMO, CAT, TrpE, staphylococcal protein A, streptococcal proteins, starch-binding protein, cellulose-binding domain of endoglucanase A, cellulose-binding domain of exoglucanase Cex, biotin-binding domain, recA, Flag, c-Myc, poly(His), poly (Arg), poly(Asp), poly(Gln), poly(Phe), poly(Cys), green fluorescent protein, red fluorescent protein, yellow fluorescent protein, cyan fluorescent protein, biotin, avidin, streptavidin, antibody epitopes, and fragments thereof.

A "cleaving agent" is an agent that is useful to cleave or separate, e.g., a cleavable peptide or polypeptide from a heterologous polypeptide or protein. Exemplary cleaving agents include, but are not limited to, palladium, cyanogen bromide (CNBr), formic acid, hydroxylamine, clostripain, thrombin, chymotrypsin, trypsin, trypsin-like proteases, carboxypeptidase, enterokinase (enteropeptidase), Kex 2 protease, Omp T protease, Factor Xa protease, subtilisin, proTEV, SUMO protease, V8 protease, HIV protease, rhinovirus protease, furilisin protease, IgA proteases, human Pace protease, collagenase, Nia protease, poliovirus 2Apro protease, poliovirus 3C protease, genenase, furin, elastase, Proteinase K, pepsin, rennin (chymosin), microbial aspartic proteases, papain, calpain, chymopapain, ficin (ficain), bromelain (bromelase), cathespisin B, caspases, thermolysin, Endoprotease Arg-C, Endoprotease Glu-C, Endoprotease Lys-C, kallikrein, and plasmin.

The terms "identical" and percent "identity", in the context of two or more polynucleotide or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of nucleotides or amino acid residues that are the same, when compared and aligned for maximum correspondence, as measured using one of the following sequence comparison algorithms or by visual inspection.

The phrase "substantially homologous" or "substantially identical", in the context of two nucleic acids or polypeptides, generally refers to two or more sequences or subsequences that have at least 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 98% nucleotide or amino acid residue identity, when compared and aligned for maximum correspondence, as measured using one of the following sequence comparison algorithms or by visual inspection. In certain embodiments, the substantial homology or identity exists over regions of the sequences that are at least about 25, 50, 100 or 150 residues in length. In another embodiment, the sequences are substantially homologous or identical over the entire length of either or both comparison biopolymers.

For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are inputted into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, Adv. Appl. Math., 2: 482 (1981), by the homology alignment algorithm of Needleman & Wunsch, J. Mol. Biol., 48: 443 (1970), by the search for similarity method of Pearson & Lipman, Proc. Natl. Acad. Sci. USA, 85: 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis.), or by visual inspection. One example of a useful algorithm is PILEUP, which uses a simplification of the progressive alignment method of Feng & Doolittle, J. Mol. Evol., 35: 351-360 (1987) and is similar to the method described by Higgins & Sharp, CABIOS, 5: 151-153 (1989). Another algorithm useful for generating multiple alignments of sequences is Clustal W (Thompson et al., Nucleic Acids Research, 22: 4673-4680 (1994)). An example of an algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm (Altschul et al., J. Mol. Biol., 215: 403-410 (1990); Henikoff & Henikoff, Proc. Natl. Acad. Sci. USA, 89: 10915 (1989); Karlin & Altschul, Proc. Natl. Acad. Sci. USA, 90: 5873-5787 (1993)). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information.

A further indication that two nucleic acid sequences or polypeptides are substantially homologous or identical is that the polypeptide encoded by the first nucleic acid is immunologically cross reactive with the polypeptide encoded by the second nucleic acid, as described below. Thus, a polypeptide is typically substantially identical to a second polypeptide, for example, where the two polypeptides differ only by conservative substitutions. Another indication that two nucleic acid sequences are substantially identical is that the two molecules hybridize to each other under stringent conditions, as described herein.

"Substantially pure" or "isolated" means an object species is the predominant species present (i.e., on a molar basis, more abundant than any other individual macromolecular species in the composition), and a substantially purified fraction is a composition wherein the object species comprises at least about 50% (on a molar basis) of all macromolecular species present. In one embodiment, a substantially pure composition means that the species of interest comprises at least about 70%, 75%, 80%, 85%, 90%, 95%, 98% or more of the macromolecular species present in the composition on a molar or weight basis. The object species is purified to essential homogeneity (contaminant species cannot be detected in the composition by conventional detection methods) if the composition consists essentially of a single macromolecular species. Solvent species, small molecules (<500 Daltons), stabilizers (e.g., BSA), and elemental ion species are not considered macromolecular species for purposes of this definition. In an embodiment, the compounds of the disclosure are substantially pure or isolated. In another embodiment, the compounds of the disclosure are substantially pure or isolated with respect to the macromolecular starting materials used in their production. In yet another embodiment, the pharmaceutical compositions of the disclosure comprise a substantially pure or isolated CNP variant admixed with one or more pharmaceutically acceptable excipients, carriers or diluents, and optionally with another biologically active agent.

"Naturally occurring" as applied to an object refers to the fact that the object can be found in nature. For example, a polypeptide or polynucleotide sequence that is present in an organism (including viruses) and which has not been intentionally modified by man in the laboratory is naturally occurring. In one embodiment, a "naturally occurring" substance is of human origin.

"Wild-type" (wt) is a term referring to the natural form, including sequence, of a polynucleotide, polypeptide or protein in a species. A wild-type form is distinguished from a mutant form of a polynucleotide, polypeptide or protein arising from genetic mutation(s).

In one embodiment, a first polypeptide that is an "analog" or "variant" or "derivative" of a second polypeptide is a polypeptide having at least about 50%, 60% or 70% sequence homology, but less than 100% sequence homology, with the second polypeptide. Such analogs, variants or derivatives may be comprised of non-naturally occurring amino acid residues, including without limitation, homoarginine, ornithine, penicillamine, and norvaline, as well as naturally occurring amino acid residues. Such analogs, variants or derivatives may also be composed of one or a plurality of D-amino acid residues, and may also contain peptidomimetics or peptide bond isosteres such as non-peptide linkages between two or more amino acid or peptidomimetic residues. In another embodiment, a first polypeptide is an "analog", "variant" or "derivative" of a second polypeptide if the first polypeptide is not a known cleavage product of the second polypeptide or is not a known precursor of the second polypeptide, even if the first polypeptide has 100% sequence homology to the second polypeptide or has a wild-type sequence.

In an embodiment, the term "derived from" as used herein refers to a polypeptide or peptide sequence that is based on a wild type or naturally occurring polypeptide or peptide sequence and can have one or more deletions, additions, and/or substitutions with natural amino acids, unnatural amino acids or peptidomimetics. In one embodiment, the derivative sequence shares at least about 40%, 50%, 60% or 70%, but less than 100%, sequence similarity to the wild-type or naturally occurring sequence. In another embodiment, the

derivative may be a fragment of a polypeptide, wherein the fragment is substantially homologous (e.g., at least about 70%, 75%, 80%, 85%, 90%, or 95% homologous) to the wild-type polypeptide over a length of at least about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 amino acids. In still another embodiment, a polypeptide is "derived from" a wild-type polypeptide if it has a moiety (e.g., a polymer such as, e.g., PEG) directly or indirectly attached to it which is not present on the wild-type polypeptide, even if both polypeptides share 100% homology in their amino acid sequence.

As used herein, an "NPPC-derived" polypeptide refers to a polypeptide derived from the natriuretic peptide precursor C(NPPC) polypeptide, which is a single chain 126-amino acid pre-pro polypeptide, and which upon cleavage ultimately results in wtCNP22. Removal of the signal peptide from NPPC yields pro-CNP, and further cleavage by the endoprotease furin generates an active 53-amino acid peptide (CNP-53), which is secreted and cleaved again by an unknown enzyme to produce the mature 22-amino acid peptide (CNP, or CNP-22). Therefore, CNP22 itself is an "NPPC-derived" polypeptide by virtue of being derived from NPPC. In one embodiment, an NPPC-derived polypeptide is at least about 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, or 95% homologous to the wild type NPPC over the same number of amino acid residues. It is further contemplated that an NPPC-derived peptide may comprise from about 1 to about 53, or 1 to 37, or 1 to 35, or 1 to 31, or 1 to 27, or 1 to 22, or to 35, or about 15 to about 37 residues of the NPPC polypeptide. In one embodiment, an NPPC-derived peptide may comprise a sequence of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, or 53 amino acids derived from the NPPC polypeptide.

The term "effective amount" means a dosage sufficient to produce a desired result on a health condition, pathology, or disease of a subject or for a diagnostic purpose. The desired result may comprise a subjective or objective improvement in the recipient of the dosage. "Therapeutically effective amount" refers to that amount of an agent effective to produce the intended beneficial effect on health. An appropriate "effective" amount in any individual case may be determined by one of ordinary skill in the art using routine experimentation. It will be understood that the specific dose level and frequency of dosage for any particular patient may be varied and will depend upon a variety of factors, including the activity of the specific compound employed; the bioavailability, metabolic stability, rate of excretion and length of action of that compound; the mode and time of administration of the compound; the age, body weight, general health, sex, and diet of the patient; and the severity of the particular condition.

"Treatment" refers to prophylactic treatment or therapeutic treatment or diagnostic treatment. In certain embodiments, "treatment" refers to administration of a compound or composition to a subject for therapeutic, prophylactic or diagnostic purposes.

A "prophylactic" treatment is a treatment administered to a subject who does not exhibit signs of a disease or exhibits only early signs of the disease, for the purpose of decreasing the risk of developing pathology. The compounds or compositions of the disclosure may be given as a prophylactic treatment to reduce the likelihood of developing a pathology or to minimize the severity of the pathology, if developed.

A "therapeutic" treatment is a treatment administered to a subject who exhibits signs or symptoms of pathology for the purpose of diminishing or eliminating those signs or symptoms. The signs or symptoms may be biochemical, cellular, histological, functional or physical, subjective or objective. The compounds of the disclosure may also be given as a therapeutic treatment or for diagnosis.

"Diagnostic" means identifying the presence, extent and/or nature of a pathologic condition. Diagnostic methods differ in their specificity and selectivity. While a particular diagnostic method may not provide a definitive diagnosis of a condition, it suffices if the method provides a positive indication that aids in diagnosis.

"Bone- or cartilage-associated biomarker" or "bone- or cartilage-associated marker" refers to a growth factor, enzyme, protein, or other detectable biological substance or moiety whose level is increased or decreased in association with, e.g., cartilage turnover, cartilage formation, cartilage growth, bone resorption, bone formation, bone growth, or combinations thereof. Such biomarkers may be measured before, during and/or after administration of a CNP variant as described herein. Exemplary bone- or cartilage-associated biomarkers include, but are not limited to, CNP, cGMP, propeptides of collagen type II and fragments thereof, collagen type II and fragments thereof, propeptides of collagen type I and fragments thereof, collagen type 1 and fragments thereof, osteocalcin, proliferating cell nuclear antigen (PCNA), aggrecan chondroitin sulfate, and alkaline phosphatase. Cartilage- and bone-associated biomarkers can be measured in any appropriate biological sample, including but not limited to tissues, blood, serum, plasma, cerebrospinal fluid, synovial fluid and urine. In some embodiments, the biomarkers are measured in blood, plasma or serum from animals undergoing efficacy/pharmacodynamic in vivo studies and/or from the conditioned media of ex vivo studies.

In certain embodiments, the level of at least one bone- or cartilage-associated biomarker is measured and the amount or frequency of administration of CNP variant administered to a subject can be adjusted according to the level of the biomarker measured. In some embodiments, the level of biomarker is "below a target level" or "above a target level." A target level of a biomarker is a level or range of levels of the biomarker at which a therapeutic effect is observed in the subject receiving the CNP variant. In certain embodiments, the target level of a biomarker for a subject having a CNP-responsive disorder or condition is the level or range of levels of the biomarker observed in a normal, non-affected subject. In other embodiments, to indicate a therapeutic effect, the target level of a biomarker need not be equivalent to the level or range of levels of the biomarker observed in a normal subject, but can be within, e.g., 100%, 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10% or 5% of the "normal" level or range of levels of the biomarker observed in a non-affected subject.

For example, if the level of a biomarker increases in association with bone or cartilage formation or growth, the target level of the biomarker indicating a therapeutic effect may be higher than the level of the biomarker in patients suffering from a CNP-responsive disorder who have not been administered a CNP variant, and may optionally be lower than the "normal" level(s), at about the "normal" level(s), or above the "normal" level(s) of the biomarker in subjects not suffering from that disorder. In one embodiment, if the level of a biomarker is below a target level, it indicates an inadequate therapeutic effect, which may require an increase in the amount or frequency of administration of CNP variant administered. In a related embodiment, if the biomarker is above a target level, it indicates that more CNP variant than necessary has been administered, which may require a decrease in the amount or frequency of administration of the CNP variant administered.

As another example, if the level of a biomarker decreases in association with bone or cartilage formation or growth, the

target level of the biomarker indicating a therapeutic effect may be lower than the level of the biomarker in patients suffering from a CNP-responsive disorder who have not been administered a CNP variant, and may optionally be higher than the "normal" level(s), at about the "normal" level(s), or below the "normal" level(s) of the biomarker in subjects not suffering from that disorder. In such a case, the converse of the above adjustments in CNP variant amount and frequency of administration may apply.

"Pharmaceutical composition" refers to a composition suitable for pharmaceutical use in subject animal, including humans and mammals. A pharmaceutical composition comprises a therapeutically effective amount of a CNP variant, optionally another biologically active agent, and optionally a pharmaceutically acceptable excipient, carrier or diluent. In an embodiment, a pharmaceutical composition encompasses a composition comprising the active ingredient(s), and the inert ingredient(s) that make up the carrier, as well as any product that results, directly or indirectly, from combination, complexation or aggregation of any two or more of the ingredients, or from dissociation of one or more of the ingredients, or from other types of reactions or interactions of one or more of the ingredients. Accordingly, the pharmaceutical compositions of the present disclosure encompass any composition made by admixing a compound of the disclosure and a pharmaceutically acceptable excipient, carrier or diluent.

"Pharmaceutically acceptable carrier" refers to any of the standard pharmaceutical carriers, buffers, and the like, such as a phosphate buffered saline solution, 5% aqueous solution of dextrose, and emulsions (e.g., an oil/water or water/oil emulsion). Non-limiting examples of excipients include adjuvants, binders, fillers, diluents, disintegrants, emulsifying agents, wetting agents, lubricants, glidants, sweetening agents, flavoring agents, and coloring agents. Suitable pharmaceutical carriers, excipients and diluents are described in Remington's Pharmaceutical Sciences, 19th Ed. (Mack Publishing Co., Easton, 1995). Preferred pharmaceutical carriers depend upon the intended mode of administration of the active agent. Typical modes of administration include enteral (e.g., oral) or parenteral (e.g., subcutaneous, intramuscular, intravenous or intraperitoneal injection; or topical, transdermal, or transmucosal administration).

A "pharmaceutically acceptable salt" is a salt that can be formulated into a compound for pharmaceutical use, including but not limited to metal salts (e.g., sodium, potassium, magnesium, calcium, etc.) and salts of ammonia or organic amines.

By "pharmaceutically acceptable" or "pharmacologically acceptable" is meant a material that is not biologically or otherwise undesirable, i.e., the material may be administered to an individual without causing any undesirable biological effects or without interacting in a deleterious manner with any of the components of the composition in which it is contained or with any components present on or in the body of the individual.

The term "unit dosage form" refers to physically discrete units suitable as unitary dosages for human and animal subjects, each unit containing a predetermined quantity of a compound of the disclosure calculated in an amount sufficient to produce the desired effect, optionally in association with a pharmaceutically acceptable excipient, diluent, carrier or vehicle. The specifications for the novel unit dosage forms of the present disclosure depend on the particular compound employed and the effect to be achieved, and the pharmacodynamics associated with each compound in the host.

"Physiological conditions" refer to conditions in the body of an animal (e.g., a human). Physiological conditions include, but are not limited to, body temperature and an aqueous environment of physiologic ionic strength, pH and enzymes. Physiological conditions also encompass conditions in the body of a particular subject which differ from the "normal" conditions present in the majority of subjects, e.g., which differ from the normal human body temperature of approximately 37° C. or differ from the normal human blood pH of approximately 7.4.

By "physiological pH" or a "pH in a physiological range" is meant a pH in the range of approximately 7.0 to 8.0 inclusive, more typically in the range of approximately 7.2 to 7.6 inclusive.

As used herein, the term "subject" encompasses mammals and non-mammals. Examples of mammals include, but are not limited to, any member of the mammalian class: humans, non-human primates such as chimpanzees, and other apes and monkey species; farm animals such as cattle, horses, sheep, goats, swine; domestic animals such as rabbits, dogs, and cats; laboratory animals including rodents, such as rats, mice and guinea pigs, and the like. Examples of non-mammals include, but are not limited to, birds, fish, and the like. The term does not denote a particular age or gender.

The terms "polyethylene glycol", "PEG", "polyethylene oxide" and "PEO" are used interchangeably herein unless indicated otherwise. A CNP peptide (CNP22 or a variant thereof) conjugated via an amino group to a "PEOn" polymer associated with the number n, in general has the formula: CH_3—[—O—CH_2CH_2—]$_n$—C(=O)—NHR, where n is the number of ethylene oxide units and R denotes the rest of the peptide. The "PEOn" polymer can optionally have an alkylene group, $(CH_2)_m$, where m is an integer from 1 to 5, between the carbonyl carbon and the repeating ethylene oxide units. Such a "PEOn" (e.g., PEO12 or PEO24) polymer is monodispersed, i.e., is a single discrete polymer of a particular molecular weight. Similarly, a CNP peptide conjugated via an amino group to a "PEGnK" polymer associated with the number nK, in general has the formula: CH_3—[—O—CH_2CH_2—]$_p$—C(=O)—NHR, where p is an integer greater than 1. The "PEGnK" polymer also can optionally have an alkylene group, $(CH_2)_m$, where m is an integer from 1 to 5, between the carbonyl carbon and the repeating ethylene oxide units. However, such a "PEGnK" (e.g., PEG1K, PEG2K, PEG5K or PEG20K) polymer is polydispersed, i.e., contains a mixture of polymers having a distribution of molecular weights, where the number nK denotes the polymer number-average molecular weight (M_n) in kilo Daltons. For example, "PEG2K" conjugated to a CNP peptide denotes a polydispersed PEG polymer having a polymer number-average molecular weight of around 2 kDa.

When a range of the mass of a polymer (e.g., PEG) is given (e.g., in units of kDa), the range refers to a range of polymer number-average molecular weights, not to a range of molecular weights of multiple polymers in a polydispersed mixture, unless expressly indicated otherwise.

The term "halogen", "halide" or "halo" refers to fluorine, chlorine, bromine, and/or iodine.

The term "alkyl" refers to a linear or branched saturated monovalent hydrocarbon radical, wherein the alkyl may optionally be substituted with one or more substituents Q as described herein. In certain embodiments, the alkyl is a linear saturated monovalent hydrocarbon radical that has 1 to 20 (C_{1-20}), 1 to 15 (C_{1-15}), 1 to 12 (C_{1-12}), 1 to 10 (C_{1-10}), or 1 to 6 (C_{1-6}) carbon atoms, or a branched saturated monovalent hydrocarbon radical of 3 to 20 (C_{3-20}), 3 to 15 (C_{3-15}), 3 to 12 (C_{3-12}), 3 to 10 (C_{3-10}), or 3 to 6 (C_{3-6}) carbon atoms. As used herein, linear C_{1-6} and branched C_{3-6} alkyl groups are also referred as "lower alkyl." Examples of alkyl groups include,

but are not limited to, methyl, ethyl, propyl (including all isomeric forms, including n-propyl and isopropyl), butyl (including all isomeric forms, including n-butyl, isobutyl, sec-butyl and tert-butyl), pentyl (including all isomeric forms), and hexyl (including all isomeric forms). For example, C_{1-6} alkyl refers to a linear saturated monovalent hydrocarbon radical of 1 to 6 carbon atoms or a branched saturated monovalent hydrocarbon radical of 3 to 6 carbon atoms.

The term "alkoxy" refers to an —O-alkyl group. In certain embodiments, an alkoxy group may optionally be substituted with one or more substituents Q as described herein.

The term "haloalkyl" refers to an alkyl group that is substituted with one or more halide atoms. In certain embodiments, a haloalkyl group is substituted with one, two, three, four, five or six halide atoms. In certain embodiments, a haloalkyl group may optionally be substituted with one or more additional substituents Q as described herein.

The term "cycloalkyl" refers to a cyclic saturated bridged and/or non-bridged monovalent hydrocarbon radical, which may be optionally substituted with one or more substituents Q as described herein. In certain embodiments, the cycloalkyl has from 3 to 20 (C_{3-20}), from 3 to 15 (C_{3-15}), from 3 to 12 (C_{3-12}), from 3 to 10 (C_{3-10}), or from 3 to 7 (C_{3-7}) carbon atoms. Examples of cycloalkyl groups include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, decalinyl, and adamantyl.

The term "heterocyclyl" or "heterocyclic" refers to a monocyclic non-aromatic ring system or a multicyclic ring system that contains at least one non-aromatic ring, wherein one or more of the non-aromatic ring atoms are heteroatoms independently selected from O, S, or N, and the remaining non-aromatic ring atoms are carbon atoms. In certain embodiments, the heterocyclyl or heterocyclic group has from 3 to 20, from 3 to 15, from 3 to 10, from 3 to 8, from 4 to 7, or from 5 to 6 ring atoms. In certain embodiments, the heterocyclyl is a monocyclic, bicyclic, tricyclic, or tetracyclic ring system, which may include a fused or bridged ring system, and in which the nitrogen or sulfur atoms may be optionally oxidized, the nitrogen atoms may be optionally quaternized, and some rings may be partially or fully saturated, or aromatic. The heterocyclyl may be attached to the main structure at any heteroatom or carbon atom which results in the creation of a stable compound. Examples of heterocyclic groups include, but are not limited to, acridinyl, azepinyl, benzimidazolyl, benzindolyl, benzoisoxazolyl, benzisoxazinyl, benzodioxanyl, benzodioxolyl, benzofuranonyl, benzofuranyl, benzonaphthofuranyl, benzopyranonyl, benzopyranyl, benzotetrahydrofuranyl, benzotetrahydrothienyl, benzothiadiazolyl, benzothiazolyl, benzothiophenyl, benzotriazolyl, benzothiopyranyl, benzoxazinyl, benzoxazolyl, benzothiazolyl, β-carbolinyl, carbazolyl, chromanyl, chromonyl, cinnolinyl, coumarinyl, decahydroisoquinolinyl, dibenzofuranyl, dihydrobenzisothiazinyl, dihydrobenzisoxazinyl, dihydrofuryl, dihydropyranyl, dioxolanyl, dihydropyrazinyl, dihydropyridinyl, dihydropyrazolyl, dihydropyrimidinyl, dihydropyrrolyl, dioxolanyl, 1,4-dithianyl, furanonyl, furanyl, imidazolidinyl, imidazolinyl, imidazolyl, imidazopyridinyl, imidazothiazolyl, indazolyl, indolinyl, indolizinyl, indolyl, isobenzotetrahydrofuranyl, isobenzotetrahydrothienyl, isobenzothienyl, isochromanyl, isocoumarinyl, isoindolinyl, isoindolyl, isoquinolinyl, isothiazolidinyl, isothiazolyl, isoxazolidinyl, isoxazolyl, morpholinyl, naphthyridinyl, octahydroindolyl, octahydroisoindolyl, oxadiazolyl, oxazolidinonyl, oxazolidinyl, oxazolopyridinyl, oxazolyl, oxiranyl, perimidinyl, phenanthridinyl, phenathrolinyl, phenarsazinyl, phenazinyl, phenothiazinyl, phenoxazinyl, phthalazinyl, piperazinyl, piperidinyl, 4-piperidonyl, pteridinyl, purinyl, pyrazinyl, pyrazolidinyl, pyrazolyl, pyridazinyl, pyridinyl, pyridopyridinyl, pyrimidinyl, pyrrolidinyl, pyrrolinyl, pyrrolyl, quinazolinyl, quinolinyl, quinoxalinyl, quinuclidinyl, tetrahydrofuryl, tetrahydrofuranyl, tetrahydroisoquinolinyl, tetrahydropyranyl, tetrahydrothienyl, tetrazolyl, thiadiazolopyrimidinyl, thiadiazolyl, thiamorpholinyl, thiazolidinyl, thiazolyl, thienyl, triazinyl, triazolyl, and 1,3,5-trithianyl. In certain embodiments, a heterocyclic group may optionally be substituted with one or more substituents Q as described herein.

The term "aryl" refers to a monocyclic aromatic group or a multicyclic monovalent aromatic group that contain at least one aromatic hydrocarbon ring. In certain embodiments, the aryl has from 6 to 20 (C_{6-20}), from 6 to 15 (C_{6-15}), or from 6 to 10 (C_{6-10}) ring atoms. Examples of aryl groups include, but are not limited to, phenyl, naphthyl, fluorenyl, azulenyl, anthryl, phenanthryl, pyrenyl, biphenyl, and terphenyl. Aryl also refers to bicyclic or tricyclic carbon rings, where at least one of the rings is aromatic and the others may be saturated, partially unsaturated, or aromatic, for example, dihydronaphthyl, indenyl, indanyl, and tetrahydronaphthyl (tetralinyl). In certain embodiments, an aryl group may optionally be substituted with one or more substituents Q as described herein.

The term "heteroaryl" refers to a monocyclic aromatic group or a multicyclic aromatic group that contain at least one aromatic ring, wherein at least one aromatic ring contains one or more heteroatoms independently selected from O, S, and N. Each ring of a heteroaryl group can contain one or two O atoms, one or two S atoms, and/or one to four N atoms, provided that the total number of heteroatoms in each ring is four or less and each ring contains at least one carbon atom. The heteroaryl may he attached to the main structure at any heteroatom or carbon atom which results in the creation of a stable compound. In certain embodiments, the heteroaryl has from 5 to 20, from 5 to 15, or from 5 to 10 ring atoms. Examples of monocyclic heteroaryl groups include, but are not limited to, pyrrolyl, pyrazolyl, pyrazolinyl, imidazolyl, oxazolyl, isoxazolyl, thiazolyl, thiadiazolyl, isothiazolyl, furanyl, thienyl, oxadiazolyl, pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, and triazinyl. Examples of bicyclic heteroaryl groups include, but are not limited to, indolyl, benzothiazolyl, benzoxazolyl, benzothienyl, quinolinyl, tetrahydroisoquinolinyl, isoquinolinyl, benzimidazolyl, benzopyranyl, indolizinyl, benzofuranyl, isobenzofuranyl, chromonyl, coumarinyl, cinnolinyl, quinoxalinyl, indazolyl, purinyl, pyrrolopyridinyl, furopyridinyl, thienopyridinyl, dihydroisoindolyl, and tetrahydroquinolinyl. Examples of tricyclic heteroaryl groups include, but are not limited to, carbazolyl, benzindolyl, phenanthrollinyl, acridinyl, phenanthridinyl, and xanthenyl. In certain embodiments, a heteroaryl group may optionally be substituted with one or more substituents Q as described herein.

The term "optionally substituted" is intended to mean that a group, including alkyl, alkoxy, haloalkyl, cycloalkyl, heterocyclyl, aryl and heteroaryl, may be substituted with one or more substituents Q (in one embodiment, one, two, three or four substituents Q), where each Q is independently selected from the group consisting of cyano, halo, oxo, nitro, C_{1-6} alkyl, C_{1-6} alkoxy, halo-C_{1-6} alkyl, C_{3-7} cycloalkyl, heterocyclyl, C_{6-14} aryl, heteroaryl, $—C(O)R^e$, $—C(O)OR^e$, $—C(O)NR^fR^g$, $—C(NR^e)NR^fR^g$, $—OR^e$, $—OC(O)R^e$, $—OC(O)OR^e$, $—OC(O)NR^fR^g$, $—OC(=NR^e)NR^fR^g$, $—OS(O)R^e$, $—OS(O)_2R^e$, $—OS(O)NR^fR^g$, $—OS(O)_2NR^fR^g$, $—NR^fR^g$, $—NR^eC(O)R^f$, $—NR^eC(O)OR^f$, $—NR^eC(O)NR^fR^g$, $—NR^eC(=NR^h)NR^fR^g$, $—NR^eS(O)R^f$, $—NR^eS(O)_2R^f$, $—NR^eS(O)NR^fR^g$, $—NR^eS(O)_2NR^fR^g$, $—SR^e$, $—S(O)R^e$, $—S(O)_2R^e$, and $—S(O)_2NR^fR^g$, wherein each R^e, R^f, R^g, and R^h is independently hydrogen, C_{1-6} alkyl, C_{3-7} cycloalkyl, heterocyclyl, C_{6-14} aryl, or heteroaryl; or R^f and R^g, together with the N atom to which they are attached, form heterocyclyl.

B. CNP Variants

The use of CNP22 as a therapeutic is limited by its short half-life in plasma (J. Clin. Endocrinol. Metab., 78: 1428-35 (1994)). In human plasma, the concentration of CNP22 typically is less than five picomolar. CNP22 is degraded and cleared from circulation by NEP and NPR-C in humans (Growth Hormone & IGF Res., 16: S6-S14). In all human and animal studies using systemically administered CNP22, continuous infusion has been used to increase the CNP22 concentration in the subjects. A CNP peptide having a longer half-life and at least a similar level of functionality would be beneficial to a CNP-based therapeutic strategy. CNP variants are also disclosed in related International Application No. PCT/US08/84270, specifically incorporated herein by reference.

The present disclosure provides CNP variants which have reduced affinity to NEP and/or NPR-C, and reduced susceptibility to cleavage by NEP and/or clearance by NPR-C, but which have substantially similar or better functionality than wild-type CNP22. Reduced susceptibility of CNP variants to cleavage by NEP and/or clearance by NPR-C would increase the plasma or serum half-life of the variants, thereby increasing the opportunity for the variants to distribute to the target tissues and sites and effectuate the desired pharmacological effects. In certain embodiments, the CNP variants described herein have reduced susceptibility to cleavage by NEP and/or clearance by NPR-C in vitro or in vivo by at least about 1.5-fold, 2-fold, 2.5-fold, 3-fold, 3.5-fold, 4-fold, 4.5-fold, or 5-fold compared to wtCNP22, and have increased plasma or serum half-life in vivo by at least about 1.5-fold, 2-fold, 2.5-fold, 3-fold, 3.5-fold, 4-fold, 4.5-fold, or 5-fold compared to wtCNP22, while retaining at least about 50%, 60%, 70%, 80%, 90% or 100% of the functionality of wtCNP22, or having at least about 1.5-fold, 2-fold, 2.5-fold, 3-fold, 3.5-fold, 4-fold, 4.5-fold, or 5-fold greater functionality than wtCNP22. CNP functionality can be evaluated in terms of, e.g., the level of one or more biomarkers (e.g., cGMP) associated with cartilage or bone formation or growth in an in vitro or in vivo study, the length of particular bones in an ex vivo or in vivo study, etc.

Natural substrates of NEP are small and natriuretic peptides (about 2.2 to about 3.2 kDa) are the largest of the natural substrates. According to X-ray crystallographic analyses, the NEP active-site is buried deep inside a central cavity, effectively restricting the size of substrate molecules to no more than about 3 kDa (Oefner et al., J. Mol. Biol., 296: 341-349 (2000)). Based on NPR-B signaling studies, variants of CNP-22, such as CNP-17 (retaining only the cyclic domain, Cys6-Cys22, of CNP22) and CNP-53 (CNP-22 with a 31-amino acid extension at the N-terminus), can still bind and activate NPR-B similarly to the 2.2 kDa wtCNP-22. Accordingly, the disclosure encompasses CNP variants conjugated to a natural (e.g., peptide) and/or synthetic (e.g., PEG) polymer at the N-terminus and/or C-terminus of CNP22 or variants thereof, which exhibit increased NEP resistance but retain the ability to bind and activate the NPR-B signaling receptor.

In one embodiment, the disclosure encompasses CNP variants represented by the general formula:

(x)-Cys_6-Phe_7-Gly_8-Leu_9-Lys_{10}-Leu_{11}-Asp_{12}-Arg_{13}-Ile_{14}-Gly_{15}-Ser_{16}-Met_{17}-Ser_{18}-Gly_{19}-Leu_{20}-Gly_{21}-Cys_{22}-(z) (SEQ ID NO:139), or

(x)-Gly_1-Leu_2-Ser_3-Lys_4-Gly_5-Cys_6-Phe_7-Gly_8-Leu_9-Lys_{10}-Leu_{11}-Asp_{12}-Arg_{13}-Ile_{14}-Gly_{15}-Ser_{16}-Met_{17}-Ser_{18}-Gly_{19}-Leu_{20}-Gly_{21}-Cys_{22}-(z) (SEQ ID NO:140), wherein:

(x) and (z) each independently are a natural polymer (e.g., a peptide sequence containing at least one amino acid) and/or a synthetic polymer (e.g., PEG) as described herein, such that the total mass of the CNP variant is characterized by the ranges described generally herein, e.g., in the range from about 2.6 kDa or 2.8 kDa to about 6 or 7 kDa. In one embodiment, the residues from Cys6 to Cys22 form a cyclic portion. In an embodiment, (x) and/or (z) comprise an amino acid extension derived from NPPC or a non-CNP polypeptide (e.g., ANP, BNP, IgG, etc.), wherein the extension contains 1 to 40, 1 to 35, 1 to 31, 5 to 35, 5 to 31 or 5 to 15 amino acids. In another embodiment, the CNP variants comprise one or more modifications and/or substitutions with another natural amino acid, an unnatural amino acid, a peptidomimetic and/or a peptide bond isostere at one or more of the following positions of CNP22: Gly1, Lys4, Gly5, Cys6, Phe7, Gly8, Leu9, Lys10, Leu11, Ile14, Gly15, Ser16, Met17, Gly19, Leu20 and Gly21.

In another embodiment, CNP variants having a total mass characterized by the ranges described generally herein, e.g., from about 2.6 kDa or 2.8 kDa to about 6 or 7 kDa, designed for increased resistance to NEP degradation, are represented by the general formula:

(x)-Cys_6-Phe_7-Gly_8-Leu_9-$(h)_{10}$-Leu_{11}-Asp_{12}-Arg_{13}-Ile_{14}-Gly_{15}-Ser_{16}-Met_{17}-Ser_{18}-Gly_{19}-Leu_{20}-Gly_{21}-Cys_{22}-(z) (SEQ ID NO: 141), or

(x)-Gly_1-Leu_2-Ser_3-$(b)_4$-Gly_5-Cys_6-Phe_7-Gly_8-Leu_9-$(h)_{10}$-Leu_{11}-Asp_{12}-Arg_{13}-Ile_{14}-Gly_{15}-Ser_{16}-Met_{17}-Ser_{18}-Gly_{19}-Leu_{20}-Gly_{21}-Cys_{22}-(z) (SEQ ID NO: 6), wherein:

(x) is a synthetic or natural polymeric group, or a combination thereof, wherein a non-limiting example of a synthetic polymeric group is PEG (or PEO), and a non-limiting example of a natural polymeric group is an amino acid sequence containing from 1 to 35 amino acids and derived from NPPC or variants thereof with substitutions and/or deletions, ANP, BNP, or other non-CNP (poly)peptides such as, e.g., serum albumin, IgG, histidine-rich glycoproteins, fibronectin, fibrinogen, zinc finger-containing polypeptides, osteocrin or fibroblast growth factor 2 (FGF2);

(z) may be absent or may be a synthetic or natural polymeric group, or a combination thereof, wherein a non-limiting example of a synthetic polymeric group is PEG, and a non-limiting example of a natural polymeric group is an amino acid sequence derived from a natriuretic polypeptide (e.g., NPPC, CNP, ANP or BNP) or non-natriuretic polypeptide (e.g., serum albumin or IgG); and

(b) and (h) independently may be the wild type Lys at that position or may be replaced with a conservative amino acid substitution or any natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amine on a side chain, including but not limited to Arg, Gly, 6-hydroxynorleucine, citrulline (Cit), Gln, Glu or Ser. In one embodiment, (b) is Arg. In another embodiment, for improved NEP resistance, (b) is not Gly. In yet another embodiment, (h) is not Arg.

Non-limiting examples of amino acid sequences derived from NPPC or variants thereof include:

```
Arg,

Glu-Arg,

                                        (SEQ ID NO: 7)
Gly-Ala-Asn-Lys-Lys,

                                        (SEQ ID NO: 8)
Gly-Ala-Asn-Arg-Arg,

                                        (SEQ ID NO: 9)
Gly-Ala-Asn-Pro-Arg,
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-continued

(SEQ ID NO: 10)
Gly-Ala-Asn-Gln-Gln,

(SEQ ID NO: 11)
Gly-Ala-Asn-Ser-Ser,

(SEQ ID NO: 12)
Gly-Ala-Asn-Arg-Gln,

(SEQ ID NO: 13)
Gly-Ala-Asn-Arg-Met,

(SEQ ID NO: 14)
Gly-Ala-Asn-Arg-Thr,

(SEQ ID NO: 15)
Gly-Ala-Asn-Arg-Ser,

(SEQ ID NO: 16)
Ala-Ala-Trp-Ala-Arg-Leu-Leu-Gln-Glu-His-Pro-Asn-Ala,

(SEQ ID NO: 17)
Ala-Ala-Trp-Ala-Arg-Leu-Leu-Gln-Glu-His-Pro-Asn-Ala-Arg,

(SEQ ID NO: 18)
Asp-Leu-Arg-Val-Asp-Thr-Lys-Ser-Arg-Ala-Ala-Trp-Ala-Arg,

(SEQ ID NO: 19)
Gln-Glu-His-Pro-Asn-Ala-Arg-Lys-Tyr-Lys-Gly-Ala-Asn-Lys-Lys,

(SEQ ID NO: 20)
Gln-Glu-His-Pro-Asn-Ala-Arg-Lys-Tyr-Lys-Gly-Ala-Asn-Arg-Arg,

(SEQ ID NO: 21)
Asp-Leu-Arg-Val-Asp-Thr-Lys-Ser-Arg-Ala-Ala-Trp-Ala-Arg-Leu-Leu-Gln-Glu-His-Pro-Asn-Ala-Arg-Lys-Tyr-Lys-Gly-Ala-Asn-Lys-Lys,
and

(SEQ ID NO: 22)
Asp-Leu-Arg-Val-Asp-Thr-Lys-Ser-Arg-Ala-Ala-Trp-Ala-Arg-Leu-Leu-Gln-Glu-His-Pro-Asn-Ala-Arg-Lys-Tyr-Lys-Gly-Ala-Asn-Arg-Arg.

Non-limiting examples of amino acid sequences derived from non-CNP polypeptides such as, e.g., ANP, BNP, serum albumin and IgG include:

(SEQ ID NO: 23)
Ser-Leu-Arg-Arg-Ser-Ser;

(SEQ ID NO: 24)
Asn-Ser-Phe-Arg-Tyr;

(SEQ ID NO: 25)
Ser-Pro-Lys-Met-Val-Gln-Gly-Ser-Gly;

(SEQ ID NO: 26)
Met-Val-Gln-Gly-Ser-Gly;

(SEQ ID NO: 27)
Lys-Val-Leu-Arg-Arg-Tyr;

(SEQ ID NO: 28)
Lys-Val-Leu-Arg-Arg-His;

(SEQ ID NO: 29)
Gly-Gln-His-Lys-Asp-Asp-Asn-Pro-Asn-Leu-Pro-Arg;

(SEQ ID NO: 30)
Gly-Val-Pro-Gln-Val-Ser-Thr-Ser-Thr;

-continued

(SEQ ID NO: 31)
Gly-Glu-Arg-Ala-Phe-Lys-Ala-Trp-Ala-Val-Ala-Arg-Leu-Ser-Gln;

(SEQ ID NO: 32)
Gly-Gln-Pro-Arg-Glu-Pro-Gln-Val-Tyr-Thr-Leu-Pro-Pro-Ser.

In an embodiment, the N-terminal (x) group and/or the C-terminal (z) group of any of the CNP variants having an (x) and/or (z) group, as described herein, independently comprise an amino acid sequence that contains a small number of, if any, acidic natural or unnatural amino acids (e.g., Asp or Glu). In another embodiment, (x) and/or (z) are enriched in basic natural or unnatural amino acids (e.g., Lys, Arg or His) to maintain an alkaline pI similar to the pI of CNP22 (pI 8.9). In one embodiment, the pI of the CNP variants is in the range from about 8 to about 10.5, designed so that the CNP variants can diffuse more readily through the extracellular matrix surrounding chondrocytes of bone growth plates. In narrower embodiments, the pI of the CNP variants is from about 8.5 to about 10.5, or from about 8.5 to about 10, or from about 9 to about 10.

In yet another embodiment, (x) and/or (z) are enriched in polar natural or unnatural amino acids, designed for increased aqueous solubility. In still another embodiment, (x) and/or (z) contain a small number of, if any, hydrophobic natural or unnatural amino acids (e.g., Ala, Val, Leu, Ile or Met).

In a further embodiment, the N-terminus of the CNP variants terminates in at least one glycine residue, designed for increased serum half-life. In a related embodiment, to prevent pyroglutamine formation, the N-terminus of CNP variants terminates in a glycine residue if it would otherwise terminate in glutamine. In one embodiment, the (x) group contains an amino acid extension whose N-terminus terminates in at least one glycine residue. In another embodiment, (x) and/or (z) do not contain two adjacent basic natural or unnatural amino acids (e.g., Lys-Lys or Arg-Arg), designed to reduce susceptibility to cleavage by the protease furin. In an embodiment, (x) does not contain two adjacent basic amino acids immediately preceding the position corresponding to Gly1 of CNP22.

In still another embodiment, the (x) group and/or the (z) group of the CNP variants comprise an amino acid sequence derived from NPPC (e.g., derived from CNP53). In an embodiment, (x) comprises an amino acid sequence derived from the N-terminal tail of ANP or BNP. In another embodiment, (z) comprises an amino acid sequence derived from the C-terminal tail of ANP or BNP. In a further embodiment, (x) and/or (z) comprise an amino acid sequence derived from a non-natriuretic polypeptide such as, e.g., IgG, human serum albumin (HSA), histidine-rich glycoproteins, fibronectin, fibrinogen, zinc finger-containing polypeptides, FGF-2, and bone-targeting proteins (e.g., osteocrin, osteopontin, osteocalcin, and sialoprotein).

In any embodiment described herein in which CNP22 or a variant thereof can have an N-terminal (x) group and/or a C-terminal (z) group, (x) and/or (z) independently can contain an amino acid sequence derived from the functional domain of a bone morphogenetic protein (BMP). An N-terminal and/or C-terminal amino acid extension derived from the functional domain of a BMP can increase the NEP resistance, and hence the serum half-life of the CNP variant, by increasing the total mass of the CNP variant to characterized by the ranges described generally herein, e.g., a range from about 2.6 kDa or 2.8 kDa to about 6 or 7 kDa. In addition, since certain BMPs are growth factors and cytokines that

induce the formation of bone and cartilage, a fragment derived from the functional domain of a BMP can promote chondrocyte, cartilage or bone growth by a mechanism distinct from activation of the guanylyl cyclase function of NPR-B by the cyclic domain of CNP22 or a variant thereof. Non-limiting examples of BMPs that promote bone formation and development, cartilage formation and development, and/or osteoblast differentiation include BMP1, BMP2, BMP3, BMP5, BMP7 and BMP8a. In an embodiment, the N-terminus and/or C-terminus of CNP22 or a variant thereof independently are conjugated to an amino acid sequence derived from the last 140 amino acids in the C-terminal portion of BMP1, BMP2, BMP3, BMP5, BMP7 or BMP8a.

In one embodiment, the CNP variants contain an amino acid extension at the N-terminus and/or C-terminus of CNP22 or CNP17, including but not limited to:

(SEQ ID NO: 4)
DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMS GLGC (CNP-53);

(SEQ ID NO: 60)
QEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-37, Analog BL);

(SEQ ID NO: 61)
AAWARLLQEHPNAGLSKGCFGLKLDRIGSMSGLGC (Analog CA);

(SEQ ID NO: 62)
AAWARLLQEHPNARGLSKGCFGLKLDRIGSMSGLGC (Analog CB);

(SEQ ID NO: 63)
DLRVDTKSRAAWARGLSKGCFGLKLDRIGSMSGLGC (Analog CC);

(SEQ ID NO: 40)
RGLSKGCFGLKLDRIGSMSGLGC;

(SEQ ID NO: 38)
ERGLSKGCFGLKLDRIGSMSGLGC;

(SEQ ID NO: 64)
GANQQGLSKGCFGLKLDRIGSMSGLGC;

(SEQ ID NO: 65)
GANRRGLSKGCFGLKLDRIGSMSGLGC;

(SEQ ID NO: 66)
GANPRGLSKGCFGLKLDRIGSMSGLGC;

(SEQ ID NO: 67)
GANSSGLSKGCFGLKLDRIGSMSGLGC;

(SEQ ID NO: 144)
GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC;
and

(SEQ ID NO: 68)
SPKMVQGSG-CNP17-KVLRRH (Analog CD) (CNP17 having N-terminal and C-terminal tails derived from BNP).

In another embodiment, the CNP variants have a K4R substitution at position 4 of CNP22. Non-limiting examples of CNP(K4R) variants include:

(SEQ ID NO: 36)
GANRRGLSRGCFGLKLDRIGSMSGLGC (Analog AY);

(SEQ ID NO: 37)
GANPRGLSRGCFGLKLDRIGSMSGLGC (Analog CI);

(SEQ ID NO: 41)
RGLSRGCFGLKLDRIGSMSGLGC (Analog AZ);

(SEQ ID NO: 39)
ERGLSRGCFGLKLDRIGSMSGLGC (Analog BA);

-continued

(SEQ ID NO: 69)
GANQQGLSRGCFGLKLDRIGSMSGLGC (Analog CH);
and

(SEQ ID NO: 70)
GANSSGLSRGCFGLKLDRIGSMSGLGC (Analog CG).

In further embodiments, the CNP variants are chimeras comprising CNP22, or a variant thereof having amino acid addition(s), deletion(s) and/or substitution(s), and a peptide fragment derived from a polypeptide or protein other than CNP, or the whole non-CNP polypeptide or protein, to the N-terminus of the CNP peptide, wherein CNP22 or the variant thereof may optionally have an N-terminal amino acid extension of one or more amino acid residues. In certain embodiments, the CNP chimeras comprise CNP22 or a variant thereof that has an N-terminal amino acid extension of one or more amino acid residues. In certain embodiments, the CNP chimeras contain lysine-lysine (KK) residues or GANKK residues immediately preceding the first position of CNP22 (Gly in the case of CNP22) or a variant thereof. In other embodiments, the CNP chimeras contain one or two residues different from lysine-lysine immediately preceding the first position of CNP22 or a variant thereof. Non-limiting examples of residues that can immediately precede the first position of CNP22 or a variant thereof include KP, PK, PR, PQ, QK, QQ, RR, SS, GANKP(SEQ ID NO: 200), GANPK (SEQ ID NO: 201), GANPR (SEQ ID NO: 9), GANPQ (SEQ ID NO: 202), GANQK (SEQ ID NO: 203), GANQQ (SEQ ID NO: 10), GANRR (SEQ ID NO: 8), and GANSS (SEQ ID NO: 11).

In another embodiment, the CNP variants are chimera comprising CNP22 and an N-terminal peptide fragment, including but not limited to:

(SEQ ID NO: 76)
GHHSHEQHPHGANQQGLSKGCFGLKLDRIGSMSGLGC (Analog CQ) (histidine-rich glycoprotein (HRGP) fragment-CNP22 chimera);

(SEQ ID NO: 77)
GAHHPHEHDTHGANQQGLSKGCFGLKLDRIGSMSGLGC (Analog CR) (HRGP fragment-CNP22 chimera);

(SEQ ID NO: 78)
GHHSHEQHPHGANPRGLSKGCFGLKLDRIGSMSGLGC (Analog CX) (HRGP fragment-CNP22 chimera);

(SEQ ID NO: 79)
GQPREPQVYTLPPSGLSKGCFGLKLDRIGSMSGLGC (Analog CF) ($IgG_1(F_c)$ fragment-CNP22 chimera);

(SEQ ID NO: 80)
GQHKDDNPNLPRGANPRGLSKGCFGLKLDRIGSMSGLGC (Analog CY) (human serum albumin (HSA) fragment-CNP22 chimera);

(SEQ ID NO: 81)
GERAFKAWAVARLSQGLSKGCFGLKLDRIGSMSGLGC (Analog CE) (HSA fragment-CNP22 chimera);

(SEQ ID NO: 82)
FGIPMDRIGRNPRGLSKGCFGLKLDRIGSMSGLGC (Analog CZ) (osteocrin "NPR C inhibitor" fragment-CNP22 chimera);
and

(SEQ ID NO: 83)
GKRTGQYKLGSKTGPGPKGLSKGCFGLKLDRIGSMSGLGC (Analog DA) (FGF2 "heparin-binding domain" fragment-CNP22 chimera).

In yet another embodiment, the CNP variants are chimera comprising an N-terminal peptide fragment and CNP22 in which arginine is substituted for Lys4 of CNP22 ("CNP22 (K4R)"), including but not limited to:

(SEQ ID NO: 84)
GQPREPQVYTGANQQGLSRGCFGLKLDRIGSMSGLGC (Analog CK) ($IgG_1(F_c)$ fragment-CNP22(K4R) chimera);

(SEQ ID NO: 85)
GVPQVSTSTGANQQGLSRGCFGLKLDRIGSMSGLGC (Analog CL) (HSA fragment-CNP22(K4R) chimera);

(SEQ ID NO: 86)
GQPSSSSQSTGANQQGLSRGCFGLKLDRIGSMSGLGC (Analog CM) (fibronectin fragment-CNP22(K4R) chimera);

(SEQ ID NO: 87)
GQTHSSGTQSGANQQGLSRGCFGLKLDRIGSMSGLGC (Analog CN) (fibrinogen fragment-CNP22(K4R) chimera);

(SEQ ID NO: 88)
GSTGQWHSESGANQQGLSRGCFGLKLDRIGSMSGLGC (Analog CO) (fibrinogen fragment-CNP22(K4R) chimera);
and

(SEQ ID NO: 89)
GSSSSSSSSSGANQQGLSRGCFGLKLDRIGSMSGLGC (Analog CP) (zinc finger fragment-CNP22(K4R) chimera).

Chimera comprising IgG and CNP22 or a variant thereof are designed for, inter alia, increased resistance to NEP degradation and reduced binding to serum albumin. CNP chimera comprising a surface fragment of HSA are designed for, inter alia, reduced immunogenicity and reduced binding to serum albumin. HRGP-CNP22 and HRGP-CNP22(K4R) chimera containing a cationic, histidine-rich, non-lysine, non-arginine sequence at the N-terminus are designed for, inter alia, increased stability to proteases. Chimera containing an osteocrin fragment are designed to release, upon protease (e.g., furin) cleavage, the osteocrin fragment at bone growth plates, where the fragment would inhibit the clearance receptor NPR-C. With respect to chimera comprising an FGF2 heparin-binding fragment, heparin binding to the fragment is designed to protect the chimera from degradation, thereby providing a longer serum half-life. Chimera containing a fibronectin, fibrinogen, or zinc-finger fragment are designed for reduced binding to serum albumin, among other features.

Not intending to be bound by theory, a CNP variant of molecular weight from about 2.6 or 2.8 kDa to about 6 or 7 kDa which has increased resistance to NEP degradation and has similar or improved functionality (e.g., binding to NPR-B and stimulation of cGMP signaling) as compared to wtCNP22, may be more effective if it does not bind tightly to plasma proteins such as serum albumin. A CNP variant that does not bind tightly to plasma proteins (e.g., serum albumin) may be more effective in diffusing through cartilage, getting to chondrocytes of bone growth plates, and binding to and activating NPR-B for cGMP signaling. In one embodiment, CNP variants designed for reduced binding to plasma proteins (e.g., serum albumin) are chimeras comprising CNP22 or a variant thereof and a peptide fragment from IgG. In another embodiment, CNP variants designed for reduced binding to plasma proteins are chimeras comprising CNP22 or CNP22(K4R) and a fragment from a polypeptide (e.g., IgG, HSA, fibronectin, fibrinogen, a zinc finger-containing polypeptide, etc.). In yet another embodiment, CNP variants designed for reduced binding to plasma proteins comprise CNP22 or a variant thereof conjugated to a hydrophilic or water-soluble polymer. In one embodiment, the hydrophilic or water-soluble polymer is PEG (or PEO). In another embodiment, the hydrophilic or water-soluble polymer (e.g., PEG) is functionalized with one or more functional groups that impart a negative charge to the polymer under physiological conditions, such as, e.g, carboxyl, sulfate or phosphate groups, or a combination thereof.

In a further embodiment, CNP variants of the disclosure include truncated CNP peptides ranging from human CNP-17 (hCNP-17) to human CNP-53 (hCNP-53), and having wild-type amino acid sequences derived from hCNP-53. Such truncated CNP peptides include:

(SEQ ID NO: 4)
DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSG LGC (CNP-53);

(SEQ ID NO: 146)
LRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGL GC (CNP-52);

(SEQ ID NO: 147)
RVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGL GC (CNP-51);

(SEQ ID NO: 148)
VDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-50);

(SEQ ID NO: 149)
DTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-49);

(SEQ ID NO: 150)
TKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-48);

(SEQ ID NO: 151)
KSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-47);

(SEQ ID NO: 152)
SRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-46);

(SEQ ID NO: 153)
RAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-45);

(SEQ ID NO: 154)
AAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-44);

(SEQ ID NO: 155)
AWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-43);

(SEQ ID NO: 156)
WARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-42);

(SEQ ID NO: 157)
ARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-41);

(SEQ ID NO: 158)
RLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-40);

(SEQ ID NO: 159)
LLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-39);

(SEQ ID NO: 160)
LQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-38);

(SEQ ID NO: 60)
QEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-37);

(SEQ ID NO: 161)
EHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-36);

-continued

(SEQ ID NO: 162)
HPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-35);

(SEQ ID NO: 163)
PNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-34);

(SEQ ID NO: 164)
NARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-33);

(SEQ ID NO: 165)
ARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-32);

(SEQ ID NO: 166)
RKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-31);

(SEQ ID NO: 167)
KYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-30);

(SEQ ID NO: 168)
YKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-29);

(SEQ ID NO: 169)
KGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-28);

(SEQ ID NO: 170)
GANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-27);

(SEQ ID NO: 171)
ANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-26);

(SEQ ID NO: 172)
NKKGLSKGCFGLKLDRIGSMSGLGC (CNP-25);

(SEQ ID NO: 173)
KKGLSKGCFGLKLDRIGSMSGLGC (CNP-24);

(SEQ ID NO: 174)
KGLSKGCFGLKLDRIGSMSGLGC (CNP-23);

(SEQ ID NO: 1)
GLSKGCFGLKLDRIGSMSGLGC (CNP-22);

(SEQ ID NO: 175)
LSKGCFGLKLDRIGSMSGLGC (CNP-21);

(SEQ ID NO: 176)
SKGCFGLKLDRIGSMSGLGC (CNP-20);

(SEQ ID NO: 177)
KGCFGLKLDRIGSMSGLGC (CNP-19);

(SEQ ID NO: 178)
GCFGLKLDRIGSMSGLGC (CNP-18);
and

(SEQ ID NO: 2)
CFGLKLDRIGSMSGLGC (CNP-17).

In certain embodiments, CNP variants do not include CNP-17, CNP-22 or CNP-53.

In another embodiment, the truncated CNP peptides ranging from hCNP-17 to hCNP-53 can contain amino acid addition(s), deletion(s) and/or substitution(s) with natural or unnatural amino acid(s) or peptidomimetic(s) (e.g., peptide bond isostere(s)), as described herein, at any one or more of the amino acid positions of the particular truncated CNP peptides. In yet another embodiment, the truncated CNP peptides having wild-type sequences or amino acid addition(s), deletion(s) and/or substitution(s), can be conjugated at the N-terminus, C-terminus and/or internal site(s) to any of the moieties described herein, including but not limited to bone- or cartilage-targeting moieties (e.g., bisphosphonates, bone- or cartilage-targeting peptide sequences (e.g., polyAsp, polyGlu), peptide sequences derived from bone-targeting domains of bone proteins (e.g., osteopontin, osteocalcin, sialoprotein)), peptide sequences derived from the functional domains of bone morphogenetic proteins (e.g., BMP2, BMP3, BMP5, BMP7, BMP8a), peptide sequences derived from natriuretic polypeptides (e.g., NPPC, ANP, BNP), peptide sequences derived from polypeptides of non-natriuretic origin (e.g., serum albumin, IgG, histidine-rich glycoproteins, fibronectin, fibrinogen, zinc finger-containing polypeptides, FGF-2, osteocrin), moieties that reduce renal clearance (e.g., negatively charged PEG moieties), hydrophilic polymers (e.g., PEG), carbohydrates (e.g., carbohydrates recognized by receptors on the surface of cells at bone growth plates), hydrophobic acids (e.g., C_5-C_{12} carboxylic acids, natural fatty acids), phospholipids, and combinations thereof. In an embodiment, the truncated CNP peptides having wild-type sequences or amino acid addition(s), deletion(s) and/or substitution(s), and optionally conjugated to one or more moieties at the N-terminus, C-terminus and/or internal site(s), have a total mass characterized by the ranges described generally herein, e.g., from about 2.6 kDa or 2.8 kDa to about 6 or 7 kDa.

In a further embodiment, the CNP variants are derivatives of CNP37, which is QEHPNARKYKGANKK-CNP22 (SEQ ID NO: 60). The CNP37 variants can contain amino acid addition(s), deletion(s), and/or substitution(s) with natural or unnatural amino acid(s) or peptidomimetic(s) (e.g., peptide bond isostere(s)) at any one or more of the 37 positions of CNP37. Non-limiting examples of substitutions that can be made in CNP37, based on the numbering of CNP22, include K4R, G5S, G5R, G8S, K10R, G15S, S16Q, M17N, G19R, and combinations thereof. In an embodiment, the CNP37 derivatives contain a substitution of Met17 to a natural (e.g., asparagine) or unnatural amino acid or peptidomimetic, designed in part to avoid oxidation of the sulfur atom of methionine. In another embodiment, the CNP37 variants contain substitution(s) of Lys8, Lys10, Lys14 and/or Lys15 (based on numbering from the N-terminus of CNP37) to non-basic natural or unnatural amino acid(s) or peptiomimetic(s), designed in part to reduce albumin binding.

In addition or alternatively to amino acid addition(s), deletion(s) and/or substitution(s), the CNP37 derivatives can be conjugated at the N-terminus, C-terminus, and/or an internal site to any of the moieties described herein, including but not limited to bone- or cartilage-targeting moieties (e.g., bone-targeting peptide domains), moieties that reduce renal clearance (e.g., negatively charged PEG moieties), hydrophilic polymers (e.g., PEG), amino acid sequences comprising one or more amino acids (e.g., osteocrin "NPR-C inhibitor" fragment), carbohydrates (e.g., carbohydrates recognized by receptors on the surface of cells at bone growth plates), hydrophobic acids (e.g., C_5-C_{12} carboxylic acids and natural fatty acids), and combinations thereof.

In one embodiment, the CNP variants are modified CNP37 peptides having mutation(s)/substitution(s) at the furin cleavage site (underlined), designed to improve in vivo resistance to the furin protease, and/or containing glycine (underlined) at the N-terminus, designed to improve plasma stability and prevent pyroglutamine formation. Such CNP37 variants include but are not limited to:

(SEQ ID NO: 71)
GQEHPNARKYKGANPKGLSKGCFGLKLDRIGSMSGLGC (An. CS);

(SEQ ID NO: 72)
GQEHPNARKYKGANQKGLSKGCFGLKLDRIGSMSGLGC (An. CT);

(SEQ ID NO: 73)
GQEHPNARKYKGANQQGLSKGCFGLKLDRIGSMSGLGC (An. CU);

-continued

(SEQ ID NO: 74)
GQEHPNARKYKGANKPGLSKGCFGLKLDRIGSMSGLGC (An. CW);

(SEQ ID NO: 75)
GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC
(Gly-CNP37, An. DB);
and

(SEQ ID NO: 145)
PGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC
(Pro-Gly-CNP37)

In a further embodiment, the CNP variants of the disclosure include CNP peptides and variants thereof that can be produced by the fusion protein process described herein. Non-limiting examples of CNP variants that can be produced by the fusion protein process described herein, using chemical or proteolytic cleavage or protein self-cleavage, include:

(SEQ ID NO: 179)
GDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMS
GLGC (Gly-wtCNP53);

(SEQ ID NO: 75)
GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC
(Gly-wtCNP37);

(SEQ ID NO: 60)
QEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (wtCNP37);

(SEQ ID NO: 144)
GHKSEVAHRFK-GANKKGLSKGCFGLKLDRIGSMSGLGC
(HSA fragment-wtCNP27);

(SEQ ID NO: 36)
GANRRGLSRGCFGLKLDRIGSMSGLGC [CNP27(K4,5,9R)];

(SEQ ID NO: 180)
DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSG
LGC [CNP53(M48N)];

(SEQ ID NO: 181)
GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC
[Gly-CNP37(M32N)];

(SEQ ID NO: 182)
QEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC
[CNP37(M32N)];

(SEQ ID NO: 183)
GHKSEVAHRFK-GANKKGLSKGCFGLKLDRIGSNSGLGC
[HSA-CNP27(M22N)];

(SEQ ID NO: 184)
GANRRGLSRGCFGLKLDRIGSNSGLGC [CNP27(K4,5,9R,
M22N)];

(SEQ ID NO: 185)
PDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMS
GLGC (Pro-wtCNP53);

(SEQ ID NO: 145)
PGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC
(Pro-Gly-wtCNP37);

(SEQ ID NO: 186)
PQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC
(Pro-wtCNP37);

(SEQ ID NO: 187)
PNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (wtCNP34);

(SEQ ID NO: 188)
P-GHKSEVAHRFK-GANKKGLSKGCFGLKLDRIGSMSGLGC
(Pro-HSA-wtCNP27);

(SEQ ID NO: 189)
PGANRRGLSRGCFGLKLDRIGSMSGLGC [Pro-CNP27(K4,5,9R)];

-continued

(SEQ ID NO: 190)
MDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMS
GLGC (Met-wtCNP53);

(SEQ ID NO: 191)
MGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC
(Met-Gly-wtCNP37);

(SEQ ID NO: 192)
MQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC
(Met-wtCNP37);

(SEQ ID NO: 193)
M-GHKSEVAHRFK-GANKKGLSKGCFGLKLDRIGSMSGLGC
(Met-HSA-wtCNP27);

(SEQ ID NO: 194)
MGANRRGLSRGCFGLKLDRIGSMSGLGC [Met-CNP27(K4,5,9R)].

Other CNP variants, including truncated CNP peptides ranging from hCNP-17 to hCNP-53 and having wild-type sequences or amino acid addition(s), deletion(s) and/or substitution(s), can also be produced by the fusion protein process described herein, so long as the intended site of chemical or proteolytic cleavage of the fusion protein is not present within the amino acid sequence of the target CNP variant itself. As a non-limiting example, the fusion protein process described herein can be employed to produce truncated wtCNP34 using formic acid cleavage.

In additional embodiments, for any of the CNP peptides and CNP variants described herein that have asparagine (Asn/N) residue(s) and/or glutamine (Gln/Q) residue(s), whether they have a wild-type sequence or a non-natural amino acid sequence, any Asn residue(s) and/or any Gln residue(s) can independently be substituted with any other natural or unnatural amino acids, including conservative substitutions such as Asn to Gln. Such substitution(s) are designed in part to minimize or avoid any potential deamidation of asparagine and/or glutamine. Non-limiting examples of CNP peptides and variants in which any Asn residue(s) and/or any Gln residue(s) can independently be substituted with any other natural or unnatural amino acids, including conservative substitutions such as Asn to Gln, include wtCNP34, wtCNP37, Gly-wtCNP37, Pro-wtCNP37, Pro-Gly-wtCNP37, GHKSEVAHRFK-wtCNP27 (SEQ ID NO: 144), Pro-GHKSEVAHRFK-wtCNP27 (SEQ ID NO: 188), PEO12-GANRR-CNP22(K4R) (SEQ ID NO: 36), and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36). In certain embodiments, an asparagine residue of the CNP peptides and CNP variants described herein is not substituted with glutamine, aspartic acid or glutamic acid. In certain embodiments, a glutamine residue of the CNP peptides and CNP variants described herein is not substituted with asparagine, aspartic acid or glutamic acid. As a non-limiting example, asparagine residues 7 and/or 15 of Pro-Gly-wtCNP37 (PGQEHP NARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC) (SEQ ID NO: 145) can independently be substituted with any other natural or unnatural amino acids, including glutamine, to avoid any potential deamidation of the asparagine residue(s) to aspartic acid or isoaspartic acid. In certain embodiments, asparagine residues 7 and/or 15 of Pro-Gly-wtCNP37 are not substituted with glutamine, aspartic acid or glutamic acid.

It is understood, however, that the present disclosure encompasses CNP variants in which any one or more, up to all, residues susceptible to deamidation or a deamidation-like reaction (e.g., isomerization) may be converted to other residue(s) via deamidation or a deamidation-like reaction to any

extent, up to 100% conversion per converted residue. In certain embodiments, the disclosure encompasses CNP variants in which:

(1) any one or more, up to all, asparagine (Asn/N) residues may be converted to aspartic acid or aspartate, and/or to isoaspartic acid or isoaspartate, via deamidation up to about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% conversion per converted residue; or

(2) any one or more, up to all, glutamine (Gln/Q) residues may be converted to glutamic acid or glutamate, and/or to isoglutamic acid or isoglutamate, via deamidation up to about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% conversion per converted residue; or

(3) any one or more, up to all, aspartic acid or aspartate (Asp/D) residues may be converted to isoaspartic acid or isoaspartate via a deamidation-like reaction (also called isomerization) up to about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% conversion per converted residue; or

(4) any one or more, up to all, glutamic acid or glutamate (Glu/E) residues may be converted to isoglutamic acid or isoglutamate via a deamidation-like reaction (also called isomerization) up to about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% conversion per converted residue; or

(5) a combination of the above.

As a non-limiting example, the disclosure encompasses CNP variants in which any one or more, up to all, asparagine, glutamine, aspartic acid, and/or glutamic acid residues of Pro-Gly-wtCNP37 [PGQEHPNARKYKGA NKKGLSKGCFGLKLDRIGSMSGLGC] (SEQ ID NO: 145) may be converted to (1) aspartic acid/aspartate and/or isoaspartic acid/isoaspartate, (2) glutamic acid/glutamate and/or isoglutamic acid/isoglutamate, (3) isoaspartic acid/isoaspartate, and/or (4) isoglutamic acid/isoglutamate, respectively, via deamidation or a deamidation-like reaction up to about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% conversion per converted residue, as described above.

As a further example, the disclosure encompasses CNP variants in which any one or more, up to all, asparagine and/or aspartic acid residues of Pro-Gly-wtCNP37 [PGQEHP NARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC] (SEQ ID NO: 145) may be converted to (1) aspartic acid/aspartate and/or isoaspartic acid/isoaspartate, and/or (2) isoaspartic acid/isoaspartate, respectively, via deamidation or a deamidation-like reaction up to about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% conversion per converted residue.

As another example, the present disclosure encompasses CNP variants in which any one or more, up to all, asparagine, glutamine, aspartic acid, and/or glutamic acid residues of Gly-wtCNP37 [GQEHPNARKYKGA NKKGLSKGCFGLKLDRIGSMSGLGC (SEQ ID NO: 75)] may be converted to (1) aspartic acid/aspartate and/or isoaspartic acid/isoaspartate, (2) glutamic acid/glutamate and/or isoglutamic acid/isoglutamate, (3) isoaspartic acid/isoaspartate, and/or (4) isoglutamic acid/isoglutamate, respectively, via deamidation or a deamidation-like reaction up to about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% conversion per converted residue.

As yet another example, the disclosure encompasses CNP variants in which any one or more, up to all, asparagine, glutamine, aspartic acid, and/or glutamic acid residues of wtCNP37 [QEHPNARKYKGANKKGLSKGCFGLKL DRIGSMSGLGC (SEQ ID NO: 60)] may be converted to (1) aspartic acid/aspartate and/or isoaspartic acid/isoaspartate, (2) glutamic acid/glutamate and/or isoglutamic acid/isoglutamate, (3) isoaspartic acid/isoaspartate, and/or (4) isoglutamic acid/isoglutamate, respectively, via deamidation or a deamidation-like reaction up to about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% conversion per converted residue.

As a further example, the present disclosure encompasses CNP variants in which any one or more, up to all, asparagine, aspartic acid, and/or glutamic acid residues of an HSA-wtCNP27 chimera, GHKSEVAHRFKGA NKKGLSKGCFGLKLDRIGSMSGLGC (SEQ ID NO: 144), may be converted to (1) aspartic acid/aspartate and/or isoaspartic acid/isoaspartate, (2) isoaspartic acid/isoaspartate, and/or (3) isoglutamic acid/isoglutamate, respectively, via deamidation or a deamidation-like reaction up to about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% conversion per converted residue.

As a still further example, the disclosure encompasses CNP variants in which any one or more, up to all, asparagine, aspartic acid, and/or glutamic acid residues of a Pro-HSA-wtCNP27 chimera, PGHKSEVAHRFKGA NKKGLSKGCFGLKLDRIGSMSGLGC (SEQ ID NO: 188), may be converted to (1) aspartic acid/aspartate and/or isoaspartic acid/isoaspartate, (2) isoaspartic acid/isoaspartate, and/or (3) isoglutamic acid/isoglutamate, respectively, via deamidation or a deamidation-like reaction up to about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% conversion per converted residue.

In addition, the present disclosure encompasses CNP variants in which any one or more, up to all, methionine (Met/M) residues may be oxidized to any chemically feasible oxidized form (e.g., sulfoxide and/or sulfone) up to about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% transformation per oxidized residue.

In another embodiment, the CNP variants comprise CNP22 or variants thereof conjugated at the N-terminus and/or C-terminus to moiet(ies) that facilitate translocation of the variants across a cell membrane or cell barrier. In one embodiment, the CNP variants are conjugated at the N-terminus and/or C-terminus to peptide sequence(s) that facilitate transport of the variants across a cell membrane or cell barrier, including via active peptide transporters.

In a further embodiment, the N-terminus and/or C-terminus of CNP22 or a variant thereof are conjugated to chemical moieties such as, e.g., natural and/or synthetic polymers, to increase the total mass of the modified CNP peptide to the ranges described generally herein, e.g., a range from about 2.6 or 2.8 kDa to about 6 or 7 kDa. In one embodiment, the chemical moieties are biocompatible hydrophilic or water-soluble natural (e.g., peptides, carbohydrates) or synthetic (e.g., PEG (or PEO)) polymers.

In a particular embodiment, the N-terminus and/or C-terminus of CNP22 or a variant thereof are conjugated to PEG (or PEO) polymers to result in a total mass characterized by the ranges described generally herein, e.g., from about 2.6 or 2.8 kDa to about 6 or 7 kDa. Pegylation of CNP22 or a variant thereof is designed, inter alia, to reduce immunogenicity and improve half-life by reducing renal clearance and increasing protease resistance. A PEG moiety can be attached to the N- and/or C-terminus of CNP22 or any variant described herein, including but not limited to CNP-17 (the Cys6-Cys22 cyclized portion of CNP22), CNP37, and variants of CNP17, CNP22 or CNP37 having N- and/or C-terminal amino acid extension(s), amino acid substitution(s) and/or amino acid deletion(s). In an embodiment, the Lys4 and/or Lys10 residues of CNP17, CNP22 or CNP37, or variants thereof, are substituted with a natural or unnatural amino acid (e.g., Arg,

Gly, Ser, Gln, Glu or Cit) or peptidomimetic that does not contain a reactive primary amine on a side chain, to preclude any potential PEGylation of these lysine residues. In one embodiment, the Lys4 and/or Lys10 residues of the CNP peptides are substituted with Arg. In another embodiment, the Lys10 residue is not substituted with Arg.

In a further embodiment, CNP variants (including CNP22 and variants thereof) having a PEG (or PEO) moiety and an amino acid extension at the N-terminus contain arginine at the position immediately preceding the position corresponding to Gly1 of CNP22. Such PEGylated CNP variants are designed for increased resistance to NEP degradation, reduced binding to serum albumin, and enhanced CNP functional activity (e.g., activation of cGMP signaling). Non-limiting examples of PEGylated CNP variants include PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36), PEO12-GANRR-CNP22(K4R) (SEQ ID NO: 36), PEO24-GANRR-CNP22(SEQ ID NO: 36), PEO12-GANRR-CNP22(SEQ ID NO: 36), PEO24-GANPR-CNP22(K4R) (SEQ ID NO: 37), PEO12-GANPR-CNP22(K4R) (SEQ ID NO: 37), PEO24-GANPR-CNP22(SEQ ID NO: 37), PEO12-GANPR-CNP22 (SEQ ID NO: 37), PEO24-GANQQ-CNP22(SEQ ID NO: 64), PEO12-GANQQ-CNP22(SEQ ID NO: 64), PEO24-ER-CNP22(K4R) (SEQ ID NO: 39), PEO12-ER-CNP22(K4R) (SEQ ID NO: 39), PEO24-ER-CNP22(SEQ ID NO: 39), PEO12-ER-CNP22(SEQ ID NO: 39), PEO24-R-CNP22 (K4R) (SEQ ID NO: 41), PEO12-R-CNP22(K4R) (SEQ ID NO: 41), PEO24-R-CNP22(SEQ ID NO: 41), and PEO12-R-CNP22(SEQ ID NO: 41), wherein PEO24 is a monodispersed 1.2 kDa PEG polymer and PEO12 is a monodispersed 0.6 kDa PEG polymer. In one embodiment, the PEG (or PEO) polymer is conjugated to the N-terminus of the CNP variants.

The disclosure contemplates use of hydrophilic or water soluble polymers (e.g., PEG) that can vary in type (e.g., homopolymer or copolymer; random, alternating or block copolymer; linear or branched; monodispersed or polydispersed), linkage (e.g., hydrolysable or stable linkage such as, e.g., amide, imine, aminal, alkylene, or ester bond), conjugation site (e.g., at the N-terminus and/or C-terminus, preferably not at any of the residues in the cyclized region of CNP (corresponding to residues 6-22 of CNP22)), and length (e.g., from about 0.2, 0.4 or 0.6 kDa to about 2, 3, 4 or 5 kDa). The hydrophilic or water-soluble polymer can be conjugated to the CNP peptide by means of N-hydroxy succinimide (NHS)- or aldehyde-based chemistry or other chemistry, as is known in the art. Such CNP variants can be generated using, e.g., wtCNP22 (2.2 kDa), CNP17 retaining only the cyclized region (residues 6-22) of wtCNP22, CNP variants having an amino acid extension at the N-terminus and/or C-terminus of CNP22 or CNP17, or variants having amino acid substitutions, additions and/or deletions such as, e.g., GANRR-CNP22(K4R) (SEQ ID NO: 36), GANPR-CNP22(K4R) (SEQ ID NO: 37), R-CNP22(SEQ ID NO: 40), R-CNP22 (K4R) (SEQ ID NO: 41), ER-CNP22(SEQ ID NO: 38) and ER-CNP22(K4R) (SEQ ID NO: 39). In an embodiment, the PEG-CNP variants having a total mass characterized by the ranges described generally herein, e.g., from about 2.6 or 2.8 kDa to about 6 or 7 kDa, contain a monodispersed, linear PEG (or PEO) moiety conjugated at the N-terminus and/or C-terminus via NHS- or aldehyde-based chemistry, or a two-arm or three-arm branched PEG moiety conjugated at the N-terminus and/or C-terminus via NHS-based chemistry. The disclosure also encompasses negatively charged PEG-CNP variants designed for reduced renal clearance, including but not limited to carboxylated, sulfated and phosphorylated compounds (Caliceti, Adv. Drug Deliv. Rev., 55: 1261-77 (2003); Perlman, J. Clin. Endo. Metab., 88: 3227-35 (2003); Pitkin, Antimicrob. Ag. Chemo., 29: 440-444 (1986); Vehaskari, Kidney Int'l, 22: 127-135 (1982)). In one embodiment, the PEG (or PEO) moiety contains carboxyl group(s), sulfate group(s), and/or phosphate group(s).

In another embodiment, the PEG (or PEO) moieties conjugated to the N-terminus, C-terminus and/or internal site(s) of CNP variants described herein contain one or more functional groups that are positively charged under physiological conditions. Such PEG moieties are designed, inter alia, to improve distribution of such PEGylated CNP variants to cartilage tissues. In one embodiment, such PEG moieties contain one or more primary, secondary or tertiary amino groups, quaternary ammonium groups, and/or other amine-containing (e.g., urea) groups.

In an embodiment, the disclosure encompasses CNP22 or variants thereof conjugated via NHS- or aldehyde-based chemistry to PEG (or PEO) of the formula $(CH_2CH_2O)_n$, wherein n is an integer from about 6 to about 100, and the PEG polymer is from about 0.3 kDa to about 5 kDa. In another embodiment, n is an integer from about 12 to about 50, and the PEG polymer is from about 0.6 kDa to about 2.5 kDa. In yet another embodiment, n is from about 12 to about 24, and the PEG polymer is from about 0.6 kDa to about 1.2 kDa. In still another embodiment, the terminal hydroxyl group of the PEG polymer is capped with a non-reactive group. In a particular embodiment, the end-capping group is an alkyl group, e.g., a lower alkyl group such as methyl.

In a further embodiment, the disclosure provides CNP variants having one or more peptide bonds or peptide bond isosteres that have reduced susceptibility to cleavage by peptidases including neutral endopeptidase (NEP). NEP is a membrane-bound zinc-dependent endopeptidase that cleaves a substrate peptide bond at the amino end of large hydrophobic residues. Thus, modification of a peptide bond at a cleavage site for NEP to an unnatural peptide or non-peptide bond may preclude or decrease the efficiency of NEP cleavage.

For ANP and CNP, NEP cleavage is reported to occur first at the Cys6-Phe7 bond within the cyclized region, then elsewhere throughout the remainder of the structures. For BNP, cleavage is reported to occur first at the peptide N-terminus, then within the cyclic structure. Although the primary NEP cleavage site on CNP is reported to be the Cys6-Phe7 bond, when wtCNP22 was exposed to NEP digestion for 2.5 minutes in vitro, all possible sites were unexpectedly hydrolyzed, with the Cys6-Phe7 and Gly8-Leu9 peptide bonds being slightly most labile, as described in Example 2.

Substrate specificity of NEP is primarily determined by two substrate-binding subsites, S1' and S2' (Oefner et al., J. Mol. Biol. 296:341-349 (2000)). The S1' site accepts a large hydrophobic P1' residue of which the N-terminal peptide bond is subjected to hydrolysis (e.g., Phe, Leu, Be and Met). The S2' site generally prefers a smaller residue, termed P2' (e.g., Gly or Ser). In the case of CNP, Phe7 is reported to be the preferred P1' residue for the NEP S1' site, while Gly8 is the preferred P2' residue for the S2' site. Because these two subsites can together accommodate only a certain total side chain size, any increase in the total size of the P1'-P2' residues of CNP can potentially disrupt NEP binding. For example, addition of a chloride atom at the 3-position of the P1' Phe7 aromatic ring (i.e., 3-Cl-Phe7) can potentially modify (e.g., destabilize) interactions between CNP and the NEP cleavage sites, for example at the S1' subsite. Addition of a tertiary butyl group to the smaller P2' residue Gly8 (i.e., tBu-Gly8) can potentially disrupt the interaction between CNP and the S2' subsite.

Accordingly, in one embodiment, CNP variants of the disclosure include CNP having an increase in the size of the

P1'-P2' residues, such as Phe7-Gly8, to interfere with substrate recognition at the active site, thereby reducing susceptibility to NEP cleavage. Natural amino acids, unnatural amino acids and/or peptidomimetic moieties are substituted for one or more large P1' hydrophobic residues, including but not limited to Phe7, Leu9, Leu11, Ile14, Met17 and Leu20, and/or for one or more smaller P2' residues, including but not limited to Cys6, Gly8, Gly15, Ser16 and Gly19.

The disclosure encompasses CNP variants comprising at least one modified amino acid and/or at least one modified peptide bond, at at least one residue involved in substrate recognition and/or cleavage by NEP, wherein the modified amino acids and modified peptide bonds can be natural amino acids, unnatural amino acids, peptidomimetics and/or peptide bond isosteres. In one embodiment, the NEP cleavage site on CNP between Cys6 and Phe7 is modified. In a related embodiment, the peptide bond (—C(═O)—NH—) between Cys6 and Phe7 is replaced with one of the following peptide bond isosteres:

—CH_2—NH—,

—C(═O)—N(R)—, where the amide group is alkylated with any of the following R groups: methyl, ethyl, n-propyl, isopropyl, cyclopropyl, n-butyl, isobutyl, sec-butyl or tert-butyl,

—C(═O)—NH—CH_2—,

—CH_2—S—,

—CH_2—$S(O)_n$—, where n is 1 or 2,

—CH_2—CH_2—,

—CH═CH—,

—C(═O)—CH_2—,

—CH(CN)—NH—,

—CH(OH)—CH_2—,

—O—C(═O)—NH—, or

—NHC(═O)NH—.

In another embodiment, the CNP variants are represented by the formula:

(x)-Gly_1-Leu_2-Ser_3-Lys_4-Gly_5-$(b)_6$-$(c)_7$-$(d)_8$-Leu_9-Lys_{10}-Leu_{11}-Asp_{12}-Arg_{13}-Ile_{14}-Gly_{15}-Ser_{16}-Met_{17}-Ser_{18}-Gly_{19}-Leu_{20}-Gly_{21}-Cys_{22}-(z) (SEQ ID NO: 90), wherein:

(x) and (z) independently may be absent or may be selected from the group consisting of synthetic bone-targeting compounds such as, e.g., bisphosphonates; amino acid sequences useful in bone or cartilage targeting such as, e.g., polyAsp and polyGlu; amino acid sequences derived from bone-targeting domains of bone proteins such as, e.g., osteopontin, osteocalcin, and sialoprotein (Wang et al., Adv. Drug Delivery Rev., 57: 1049-76 (2005)); polymeric and non-polymeric molecules that reduce renal clearance such as, e.g., negatively charged PEGs; and natural polymers (e.g., those containing amino acids, fatty acids and/or carbohydrates) and synthetic polymers (e.g., PEGs) that increase resistance of the CNP variant to NEP degradation by increasing the total mass of the CNP variant to the ranges described generally herein, e.g., from about 2.6 or 2.8 kDa to about 6 or 7 kDa;

(b) and (c) may be the wild-type Cys6 and Phe7, another natural amino acid or an unnatural amino acid, or may contain a peptide bond isostere as described herein to increase resistance to NEP cleavage; and

(d) may be the wild-type Gly8, or may be a larger natural or unnatural (e.g., t-Bu-Gly) amino acid or peptidomimetic to reduce binding to NEP.

In one embodiment, such CNP variants contain at least one modified amino acid at (b), (c) and/or (d).

Other peptide bonds within CNP may be cleaved even if CNP22 or a variant thereof has an NEP-resistant peptide bond or peptide bond isostere at Cys6-Phe7, including the Gly8-Leu9, Lys10-Leu11, Arg13-Ile14, Ser16-Met17, and Gly19-Leu20 bonds. Therefore, the disclosure encompasses CNP variants having peptide bond isostere(s) at one or more other NEP cleavages sites in addition to the Cys6-Phe7 bond, wherein the peptide bond isosteres include those described herein.

In another embodiment, the disclosure encompasses CNP variants having a cysteine analog at Cys6 and/or Cys22, including but not limited to homocysteine, penicillamine, 2-mercaptopropionic acid, and 3-mercaptopropionic acid. In an embodiment, such CNP variants have a cyclic domain formed by a disulfide bond between the wild-type Cys6 or analog and Cys22 or analog.

In yet another embodiment, one or more residues of CNP22 or a variant thereof, up to all the residues, are substituted with a D-amino acid. Substitution of an L-amino acid with a D-amino acid essentially moves the side chain about 120 degrees from its original position, thereby potentially disrupting the binding of the CNP peptide to NEP. In a specific embodiment, L-Phe at Phe7 is substituted with its D-enantiomer, D-Phe.

In still another embodiment, a beta amino acid such as, e.g., 3-amino-2-phenylpropionic acid (or 2-phenyl-beta-alanine), replaces the wild-type alpha-amino acid Phe7. Use of a beta-amino acid effectively increases the peptide backbone length by one methylene unit. Protease resistance can result from the change in substrate conformation or the increased distance between amino acid side chains.

Non-limiting examples of variants of CNP22 having an unnatural alpha-amino acid, a beta-amino acid or a peptide bond isostere include:

(SEQ ID NO: 56)
GLSKGC(CH_2NH)FGLKLDRIGSMSGLGC (Analog A),

(SEQ ID NO: 57)
GLSKGC-(N-Me-Phe)-GLKLDRIGSMSGLGC (Analog B),

(SEQ ID NO: 136)
GLSKGC-(D-Phe)-GLKLDRIGSMSGLGC (Analog E),

(SEQ ID NO: 58)
GLSKGCF-(tBu-Gly)-LKLDRIGSMSGLGC (Analog F),

(SEQ ID NO: 137)
GLSKGC-(3-Cl-Phe)-GLKLDRIGSMSGLGC (Analog G), and

(SEQ ID NO: 59)
GLSKGC-[NHCH_2CH(Ph)CO]-GLKLDRIGSMSGLGC (Analog H, formed using 3-amino-2-phenylpropionic acid).

In a further embodiment, the CNP variants have a total mass characterized by the ranges described generally herein, e.g., from about 2.6 or 2.8 kDa to about 6 or 7 kDa, designed for increased resistance to NEP degradation, and are represented by the formula:

(x)-Gly_1-Leu_2-Ser_3-$(a)_4$-Gly_5-$(b)_6$-$(c)_7$-$(d)_8$-$(e)_9$-$(f)_{10}$-$(g)_{11}$-Asp_{12}-Arg_{13}-$(h)_{14}$-Gly_{15}-Ser_{16}-$(i)_{17}$-Ser_{18}-Gly_{19}-$(j)_{20}$-Gly_{21}-Cys_{22}-(z) (SEQ ID NO: 46), wherein:

(x) and (z) independently may be absent or may be selected from the group consisting of synthetic bone-targeting compounds such as, e.g., bisphosphonates; amino acid sequences useful in bone or cartilage targeting such as, e.g., polyAsp and polyGlu; amino acid sequences derived from bone-targeting domains of bone proteins such as, e.g., osteopontin, osteocalcin, and sialoprotein; polymeric and non-polymeric moieties that reduce renal clearance such as, e.g., negatively charged PEGs; polymers containing, e.g., amino acids, hydrophobic acids, and/or carbohydrates; and synthetic hydrophilic polymers such as, e.g., PEGs;

(a) may be the wild-type Lys at that position or may be replaced with a conservative amino acid substitution or a natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amine on a side chain, including but not limited to Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu, wherein in one embodiment (a) is Arg;

(b) is selected from the group consisting of Cys and peptide-bond isosteres between Cys6 and Phe7 such as, e.g., Cys-CH_2—NH;

(c) is selected from the group consisting of L-Phe; D-Phe; 3-amino-2-phenylpropionic acid; N-alkylated derivatives of Phe, wherein the N-alkyl group is methyl, ethyl, n-propyl, isopropyl, cyclopropyl, n-butyl, isobutyl, sec-butyl or tert-butyl; and Phe analogs, wherein one or more ortho-, meta-, and/or para-positions of the benzene ring of the Phe analog are substituted with one or more substituents selected from the group consisting of halogen, hydroxyl, cyano, straight or branched C_{1-6} alkyl, straight or branched C_{1-6} alkoxy, straight or branched halo-C_{1-6} alkyl, C_{3-10} cycloalkyl, heterocyclyl, C_{6-14} aryl and heteroaryl (examples include, but are not limited to, tyrosine, 3-chlorophenylalanine, 2,3-chloro-phenylalanine, 3-chloro-5-fluoro-phenylalanine, 2-chloro-6-fluoro-3-methyl-phenylalanine), or wherein the benzene ring of the Phe analog can be replaced with another aryl group (non-limiting examples include 1- and 2-naphthylalanine) or with a heteroaryl group (non-limiting examples include pyridylalanine, thienylalanine and furylalanine);

(d) is selected from the group consisting of Gly, tert-butyl-Gly (tBu-Gly), Thr, Ser, Val and Asn;

(e) is selected from the group consisting of Leu, Ser, Thr and peptide-bond isosteres such as, e.g., N-Me-Leu;

(f) may be the wild type Lys at that position or may be replaced with a conservative amino acid substitution or a natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amine on a side chain, including but not limited to Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu, wherein in one embodiment (f) is not Arg;

(g) is selected from the group consisting of Leu and peptide-bond isosteres such as, e.g., N-Me-Leu;

(h) is selected from the group consisting of Ile, tBu-Gly, and peptide-bond isosteres such as, e.g., N-Me-Ile;

(i) is selected from the group consisting of Met, Val, Asn, beta-Cl-Ala, 2-aminobutyric acid (Abu) and 2-amino-isobutyric acid (Aib); and

(j) is selected from the group consisting of Leu, norleucine (Nle), homoleucine (Hleu), Val, tert-butyl-Ala (tBu-Ala), Ser, Thr, Arg, and peptide-bond isosteres such as, e.g., N-Me-Leu.

In another embodiment, the CNP variants have a total mass characterized by the ranges described generally herein, e.g., from about 2.6 or 2.8 kDa to about 6 or 7 kDa, designed for increased resistance to NEP cleavage, and are represented by the formula:

(x)-Gly_1-Leu_2-Ser_3-$(a)_4$-Gly_5-$(b)_6$-$(c)_7$-$(d)_8$-$(e)_9$-$(f)_{10}$-$(g)_{11}$-Asp_{12}-Arg_{13}-$(h)_{14}$-$(i)_{15}$-Ser_{16}-$(j)_{17}$-Ser_{18}-Gly_{19}-$(k)_{20}$-Gly_{21}-Cys_{22}-(z) (SEQ ID NO:143), wherein:

(x) and (z) independently may be absent or may be selected from the group consisting of synthetic bone-targeting compounds such as, e.g., bisphosphonates; amino acid sequences useful in bone or cartilage targeting such as, e.g., polyAsp and polyGlu; amino acid sequences derived from bone-targeting domains of bone proteins and derivatives thereof, such as, e.g., fusion proteins or peptide sequences of osteopontin, osteocalcin, sialoprotein, etc.; moieties that reduce renal clearance, including but not limited to hydrophilic or water-soluble polymers such as, e.g., charged PEG molecules; and moieities comprising, e.g., PEGs, amino acids, carbohydrates, and/or hydrophobic acids;

(a) may be the wild type Lys at that position or may be replaced with a conservative amino acid substitution or a natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amine on a side chain, including but not limited to Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu, wherein in one embodiment (a) is Arg;

(b) is selected from the group consisting of Cys and peptide bond isosteres between Cys6 and Phe7 such as, e.g., Cys-CH_2—NH;

(c) is selected from the group consisting of L-Phe; D-Phe; 3-amino-2-phenylpropionic acid; N-alkylated derivatives of Phe, wherein the N-alkyl group is methyl, ethyl, n-propyl, isopropyl, cyclopropyl, n-butyl, isobutyl, sec-butyl or tert-butyl; and Phe analogs, wherein one or more ortho-, meta-, and/or para-positions of the henzene ring of the Phe analog are substituted with one or more substituents selected from the group consisting of halogen, hydroxyl, cyano, straight or branched C_{1-6} alkyl, straight or branched C_{1-6} alkoxy, straight or branched halo-C_{1-6} alkyl, C_{3-10} cycloalkyl, C_{6-14} aryl, heterocyclyl and heteroaryl (examples include, but are not limited to, tyrosine, 3-chlorophenylalanine, 2,3-chloro-phenylalanine. 3-chloro-5-fluoro-phenylalanine, 2-chloro-6-fluoro-3-methyl-phenylalanine), or wherein the benzene ring of the Phe analog can be replaced with another aryl group (non-limiting examples include 1- and 2-naphthylalanine) or with a heteroaryl group (non-limiting examples include pyridylalanine, thienylalanine and furylalanine);

(d) is selected from the group consisting of Gly, tert-butyl-Gly, Thr, Ser, Val and Asn;

(e) is selected from the group consisting of Leu, Ser, Thr, and peptide bond isosteres such as, e.g., N-Me-Leu;

(f) is selected from the group consisting of Lys, Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln and Ser;

(g) is selected from the group consisting of Leu, Asn, and peptide bond isosteres such as, e.g., N-Me-Leu;

(h) is selected from the group consisting of Ile, tert-butyl-Gly (tBu-Gly), Asn, and peptide bond isosteres such as, e.g., N-Me-Ile;

(i) is selected from the group consisting of Gly, Arg, Ser and Asn;

(j) is selected from the group consisting of Met, Val, Asn, beta-Cl-Ala, 2-aminobutyric acid (Abu) and 2-amino-isobutyric acid (Aib); and

(k) is selected from the group consisting of Leu, norleucine (Nle), homoleucine (Hleu), Val, tert-butyl-Ala (tBu-Ala), Arg, Thr, Ser, and peptide bond isosteres such as, e.g., N-Me-Leu.

To improve the delivery of the CNP variants to the target sites of bone-related disorders (e.g., skeletal dysplasias), the CNP variants can be attached (e.g., at the N-terminus and/or C-terminus) to bone- or cartilage-targeting moieties. Non-limiting examples of bone- or cartilage-targeting moieties include bisphosphonates; hydroxyapatite; glucosamine; collagen (e.g., collagen type X); polyAsp; polyGlu; and amino acid sequences derived from bone-targeting domains of bone proteins such as, e.g., osteocrin, osteopontin, osteocalcin, and sialoprotein.

In addition to being less susceptible to NEP cleavage, the CNP variants potentially have reduced affinity to the NPR-C clearance receptor, while retaining CNP functionality. Besides NEP-mediated degradation, the half-life of CNP22 is influenced by the clearance receptor, NPR-C, which shares 58% sequence homology with the extracellular peptide-binding domain of NPR-B. CNP22 binds tightly to not only NPR-B (7-30 pM affinity), but also NPR-C (11-140 pM)

(Bennett, B. D. et al., J. Biol. Chem., 266: 23060-67 (1991); Koller K. J. & Goeddel, D. V., Circulation, 86: 1081-88 (1992); Suga, S. et al., Endocrinology, 130: 229-39 (1992)). Even though the NPR-B crystal structure has yet to be reported, sequence homology as well as similarities between the NPR-C and NPR-A crystal structures (He, X.-L. et al., Science, 293(5535): 1657-62 (2001); Ogawa, H. et al., J. Biol. Chem., 279(27): 28625-31 (2004); He, X.-L., J. Mol. Biol., 361(4): 698-714 (2006)) suggest that NPR-B likely assumes a similar overall structural fold.

Therefore, an NPR-B homology model was built based on structure-based sequence alignment and crystallographic structures of the following related systems: CNP bound to NPR-C, ANP bound to NPR-A, and ANP bound to NPR-C (He, X.-L. et al., Science, 293(5535): 1657-62 (2001); Ogawa, H. et al., J. Biol. Chem., 279(27): 28625-31 (2004); He, X.-L., J. Mol. Biol., 361(4): 698-714 (2006)). Based on observations that the receptor appears to determine the bound peptide conformation, and that NPR-B most closely resembles NPR-A with respect to primary structure and functional properties, the NPR-B/CNP homology model was built with the NPR-A/ANP crystal structure as a model. Published signaling data of CNP variants (U.S. Pat. No. 5,434,133 and US Patent Application Publication No. 2004/0138134 A1), and of functional ANP variants that no longer bind to NPR-C (Cunningham, EMBO 13(11) 2508-15, 1994) were used to refine and interpret the NPR-B/CNP model.

The present disclosure encompasses CNP variants designed for improved NPR-B selectivity based on a homology-based structural model of the NPR-B/CNP complex. Combining the experimental and computational structure data of natriuretic peptides bound to the various receptors with the published functional data, CNP variants were generated that continue to bind to NPR-B, but can potentially have reduced affinity to the NPR-C clearance receptor. For example, NPR-C has a unique insertion in a loop structure in the peptide-binding site, placing its loop residues closer to such peptide residues as CNP Gly8 (or ANP Gly9), compared to respective loop residues in NPR-A and NPR-B. Earlier studies indicated that the G9T mutation in ANP contributes to reduce affinity to NPR-C, thereby improving NPR-A selectivity (Cunningham, EMBO J., 13(11): 2508-15 (1994)). Accordingly, CNP variants were generated to replace the corresponding Gly8 residue with a larger residue (Ser, Val, Thr or Asn) to disrupt the CNP binding to NPR-C without affecting its binding to NPR-B. Further, one or more mutations were introduced at the C-terminal end of CNP, encompassing Gly15 to Gly21, which is predicted to interact with receptor-specific residues, based on the detailed structural analyses of the receptor/peptide complexes. For example, a G19R mutation in CNP22 does not result in a significant loss of NPR-B signaling activity. This mutation, however, cannot be modeled into the available crystal structure of NPR-C/CNP without altering the conformations of neighboring residues. These observations suggest that the G19R mutation may selectively disrupt the binding of CNP to a particular receptor, such as NPR-C.

In an embodiment, the CNP variants have substitution(s) at one or more Gly sites at positions 1, 5, 8, 15, 19 and 21, to reduce conformational flexibility and thereby increase receptor specificity. Comparative analyses of crystal structures of ANP bound to NPR-C and NPR-A (Ogawa, H. et al., J. Biol. Chem., 279: 28625-31 (2004); He, X.-L., J. Mol. Biol., 361: 698-714 (2006)) indicate that the conformational flexibility of ANP may play an important role in determining the receptor selectivity.

In one embodiment, functional CNP variants with potentially reduced affinity to NPR-C have one or more of the following amino acid substitutions: G1R, G1E, G5R, G5Q, G5S, F7Y, G8T, G8S, G8V, G8N, L9S, L9T, K10Cit, K10Q, K10S, 114N, G15R, G15S, G15N, G15Cit, S16Q, M17V, M17N, G19S, G19R, G19N, L20V, L20R, L20T, L20S, G21S, G21T and G21R. In an embodiment, the CNP variants have multipoint substitutions at positions 1, 5, 7, 8, 9, 10, 14, 15, 16, 17, 19, 20 and/or 21, and may optionally have modifications at any of the other positions in the peptide sequence of the variant.

In a further embodiment, the CNP variants described herein may be conjugated to moieties, up to a total mass characterized by the ranges described generally herein, e.g., from about 2.6 or 2.8 kDa to about 6 or 7 kDa, at the N-terminus, the C-terminus and/or an internal site, to facilitate bone/cartilage targeting, reduce NPR-C and renal clearance, increase resistance to NEP degradation, and/or improve CNP functionality. In one embodiment, the CNP variants are not conjugated to a polymeric moiety at a site within the cyclic region (corresponding to Cys6 to Cys22 of CNP22). Non-limiting examples of polymeric or non-polymeric moieties that can be conjugated to the CNP variants include synthetic bone-targeting compounds such as, e.g., bisphosphonates; bone/cartilage targeting peptide sequences such as, e.g., polyAsp and polyGlu; peptide sequences derived from bone-targeting domains of bone proteins such as, e.g., osteopontin, osteocalcin and sialoprotein; peptide sequences derived from the functional domains of bone morphogenetic proteins such as, e.g., BMP2, BMP3, BMP5, BMP7 and BMP8a; peptide sequences derived from polypeptides of natriuretic origin such as, e.g., NPPC, ANP and BNP; other natural polymeric or non-polymeric moieties such as, e.g., carbohydrates, fatty acids and phospholipids; biocompatible synthetic hydrophilic polymers such as, e.g., PEG (or PEO); hydrophobic polymeric or non-polymeric moieties such as, e.g., heptanoic acid and pentanoic acid; and combinations thereof.

The CNP variants described herein can have substantially similar or better functional activity than CNP22, e.g., with respect to stimulation of cGMP production and signaling. In one embodiment, the CNP variants in vitro or in vivo stimulate the production of at least about 50% of the cGMP level produced under the same concentration of wtCNP22 (e.g., 1 uM). In certain embodiments, the CNP variants retain at least about 50%, 60%, 70%, 80%, 90%, 95% or 100% of the cGMP-stimulation activity of wild-type CNP22 in vitro or in vivo. In another embodiment, the CNP variants have improved cGMP-stimulation activity compared to CNP22. In certain embodiments, the CNP variants in vitro or in vivo stimulate the production of at least about 110%, 120%, 130%, 140%, 150%, 200%, 250%, 300%, 350%, 400%, 450%, 500% or more of the cGMP level produced under the same concentration of wtCNP22 (e.g., 1 uM).

Optionally excluded from the present disclosure are any of the natriuretic (e.g., CNP) peptides, fragments and variants specifically disclosed, and any of the natriuretic (e.g., CNP) peptides, fragments and variants actually produced, in any of the prior publications referenced herein, including but not limited to, U.S. Pat. Nos. 5,434,133, 6,034,231, 6,020,168, 6,743,425, 7,276,481, WO 94/20534, WO 02/047871, WO 2005/098490, WO 2004/047871, EP 0497368, EP 0466174, and Furuya et al., Biochem. Biophys. Res. Comm. 183: 964-969 (1992)). All such documents are incorporated by reference herein in their entirety.

In one embodiment, the present disclosure optionally excludes all known wild-type CNP-53, wild-type CNP-22, wild-type CNP-17, wild-type BNP, and wild-type ANP of

human origin and non-human origin. For example, in an embodiment the disclosure optionally excludes human CNP-17, human CNP-22, chicken CNP-22 (corresponding to hCNP-22(Leu9Val)), trout and eel CNP-22 (corresponding to hCNP-22(Leu2Trp, Ser3Asn, Lys4Arg)), frog CNP22-I (corresponding to hCNP-22(Leu2Tyr, Lys4Arg, Leu9Val, Ser16Ala, Met17Phe)), frog CNP22-II (corresponding to hCNP-22(Leu2Thr, Ser16Ala)), human CNP-53, and pig and rat CNP-53 (corresponding to hCNP-53(Gln17His, Ala28Gly)). In another embodiment, the disclosure optionally excludes fragments of NPPC, proCNP and CNP-53 which are produced by proteolytic cleavage in vivo in humans and non-human animals. In yet another embodiment, optionally excluded from the disclosure are the following truncated fragments of wild-type human CNP-53: CNP-50, CNP-46, CNP-44, CNP-39, CNP-30, CNP-29, CNP-28, CNP-27 and CNP-26.

In a further embodiment, the present disclosure optionally excludes CNP peptides and fragments thereof isolated or sought from the shark species *Triakis scyllia* and *Scyliorhinus canicula* (see, e.g., M. Takano et al., Zool. Sci., 11: 451-454 (1994)), including:

(SEQ ID NO: 204)
RLLKDLSNNPLRFRGRSKKGPSRGCFGVKLDRIGAMSGLGC (CNP-41);

(SEQ ID NO: 205)
LKDLSNNPLRFRGRSKKGPSRGCFGVKLDRIGAMSGLGC (CNP-39);

(SEQ ID NO: 206)
KDLSNNPLRFRGRSKKGPSRGCFGVKLDRIGAMSGLGC (CNP-38);
and

(SEQ ID NO: 207)
GPSRGCFGVKLDRIGAMSGLGC (CNP-22).

In another embodiment, optionally excluded from the disclosure are CNP peptides and fragments thereof isolated or sought from the shark species *Lamna ditropis* (see, e.g., M. Takano et al., Zool. Sci., 11: 451-454 (1994)), including:

(SEQ ID NO: 208)
RLLKDLSNNPLRFKGRSKKGPSRGCFGVKLDRIGAMSGLGC (CNP-41);

(SEQ ID NO: 209)
LKDLSNNPLRFKGRSKKGPSRGCFGVKLDRIGAMSGLGC (CNP-39);

(SEQ ID NO: 210)
KDLSNNPLRFKGRSKKGPSRGCFGVKLDRIGAMSGLGC (CNP-38);

(SEQ ID NO: 211)
FKGRSKKGPSRGCFGVKLDRIGAMSGLGC (CNP-29);
and

(SEQ ID NO: 212)
GPSRGCFGVKLDRIGAMSGLGC (CNP-22).

In still another embodiment, optionally excluded from the disclosure are CNP peptides and fragments thereof isolated from the shark species *Squalus acanthias* (see, e.g., M. Takano et al., Zool. Sci., 11: 451-454 (1994)), including:

(SEQ ID NO: 213)
RLLQDLSNNPLRFKGRSKKGPSRSCFGLKLDRIGAMSGLGC (CNP-41);
and

(SEQ ID NO: 214)
GPSRSCFGLKLDRIGAMSGLGC (CNP-22).

In a further embodiment, the present disclosure optionally excludes CNP peptides isolated from medaka and puffer fish, designated "CNP-1", "CNP-2", "CNP-3" and "CNP-4" in K. Inoue et al., Proc. Nat. Acad. Sci., 100(17): 10079-10084 (2003):

(SEQ ID NO: 215)
GWNRGCFGLKLDRIGSMSGLGC (medaka and puffer fish CNP-1);

(SEQ ID NO: 216)
PMVAGGGCFGMKMDRIGSISGLGC (medaka CNP-2);

(SEQ ID NO: 217)
GRSSMVGGRGCFGMKIDRIGSISGLGC (puffer fish CNP-2);

(SEQ ID NO: 218)
GGMRSCFGVRLERIGSFSGLGC (medaka CNP-3);

(SEQ ID NO: 219)
GGLRSCFGVRLARIGSFSGLGC (puffer fish CNP-3);

(SEQ ID NO: 220)
GGSTSRSGCFGHKMDRIGTISGMGC (medaka CNP-4);
and

(SEQ ID NO: 221)
GGSSRSGCFGHKMDRIGTISGMGC (puffer fish CNP-4).

In a still further embodiment, optionally excluded from the disclosure are CNP-39 isolated from platypus venom and the CNP-22 fragment thereof, designated "ovCNP-39" and "ovCNP-39(18-39)" in G. de Plater et al., Toxicon., 36(6): 847-857 (1998):

(SEQ ID NO: 222)
LLHDHPNPRKYKPANKKGLSKGCFGLKLDRIGSTSGLGC (ovCNP-39);
and

(SEQ ID NO: 223)
GLSKGCFGLKLDRIGSTSGLGC (ovCNP-39(18-39).

In another embodiment, the present disclosure optionally excludes the following peptides as specifically disclosed in US 2007/0197434:

(SEQ ID NO: 224)
Gly-Leu-Ser-Lys-Gly-Cys-Phe-Gly-Leu-Lys-Leu-Asp-Arg-Ile-Gly-Ala-Met-Ser-Gly-Leu-Gly-Cys;

(SEQ ID NO: 225)
Gly-Leu-Ser-Lys-Gly-Cys-Phe-Gly-Leu-Lys-Leu-Asp-Arg-Ile-Gly-Ser-Gln-Ser-Gly-Leu-Gly-Cys;

(SEQ ID NO: 226)
Gly-Leu-Ser-Lys-Gly-Cys-Phe-Gly-Leu-Lys-Leu-Asp-Arg-Ile-Gly-Ser-Ala-Ser-Gly-Leu-Gly-Cys;

(SEQ ID NO: 227)
Ser-Leu-Arg-Arg-Ser-Ser-Cys-Phe-Gly-Leu-Lys-Leu-Asp-Arg-Ile-Gly-Ser-Met-Ser-Gly-Leu-Gly-Cys;

(SEQ ID NO: 228)
Gly-Leu-Ser-Lys-Gly-Cys-Phe-Gly-Leu-Lys-Leu-Asp-Arg-Ile-Gly-Ser-Met-Ser-Gly-Leu-Gly-Cys-Asn-Ser-Phe-Arg-Tyr;
and

(SEQ ID NO: 229)
Cys-Phe-Gly-Leu-Lys-Leu-Asp-Arg-Ile-Gly-Ser-Gln-Ser-Gly-Leu-Gly-Cys-Asn-Ser-Phe-Arg-Tyr.

In yet another embodiment, the disclosure optionally excludes peptides of SEQ ID NO:10, disclosed generically in US 2007/0197434, wherein such peptides are CNP-17 vari-

ants having certain natural amino acid substitution(s) at position(s) 4, 5, 6, 11, 12, 14 and/or 15. In still another embodiment, optionally excluded from the disclosure are peptides corresponding to hCNP-53(Ser47Ala), hCNP-53 (Met48Gln), hCNP-53(Met48Ala), and hCNP-53(C-term.)-Asn-Ser-Phe-Arg-Tyr.

In an embodiment, the present disclosure optionally excludes the peptides of SEQ ID NOs 1-4 and 6-71 as specifically disclosed in U.S. Pat. No. 7,276,481. In another embodiment, the disclosure optionally excludes peptides of SEQ ID NO 5, disclosed generically in U.S. Pat. No. 7,276,481, wherein such peptides are variants of CNP17 having at least one natural amino acid substitution at Leu9, Lys10, Leu11, Ser16, Met17, Gly19, and/or Leu20. In still another embodiment, optionally excluded are CNP17 variants in which CNP17 or variants thereof contain N-Me-Phe7, or N-Me-Phe7 and N-Me-Leu11. In a further embodiment, the disclosure optionally excludes CNP17 variants of SEQ ID NO 5, as disclosed in U.S. Pat. No. 7,276,481, which are fused or conjugated to growth hormone (GH), insulin-like growth factor 1 (IGF-1), or thyroid hormone (TH). In yet another embodiment, optionally excluded are CNP22 variants in which CNP22 is fused to GH, IGF-1 or TH, or attached to GH, IGF-1 or TH via a linker (e.g., a peptide linker). In still another embodiment, optionally excluded are CNP17 variants in which CNP17 or variants thereof are conjugated to biotin or fluorescein at the N-terminus or the C-terminus.

In a further embodiment, the present disclosure optionally excludes the peptides of Compound Nos. 1-27, and SEQ ID NOs 1-17, 22-24, 30, 31 and 40-42 as specifically disclosed in U.S. Pat. No. 5,434,133. In another embodiment, the disclosure optionally excludes peptides of SEQ ID NOs 18-21 and 25-29, disclosed generically in U.S. Pat. No. 5,434,133. In still another embodiment, the disclosure optionally excludes the peptides of SEQ ID NOs 1-4 and 9 as specifically disclosed in WO 94/20534.

In some embodiments, however, the disclosure still encompasses methods of use of the natriuretic (e.g., CNP) peptides, fragments and variants optionally excluded herein, as well as pharmaceutical compositions (including sterile pharmaceutical compositions) comprising such natriuretic (e.g., CNP) peptides, fragments and variants.

C. Synthesis and Purification of CNP Variants

In some embodiments, the CNP variants described herein are produced by recombinant expression, using certain techniques known in the art in certain embodiments. See, e.g., Sambrook, Fritsch & Maniatis, Molecular Cloning: A Laboratory Manual, Second Edition. Cold Spring Harbor Laboratory Press (Cold Spring Harbor, N.Y. (1989)); DNA Cloning: A Practical Approach, Volumes I and II, D. N. Glover, Ed. (1985); and Current Protocols in Molecular Biology, John Wiley & Sons, Inc. (1994).

In certain embodiments, the CNP variants described herein are produced by a recombinant process that comprises culturing in a medium a host cell comprising a first polynucleotide encoding a CNP variant polypeptide linked to a second polynucleotide encoding a cleavable peptide or protein under conditions that result in expression of a fusion polypeptide encoded by the polynucleotides, wherein the fusion polypeptide comprises the CNP variant polypeptide directly linked to the cleavable peptide or protein or indirectly linked thereto via a linker. In some embodiments, the host cell is transformed with an expression vector comprising the polynucleotide encoding the CNP variant polypeptide linked to the polynucleotide encoding the cleavable peptide or protein. In certain embodiments, the fusion polypeptide is expressed as a soluble protein or as an inclusion body. The expressed fusion polypeptide can be isolated from the host cell or culture medium, and the isolated fusion polypeptide can be contacted with a cleaving agent to release the CNP variant.

Host cells used to produce CNP variants can be bacterial, yeast, insect, non-mammalian vertebrate, or mammalian cells. Bacterial cells include without limitation *E. coli* cell lines and strains. Non-limiting examples of *E. coli* cell lines and strains include BL21, BL21(DE3), BL21(DE3)pLysS, BL21(DE3)pGro7, ArcticExpress(DE3), C41 [also called C41(DE3)], C43 [also called C43(DE3)], Origami B(DE3), Origami B(DE3)pLysS, KRX, and Tuner(DE3). In an embodiment, CNP variants and CNP fusion proteins are produced using BL21(DE3) cells. Mammalian cells include, but are not limited to, hamster, monkey, chimpanzee, dog, cat, bovine, porcine, mouse, rat, rabbit, sheep and human cells. The host cells can be immortalized cells (a cell line) or non-immortalized (primary or secondary) cells and can be any of a wide variety of cell types, such as, but not limited to, fibroblasts, keratinocytes, epithelial cells (e.g., mammary epithelial cells, intestinal epithelial cells), ovary cells (e.g., Chinese hamster ovary or CHO cells), endothelial cells, glial cells, neural cells, formed elements of the blood (e.g., lymphocytes, bone marrow cells), chondrocytes and other bone-derived cells, and precursors of these somatic cell types. Host cells containing the CNP variant DNA or RNA are cultured under conditions appropriate for growth of the cells, expression of the DNA or RNA and identification/selection of cells expressing the CNP variant.

In some embodiments, the host cells are grown or cultured for a time period at a temperature from about 10° C. to about 40° C., or from about 20° C. to about 40° C., or from about 30° C. to about 40° C. In certain embodiments, the host cells are grown or cultured for a time period at about 20° C., 22° C., 25° C., 28° C., 30° C., 35° C. or 37° C. In certain embodiments, the host cells are grown or cultured for a time period at about 35° C. or 37° C.

Recombinant polynucleotides encoding CNP variant polypeptides (including CNP fusion proteins) are expressed in an expression vector comprising a recombinant polynucleotide comprising expression control sequences operatively linked to a nucleotide sequence to be expressed. An expression vector comprises sufficient cis-acting elements for expression; other elements for expression can be supplied by the host cell or in vitro expression system. Expression vectors include all those known in the art, including without limitation cosmids, plasmids (e.g., naked or contained in liposomes) and viruses that incorporate the recombinant polynucleotide. The expression vector is inserted into an appropriate host cell, via transformation or transfection, for expression of the polynucleotide encoding the polypeptide (see, e.g., Sambrook et al. (supra)).

Non-limiting examples of expression vectors contemplated for production of CNP variants, including cleavable CNP fusion proteins, include pJexpress, pJexpress401, pJexpress404, pET-15b, pET-21a, pET-22b, pET-31b, pET-32a, pET-41a, pMAL, pMAL-c2X, pQE-30, pET-SUMO, and pTYB11. Expression of particular constructs can generate soluble CNP variants (including CNP fusion proteins) or insoluble CNP variants (including CNP fusion proteins) in the form of inclusion bodies.

In some embodiments, expression of the polynucleotide(s) encoding a CNP variant or CNP fusion protein is enhanced using an isopropyl β-D-1-thiogalactopyranoside (IPTG)-inducible vector. In some embodiments, the host cells are grown or cultured for a time period at a temperature from

about 10° C. to about 40° C., or from about 20° C. to about 40° C., or from about 30° C. to about 40° C., in the presence of IPTG. In certain embodiments, the host cells are grown or cultured for a time period at about 20° C., 22° C., 25° C., 28° C., 30° C., 35° C. or 37° C. in the presence of IPTG. In certain embodiments, the host cells are grown or cultured for a time period at about 35° C. or 37° C. in the presence of 1 mM IPTG.

In further embodiments, the host cells are cultured with IPTG at a concentration from about 0.4 mM to about 2 mM, or from about 0.4 mM to about 1.5 mM, or from about 0.4 mM to about 1 mM. In certain embodiments, the IPTG is at a concentration of about 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9 or 2 mM. In an embodiment, the concentration of IPTG is about 1 mM.

In certain embodiments, the CNP variants described herein are recombinantly expressed as fusion proteins comprising a CNP variant polypeptide and a cleavable carrier protein or cleavable tag (e.g., peptide tag), wherein the fusion protein comprises the CNP variant polypeptide directly linked to the cleavable carrier protein or tag or indirectly linked thereto via a linker. Use of a carrier protein or tag facilitates, e.g., detection, isolation and/or purification of the fusion protein. Cleavable carrier proteins and tags include, but are not limited to, histidine (e.g., hexa-His) tags; human transcription factor TAF12 (TAF12), TAF12 fragments, TAF12 histone fold domain, mutants of TAF12 and fragments thereof, TAF12(C/A), TAF12(D/E), TAF12(4D/4E), TAF12(6D/6E), TAF12(10D/10E), TAF12(C/A & D/E), TAF12(C/A & 4D/4E), TAF12(C/A & 6D/6E), TAF12(C/A & 10D/10E); ketosteroid isomerase (KSI); maltose-binding protein (MBP); β-galactosidase (β-Gal); glutathione-S-transferase (GST); thioredoxin (Trx); chitin binding domain (CBD); BMP-2, BMP-2 mutants, BMP-2(C/A); SUMO; and mutants and fragments thereof.

An expression construct can express a fusion protein comprising a CNP variant and a carrier protein or tag. The tag can be an amino acid sequence that confers a useful property to the fusion protein. In one embodiment, the tag is a ligand-binding domain that can be used to purify the fusion protein by applying the fusion protein to separation media containing the ligand. For example, a fusion protein comprising a glutathione-S-transferase (GST) domain can be applied to a chromatographic column containing glutathione-linked separation media. As another example, a fusion protein comprising maltose-binding protein (MBP) as a tag can be applied to separation media containing maltose. As a further example, a fusion protein comprising a polyhistidine tag may be applied to a nickel column, whereby chelation of the polyhistidine tag to the nickel column facilitates purification of the fusion protein. In another embodiment, the tag is a ligand. For example, a fusion protein can comprise glutathione as a tag and be applied to a chromatographic column containing glutathione-S-transferase-linked separation media. Non-limiting examples of carrier proteins and tags for use in fusion proteins include human transcription factor TAF12 (TAF12), ketosteroid isomerase (KSI), maltose-binding protein (MBP), β-galactosidase (β-Gal), glutathione-S-transferase (GST), thioredoxin (Trx), chitin-binding domain (CBD), BMP-2 mutation (BMPM), SUMO, CAT, TrpE, staphylococcal protein A, streptococcal proteins, starch-binding protein, cellulose-binding domain of endoglucanase A, cellulose-binding domain of exoglucanase Cex, biotin-binding domain, recA, Flag, poly(His), poly(Arg), poly(Asp), poly (Gln), poly(Phe), poly(Cys), green fluorescent protein, red fluorescent protein, yellow fluorescent protein, cyan fluorescent protein, biotin, avidin, streptavidin, antibody epitopes, and mutants and fragments thereof.

To generate the target CNP variant, the carrier protein or tag can be cleaved from the fusion protein by means of chemical cleavage, protease cleavage, or protein self-cleavage. Exemplary chemical and proteolytic cleavage agents (cleavage sites in parenthesis) include, but are not limited to, formic acid (Asp-Pro), cyanogen bromide (CNBr) (Met-X), hydroxylamine (Asn-Gly), Factor Xa (IEGR-X) (SEQ ID NO: 230), Enterokinase (DDDDK-X) (SEQ ID NO: 231), ProTEV (EXXYXQ-G) (SEQ ID NO: 232), and SUMO protease. Due to the nature of the particular kinds of chemical cleavage, cleavage using formic acid may generate Pro-CNP, CNBr may generate CNP having Met-to-Asn substitution, and hydroxylamine may generate Gly-CNP. Alternatively, chemical or protease cleavage may be avoided by using particular constructs (e.g., pET-21a-CNP) that express CNP variants not as fusion proteins. Expression of pET-21a-CNP may produce Met-CNP. Or certain fusion proteins (e.g., those containing intein-CBD) can undergo self-cleavage to generate CNP.

In further embodiments, a fusion protein comprises a cleavable peptide linker between a CNP variant and a carrier protein or tag (e.g., peptide tag). In certain embodiments, the cleavable peptide linker is selected from the group consisting of Asp-Pro, Asn-Gly, Met-X, Val-Asp-Asp-Arg (SEQ ID NO: 233), Gly-Ser-Asp-Arg (SEQ ID NO: 234), Ile-Thr-Asp-Arg (SEQ ID NO: 235), Pro-Gly-Asp-Arg (SEQ ID NO: 236), Ile-Glu-Gly-Arg-X (SEQ ID NO: 230), Asp-Asp-Asp-Asp-Lys-X (SEQ ID NO: 231), Glu-X-X-Tyr-X-Gln-Gly (SEQ ID NO: 232), Ala-Phe-Leu-Gly-Pro-Gly-Asp-Arg (SEQ ID NO: 237), and MGSSHHHHHHSSGLVPRGSHTGD-DDDKHMD (pET-15b linker) (SEQ ID NO: 95), where X denotes an amino acid. In some embodiments, the cleavable peptide linker is cleaved by a cleaving agent selected from the group consisting of palladium, cyanogen bromide (CNBr), formic acid, hydroxylamine, clostripain, thrombin, chymotrypsin, trypsin, trypsin-like proteases, carboxypeptidase, enterokinase (enteropeptidase), Kex 2 protease, Omp T protease, Factor Xa protease, subtilisin, proTEV, SUMO protease, V8 protease, HIV protease, rhinovirus protease, furilisin protease, IgA proteases, human Pace protease, collagenase, Nia protease, poliovirus 2Apro protease, poliovirus 3C protease, genenase, furin, elastase, Proteinase K, pepsin, rennin (chymosin), microbial aspartic proteases, papain, calpain, chymopapain, ficin (ficain), bromelain (bromelase), cathespisin B, caspases, thermolysin, Endoprotease Arg-C, Endoprotease Glu-C, Endoprotease Lys-C, kallikrein, and plasmin.

In certain embodiments, the cleavable carrier protein, tag (e.g., peptide tag) or peptide linker is cleaved using formic acid to release the CNP variant from the fusion protein. In some embodiments, the formic acid is at a concentration from about 1% to about 20%, or from about 1% to about 15%, or from about 2% to about 15%, or from about 1% to about 10%, or from about 2% to about 10%, or from about 1% to about 5%, or from about 2% to about 5%. In certain embodiments, the formic acid is at a concentration of about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14% or 15%. In certain embodiments, the formic acid is at a concentration of about 2%, 5% or 10%.

In further embodiments, cleavage of the CNP fusion protein in the presence of formic acid is conducted at a temperature from about 20° C. to about 80° C., or from about 30° C. to about 75° C., or from about 40° C. to about 75° C., or from about 50° C. to about 75° C., or from about 50° C. to about 70° C., or from about 55° C. to about 70° C., or from about 50° C.

to about 60° C. In some embodiments, cleavage in the presence of formic acid is conducted at about 20° C., 22° C., 25° C., 30° C., 35° C., 37° C., 40° C., 42° C., 45° C., 50° C., 55° C., 60° C., 65° C., 70° C., 75° C. or 80° C. In certain embodiments, cleavage in the presence of formic acid is conducted at about 50° C., 55° C., 60° C., 65° C. or 70° C. In certain embodiments, cleavage in the presence of formic acid is conducted at about 55° C. or 70° C.

In additional embodiments, cleavage of the CNP fusion protein in the presence of formic acid is performed for a time period from about 3 hr to about 48 hr, or from about 5 hr to about 48 hr, or from about 5 hr to about 36 hr, or from about 5 hr to about 24 hr, or from about 5 hr to about 18 hr, or from about 20 hr to about 24 hr, or for about 6 hr to about 10 hr. In certain embodiments, cleavage in the presence of formic acid is performed for about 5 hr, 6 hr, 12 hr, 15 hr, 18 hr, 20 hr or 24 hr.

In some embodiments, cleavage of the CNP fusion protein is conducted in the presence of about 2%, 5% or 10% formic acid at about 55° C. for about 20 hr to about 36 hr, or at about 60° C. for about 15 hr to about 24 hr, or at about 65° C. for about 10 hr to about 21 hr, or at about 70° C. for about 6 hr to about 18 hr. In certain embodiments, cleavage of the CNP fusion protein is conducted in the presence of about 2% formic acid at about 55° C. for about 20 hr to about 24 or 36 hr, or at about 60° C. for about 15 hr to about 24 hr, or at about 65° C. for about 10 hr to about 18 hr, or at about 70° C. for about 6 hr to about 10 hr.

The present disclosure provides mild conditions for cleavage of CNP fusion proteins using formic acid to afford high yields of CNP variants. The conditions described herein for fusion protein cleavage using formic acid are also suitable for cleavage of fusion proteins comprising polypeptides or proteins other than CNP, where the fusion proteins contain an Asp-Pro peptide bond.

In further embodiments, soluble CNP fusion proteins or CNP fusion protein inclusion bodies are treated with a buffer and/or a detergent prior to chemical cleavage (e.g., using formic acid) or proteolytic cleavage of the CNP fusion protein. Non-limiting examples of buffers include B-PER II; diluted B-PER II (e.g., 1/20 dilution); B-PER; B-PER phosphate buffer; buffers containing Tris (e.g., 25 mM Tris, pH 7.5); buffers containing Tris and NaCl (e.g., 25 mM Tris, 150 mM NaCl, pH 7.9); and PBS. In an embodiment, the buffer is B-PER II. Non-limiting examples of detergents include octyl-sucrose, Triton X-100, Tween-20, NP-40, and CA-630. The detergent can be in a buffer (e.g., 1% detergent in 25 mM Tris buffer, pH 7.5). In certain embodiments, the detergent is Triton X-100 or CA-630.

It is understood that any of the methods and conditions described above may be used in combination with any of the other methods and conditions described above to generate a CNP variant disclosed herein.

In other embodiments, the CNP variants described herein are synthesized using a peptide synthesizer and purified according to methods known in the art, e.g., according to the methods of Atherton and Sheppard, *Solid Phase Peptide Synthesis: a Practical Approach*, IRL Press (Oxford, England (1989)).

Peptides can be synthesized based on, e.g., the following peptide sequence of CNP: G^1LS(K or R)G$C^6F^7G^8$L(K or R or Nle or 6-OH-Nle)LDRIGSMSGLGC^{22}.

Exemplary CNP variants include but are not limited to:

Analog A (GLSKGC(CH2NH)FGLKLDRIGSMSGLGC) (SEQ ID NO: 56) was made by converting the backbone "—C═O" group of C^6 to a "—CH_2" group;

Analog B (GLSKGC(N—Me—Phe)GLKLDRIGSMSGLGC) (SEQ ID NO: 57) was made by converting the backbone "—NH" group of F^7 to an "—N—CH_3" group;

Analog E (GLSKGC(D-Phe)GLKLDRIGSMSGLGC) (SEQ ID NO: 136) was made using D-Phe at F^7;

Analog F (GLSKGCF(tBu-Gly)LKLDRIGSMSGLGC) (SEQ ID NO: 58) was made using a tertiary-butyl-Gly at G^8;

Analog G (GLSKGC(3-Cl-Phe)GLKLDRIGSMSGLGC) (SEQ ID NO: 137) was made by adding a chloride atom to a meta position of the phenyl ring of F^7 (similar variants can be generated by making ortho, meta and/or para substitutions of the phenyl ring of Phe7 with Cl, F, Br, OH and/or CH_3);
and

Analog H (GLSKGC[$NHCH_2CH(Ph)CO$]GLKLDRIGSMSGLGC) (SEQ ID NO: 59) was made using (±)-3-(amino)-2-phenylpropionic acid at F^7.

Examples of CNP variants having, e.g., amino acid extensions, substitutions with natural or unnatural amino acids or peptide bond isosteres, and/or conjugations to polymers or hydrophobic moieties, include without limitation:

Analog J (SEQ ID NO: 91)
C6—CH2—NH, N—Me-L9, N—Me-L20

Analog K (SEQ ID NO: 92)
N—Me-L9, N—Me-L20

Analog L (SEQ ID NO: 93)
N—Me-L9, N—Me-L11, N—Me-L20

Analog M (SEQ ID NO: 94)
N—Me-L9, N—Me-L11

Analog Z (SEQ ID NO: 95)
K4R, F7Y

Analog AA (SEQ ID NO: 96)
K4R, G8V

Analog AB (SEQ ID NO: 97)
K4R, G8S

Analog AC (SEQ ID NO: 98)
K4R, G8T

Analog AD (SEQ ID NO: 99)
K4R, L9T

Analog AE (SEQ ID NO: 100)
K4R, G15R

Analog AF (SEQ ID NO: 101)
K4R, G15Cit

-continued

Analog AG	(SEQ ID NO: 102)
K4R, M17V	
Analog AH	(SEQ ID NO: 35)
K4R	
Analog AJ	(SEQ ID NO: 103)
K4R, L20V	
Analog AK	(SEQ ID NO: 104)
K4R, L20t-Bu-Ala	
Analog AT	(SEQ ID NO: 105)
G1E, K4E	
Analog AV	(SEQ ID NO: 106)
G1E, K4E - pentanoic acid (attached at the N-terminus)	
Analog AW	(SEQ ID NO: 107)
G1E, K4E - heptanoic acid (attached at the N-terminus)	
Analog AX	(SEQ ID NO: 2)
CNP17 (delta N-term)	
Analog AY	(SEQ ID NO: 36)
GANRR-CNP22(K4R)	
Analog AZ	(SEQ ID NO: 41)
R-CNP22(K4R)	
Analog BB	(SEQ ID NO: 108)
G1E - heptanoic acid (attached at the N-terminus)	
Analog BC	(SEQ ID NO: 109)
G1E - pentanoic acid (attached at the N-terminus)	
Analog BF	(SEQ ID NO: 110)
K4R, K10Cit	
Analog BG	(SEQ ID NO: 111)
K4R, K10Q	
Analog BH	(SEQ ID NO: 112)
K4R, K10R	
Analog BJ	(SEQ ID NO: 113)
K4R, G15N	
Analog BK	(SEQ ID NO: 114)
K4R, G15S	
Analog BL	(SEQ ID NO: 60)
CNP-37	
CNP-53	(SEQ ID NO: 4)

-continued

Analog CA	(SEQ ID NO: 61)
AAWARLLQEHPNA-CNP22	
Analog CB	(SEQ ID NO: 62)
AAWARLLQEHPNAR-CNP22	
Analog CC	(SEQ ID NO: 63)
DLRVDTKSRAAWAR-CNP22	
Analog CD	(SEQ ID NO: 68)
SPKMVQGSG-CNP17-KVLRRH (N- and C-terminal BNP tails)	
Analog CE	(SEQ ID NO: 81)
GERAFKAWAVARLSQ-CNP22 (HSA-CNP22)	
Analog CF	(SEQ ID NO: 79)
GQPREPQVYTLPPS-CNP22	
PEG(24K)-CNP22	
PEG(20K)-CNP22	
PEG(5K)-CNP22	
PEG(2K)-CNP22	
PEG(2K)-CNP17	
	(SEQ ID NO: 36)
PEG(1K)-GANRR-CNP22(K4R)	
PEG(1K)-CNP22	
PEO4-(PEO12)3(branched)-CNP22	
PEO12-CNP22	
	(SEQ ID NO: 36)
PEO12-GANRR-CNP22(K4R)	
	(SEQ ID NO: 36)
PEO24-GANRR-CNP22(K4R); and	
SEQ ID NOs: 1 to 6 and 34 to 144, and variants thereof that comprise up to 1, 2, 3, 4, or 5 further modifications.	

In one embodiment, the CNP variants are cyclized via formation of a disulfide bond between Cys^6 and Cys^{22}. Cys^6 can be a cysteine analog such as, e.g., homocysteine or penicillamine. In a further embodiment, the CNP variants can be cyclized by a covalent bond formed head-to-tail, side chain-to-side chain, side chain-to-head, or side chain-to-tail. In an embodiment, the covalent bond is formed between an amino acid at or toward the N-terminus and an amino acid at or toward the C-terminus of the peptide (referred to as "terminal" amino acids in this context). In another embodiment, the covalent bond is formed between the side chains of the two terminal amino acids. In yet another embodiment, the covalent bond is formed between the side chain of one terminal amino acid and the terminal group of the other terminal amino acid, or between the terminal groups of the two terminal amino acids.

Head-to-tail cyclization of the terminal amine to the terminal carboxyl group can be carried out using a number of methods, e.g., using p-nitrophenyl ester, 2,4,5-trichlorophenyl ester, pentafluorophenyl ester, the azide method, the mixed anhydride method, HATU, a carbodimide (e.g., DIC, EDC or DCC) with a catalyst such as HOBt, HONSu or HOAt, or on-resin cyclization.

In addition, the cyclic structure can be formed via a bridging group involving the side chains of amino acid residues of the CNP variant and/or the terminal amino acid residues. A bridging group is a chemical moiety that allows cyclization of two portions of the peptide. Non-limiting examples of bridg-

ing groups include amides, thioethers, thioesters, disulfides, ureas, carbamates, sulfonamides, and the like. A variety of methods are known in the art for incorporation of units having such bridging groups. For example, a lactam bridge (i.e., a cyclic amide) can be formed between the N-terminal amino group or an amino group on a side chain and the C-terminal carboxylic acid or a carboxyl group on a side chain, e.g., the side chain of lysine or ornithine and the side chain of glutamic acid or aspartic acid. A thioester can be formed between the C-terminal carboxyl group or a carboxyl group on a side chain and the thiol group on the side chain of cysteine or a cysteine analog.

Alternatively, a cross link can be formed by incorporating a lanthionine (thio-dialanine) residue to link alanine residues that are covalently bonded together by a thioether bond. In another method, a cross-linking agent, such as a dicarboxylic acid (e.g., suberic acid (octanedioic acid)), can link the functional groups of amino acid side chains, such as free amino, hydroxyl, and thiol groups.

Enzyme-catalyzed cyclization can also be used. For example, it has been reported that the thioesterase domain of tyrocidine synthetase can be used to cyclize a thioester precursor, a subtilisin mutant can be utilized to cyclize peptide glycolate phenylalanylamide esters, and the antibody ligase 16G3 can be employed to cyclize a p-nitrophenylester. For a review of peptide cyclization, see Davies, J. Peptide Sci., 9: 471-501 (2003), incorporated herein by reference in its entirety.

In certain embodiments, the final cyclized product has a purity of at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or at least about 99%.

D. Chemically Modified CNP Variants

Chemical modification of CNP22 or variants thereof can potentially impart advantageous properties to the modified CNP peptides, such as increased stability and half-life and reduced immunogenicity (for a general discussion of chemical modification of therapeutic proteins, see Pharmazie, 57(1): 5-29 (2002)). For example, attaching natural or synthetic, polymeric or non-polymeric moieties (e.g., PEG) to CNP peptides, to increase the total mass of the CNP peptides to the ranges described generally herein, e.g., a range from about 2.6 or 2.8 kDa to about 6 or 7 kDa, can reduce the susceptibility of the modified peptides to in vivo cleavage by exopeptidases and/or endopeptidases (e.g., NEP). In addition to PEGylation, glycosylation and other chemical derivatization procedures, e.g., modification by phosphorylation, amidation, carboxylation, acetylation, methylation, and creation of acid-addition salts, amides, esters and N-acyl derivatives, may also mask potentially immunogenic regions and/or proteolytically sensitive regions (Science, 303: 480-482 (2004)).

Examples of chemical modifications include, without limitation, the polymer addition method of Bednarsaki and the cross-linking method of Altus Corporation for improving stability and protease resistance and reducing immunogenicity. Bednarsaki showed that polymer addition can improve protein temperature stability (J. Am. Chem. Soc., 114(1): 378-380 (1992)), and Altus Corporation found that glutaraldehyde cross-linking can improve enzyme stability.

Chemical modification of polypeptides can be performed in a non-specific fashion (leading to mixtures of derivatized species) or in a site-specific fashion (e.g., based on wild-type macromolecule reactivity-directed derivatization and/or site-selective modification using a combination of site-directed mutagenesis and chemical modification) or, alternatively, using expressed protein ligation methods (Curr. Opin. Biotechnol., 13(4): 297-303 (2002)).

Pegylated CNP Variants

In one embodiment, for increased stability (e.g., resistance to NEP degradation), CNP22 or variants thereof (including those having amino acid additions, substitutions and/or deletions) are conjugated to hydrophilic, natural or synthetic polymers, to increase the total mass of the modified CNP peptides to a range from about 2.6 kDa or 2.8 kDa to about 4, 5, 6, 7 or higher kDa. In certain embodiments, the added hydrophilic polymers have a total mass of about 0.6, 0.8, 1, 1.2, 1.4, 1.6, 1.8, 2, 2.2, 2.4, 2.6, 2.8, 3, 3.2, 3.4, 3.6, 3.8, 4, 4.2, 4.4, 4.6, 4.8, or about 5 kDa.

In an embodiment, the hydrophilic polymers are water-soluble so that the CNP peptides conjugated thereto do not precipitate out in an aqueous (e.g., physiological) environment. Further, the hydrophilic polymers are biocompatible, i.e., do not cause injury, toxicity or an immunological reaction in vivo.

The hydrophilic polymers can be branched or unbranched. In one embodiment, the hydrophilic polymers are not branched.

Various sites of conjugation of CNP22 or variants thereof to a hydrophilic polymer are possible, including but not limited to: (1) only at the N-terminus; (2) only at the C-terminus; (3) only at an internal site (e.g., Lys4); (4) at both the N-terminus and the C-terminus; (5) at the N-terminus and an internal site; and (6) at the C-terminus and an internal site. In one embodiment, CNP22 or variants thereof are conjugated to a hydrophilic polymer only at the N-terminus. In another embodiment, conjugation is only at an internal site (e.g., Lys4). In yet another embodiment, conjugation is at the N-terminus and an internal site (e.g., Lys4). In still another embodiment, for better functionality the CNP peptides are not conjugated to a hydrophilic polymer at a site (e.g., Lys10) within the cyclic domain (corresponding to Cys6 to Cys22 of CNP22). If conjugation to a hydrophilic polymer is based on bond formation with a reactive primary amino group on the CNP peptide, conjugation at an internal site (e.g., Lys4 and/or Lys10) can be prevented by substitution of Lys4 and/or Lys10 with a natural or unnatural amino acid or peptidomimetic that does not contain a reactive primary amino group on a side chain, such as, e.g., Gly, Ser, Arg, Asn, Gln, Asp, Glu or citrulline (Cit). In a particular embodiment, Lys4 and/or Lys10 are replaced with Arg. In another embodiment, Lys10 is not replaced with Arg.

Non-limiting examples of hydrophilic polymers include polymers formed from carboxylic acid-bearing monomers (e.g., methacrylic acid (MA) and acrylic acid (AA)), polyvinyl alcohols, polymers formed from hydroxyl-bearing monomers (e.g., hydroxyethyl methacrylate (HEMA), hydroxypropyl methacrylate (HPMA), hydroxypropyl methacrylamide, and 3-trimethylsilylpropyl methacrylate (TMSPMA)), polyalkylene oxides, polyoxyethylated polyols (e.g., glycerol), poly(ethylene glycol) (PEG), poly(propylene glycol), mono-C_1-C_{10} alkoxy-PEGs (e.g., monomethoxy-PEG), tresyl monomethoxy-PEG, aryloxy-PEGs, PEG acrylate (PEGA), PEG methacrylate, PEG propionaldehyde, bis-succinimidyl carbonate PEG, copolymers of 2-methacryloyloxyethyl-phosphorylcholine (MPC) and N-vinyl pyrrolidone (VP), hydroxy functional poly(N-vinyl pyrrolidone) (PVP), SIS-PEG (SIS is polystyrene-polyisobutylene-polystyrene block copolymer), polystyrene-PEG, polyisobutylene-PEG, PCL-PEG (PCL is polycaprolactone), PLA-PEG (PLA is polylactic acid), PMMA-PEG (PMMA is poly(methyl methacrylate)), PDMS-PEG (PDMS is polydimethyloxanone), PVDF-PEG (PVDF is polyvinylidene

fluoride), PLURONIC™ surfactants (polypropylene oxide-co-polyethylene glycol), poly(tetramethylene glycol), poly (L-lysine-g-ethylene glycol) (PLL-g-PEG), poly(L-lysine-g-hyaluronic acid) (PLL-g-HA), poly(L-lysine-g-phosphoryl choline) (PLL-g-PC), poly(L-lysine-g-vinyl pyrrolidone) (PLL-g-PVP), poly(ethylimine-g-ethylene glycol) (PEI-g-PEG), poly(ethylimine-g-hyaluronic acid) (PEI-g-HA), poly (ethylimine-g-phosphoryl choline) (PEI-g-PC), poly(ethylimine-g-vinyl pyrrolidone) (PEI-g-PVP), PLL-co-HA, PLL-co-PC, PLL-co-PVP, PEI-co-PEG, PEI-co-HA, PEI-co-PC, PEI-co-PVP, cellulose and derivatives thereof (e.g., hydroxyethyl cellulose), dextran, dextrins, hyaluronic acid and derivatives thereof (e.g., sodium hyaluronate), elastin, chitosan, acrylic sulfate, acrylic sulfonate, acrylic sulfamate, methacrylic sulfate, methacrylic sulfonate, methacrylic sulfamate, polymers and copolymers thereof, and polymers and copolymers of combinations thereof.

In a particular embodiment, the hydrophilic polymer is poly(ethylene glycol) (PEG), also called poly(ethylene oxide) (PEO). As used herein, the term "PEG" or "PEO" encompasses all the forms of PEG, branched and unbranched, which can be used to derivatize polypeptides, including without limitation mono-(C_1-C_{10}) alkoxy-PEGs and aryloxy-PEGs.

In one embodiment, the PEG-CNP conjugates comprise a PEG (or PEO) polymer of the formula $(CH_2CH_2O)_n$, wherein n is an integer from about 6 to about 100, and the PEG polymer is from about 0.3 kDa to about 5 kDa. In another embodiment, n is an integer from about 12 to about 50, and the PEG polymer is from about 0.6 kDa to about 2.5 kDa. In yet another embodiment, n is from about 12 to about 24, and the PEG polymer is from about 0.6 kDa to about 1.2 kDa. In a further embodiment, the terminal hydroxyl group of the PEG polymer is capped with a non-reactive group. In a particular embodiment, the end-capping group is an alkyl group, e.g., a lower alkyl group such as methyl, so that the PEG polymer terminates in an alkoxy group. In an embodiment, the PEG polymer is not branched. In another embodiment, CNP22 or variants thereof are conjugated to a PEG polymer only at the N-terminus.

PEGs and PEOs potentially include molecules with a distribution of molecular weights, i.e., they are potentially polydispersed, depending on the manner in which they are prepared. The size/mass distribution of a polymeric preparation can be characterized statistically by its weight average molecular weight (M_w) and its number average molecular weight (M_n), the ratio of which is called the polydispersity index (M_w/M_n). M_w and M_n can be measured by mass spectroscopy. PEG-CNP variants conjugated to a PEG moiety larger than 1.5 kDa may exhibit a range of molecular weights due to the polydispersed nature of the parent PEG molecule. For example, in the case of mPEG2K (Sunbright ME-020HS, NOF Co.), the molecular masses of the PEG molecules are distributed over a range from about 1.5 kDa to about 3 kDa, with a polydispersity index of 1.036. By contrast, the PEGs conjugated to CNP22 or variants thereof using MS $(PEG)_n$ reagents (n=4, 8, 12 or 24, denoted as, e.g., "PEO12" or "PEO24") from Pierce Biotechnology (Rockford, Ill.) are monodispersed, having discrete chain length and defined molecular weight.

Methods for generating polypeptides comprising a PEG moiety are known in the art (see, e.g., U.S. Pat. No. 5,824,784). Methods for preparing PEGylated CNP peptides generally comprise the steps of (a) reacting CNP22 or a variant thereof with a PEGylation reagent under conditions suitable for attaching PEG to the CNP peptide (e.g., at the N-terminus), and (b) obtaining the reaction product(s). Because PEGylating a CNP peptide might significantly alter its binding to NPR-B, depending on the size of the PEG moiety and the location of PEGylation, different kinds of PEG and PEGylation reaction conditions can be explored. The chemistry that can be used for PEGylation of a CNP peptide includes acylation of reactive primary amine(s) of the peptide using the NHS-ester of methoxy-PEG (O—[(N-Succinimidyloxycarbonyl)-methyl]-O'-methylpolyethylene glycol). Acylation with methoxy-PEG-NHS or methoxy-PEG-SPA results in an amide linkage that eliminates any charge of the original primary amine. PEG-CNP peptides designated with the symbol "PEO12" or "PEO24", as well as those designated with the symbol "PEG1K", "PEG2K", "PEG5K" or "PEG20K", are PEGylated via reaction of a primary amino group on the peptide with an NHS ester-activated, methoxy-end capped PEG reagent. PEG-CNP variants can also be prepared by other methods, e.g., via reductive amination involving a primary amino group on the peptide and a PEG aldehyde, such as, e.g., PEG-propionaldehyde, or mono-C_1-C_{10} alkoxy or aryloxy derivatives thereof (see U.S. Pat. No. 5,252,714).

Unlike ribosome protein synthesis, synthetic peptide synthesis proceeds from the C-terminus to the N-terminus. Accordingly, Boc-PEG (containing tert-butyloxycarbonyl (Boc)) is one method to attach PEG to the C-terminus of a peptide (R. B. Merrifield, J. Am. Chem. Soc., 85(14): 2149-2154 (1963)). Alternatively, Fmoc (fluorenylmethoxycarbonyl) chemistry can be employed (E. Atherton and R. C. Sheppard, *Solid Phase Peptide Synthesis: a Practical Approach*, IRL Press (Oxford, England (1989)).

The present methods for preparing PEG-CNP variants provide a substantially homogenous mixture of polymer-protein conjugates. After purification, discrete PEG-CNP preparations are sufficiently pure for in vitro and in vivo testing of biological properties. As demonstrated herein, certain PEG-CNP variants exhibit reduced susceptibility to NEP cleavage and substantially similar or better functionality (e.g., stimulation of cGMP production).

As described herein, PEGylation reactions of CNP22 or variants thereof, using appropriate PEGylation reagent/CNP peptide ratios and reaction conditions, provide PEG-CNP derivatives. The nature and extent of PEGylation can be determined using, e.g., PAGE and HPLC analysis. In certain embodiments, at least about 50%, 60%, 70%, 80%, 90%, 95% or 99% of CNP22 or variants thereof are mono-PEGylated at the N-terminus. To optimize the beneficial effects of PEGylation on the biological properties of a CNP peptide, the polymer length, conformation (e.g., branched or linear), and/or functionalization (e.g., adding a negatively charged group) of a PEG moiety can be varied. PEGylated CNP variants are tested for NEP resistance, pharmacokinetics and bioactivity (e.g., the ability to bind to NPR-B and stimulate the generation of cGMP). PEGylated CNP variants that show improved NEP resistance and at least about 50% of the cGMP-stimulating activity of CNP22 can be further tested, e.g., in vitro in a rat chondrosarcoma cell-based achondroplasia model and in vivo in a murine achondroplasia animal model.

E. Methods of Using CNP Variants, Pharmaceutical Compositions of CNP Variants, and Routes of Administration

Methods of Using CNP Variants

Bone-Related Disorders

Fibroblast growth factors (FGFs) play important roles in bone formation, and mutations in FGF receptor genes (FGFR 1, 2 and 3) give rise to a variety of inherited skeletal malfor-

mations (Curr. Biol., 5: 500-507 (1995)). In particular, activating mutations in FGFR-3 are responsible for disorders of the long bones, including achondroplasia, the most common form of human genetic dwarfism (Nature, 371: 252-254 (1994); Cell, 78: 335-342 (1994)), the milder disorder hypochondroplasia (Ann. N.Y. Acad. Sci., 785: 182-187 (1996)), and the more severe and neonatal lethal thanatophoric dysplasia (TD) types I and II (IIum. Mol. Genet., 5: 509-512 (1996); Nat. Genet., 9: 321-328 (1995)). Mouse models overexpressing FGF-2, and consequentially activating FGFR-3, show shortened long bones and macrocephaly (Mol. Biol. Cell, 6: 1861-73 (1995)). Consistent with this model, mice deficient in FGFR-3 show remarkable skeletal overgrowth with wider growth plates (Nature Genet., 12: 390-397 (1996)).

Complementary experiments with CNP, NPR-B and NPR-C suggest a link between the peptide ligand, the corresponding receptors, and bone growth. Activation of NPR-B by elevated plasma concentrations of CNP in transgenic mice causes skeletal overgrowth (Nat. Med., 10: 80-86 (2004)) histologically similar to that of the growth plate cartilage of FGFR-3 knockout mice (Nat. Genet., 4: 390-397 (1996)). In NPR-C knockout mice, NPR-C-mediated clearance of CNP should be eliminated; consistent with this prediction, the knockout animals show elongated long bones and elongated vertebrae with kyphosis (Proc. Natl. Acad. Sci. USA 96: 7403-08 (1999)). Conversely, CNP knockout mice are dwarfed with shorter long bones and vertebrae, a phenotype histologically similar to that of achondroplasia, and have increased mortality as a result of malocclusion and pulmonary restriction from the small rib cage (Proc. Natl. Acad. Sci. USA, 98: 4016-4021 (2001)). Consistent with the proposed role of CNP as an activator of NPR-B, the NPR-B knockout mouse has the same dwarfed skeletal phenotype and increased mortality as the CNP knockout mouse (Proc. Natl. Acad. Sci. USA, 101: 17300-05 (2004)). Furthermore, in a mouse model of achondroplasia with activated FGFR-3 in the cartilage, targeted overexpression of CNP in chondrocytes counteracts dwarfism (Yasoda et al., Nat. Med., 10: 80-86 (2004)). Additionally, CNP has been show to play a role in regulating endochondral bone growth and chondrocyte activity, including but not limited to chondrocyte proliferation and differentiation, inhibition of the mitogen activated protein (MAP) kinase/MEK (Raf-1) kinase signaling pathway, and promotion of endochondral ossification (Yasoda et al., Nat. Med., 10: 80-86 (2004)). These results suggest that activation of the CNP/NPR-B system is a potential therapeutic strategy for treatment of human achondroplasia.

By stimulating matrix production, proliferation and differentiation of chondrocytes and increasing long bone growth, the CNP variants of the disclosure are useful for treating mammals, including humans, suffering from a bone-related disorder, such as a skeletal dysplasia. Non-limiting examples of CNP-responsive bone-related disorders and skeletal dysplasias include achondroplasia, hypochondroplasia, short stature, dwarfism, osteochondrodysplasias, thanatophoric dysplasia, osteogenesis imperfecta, achondrogenesis, chondrodysplasia punctata, homozygous achondroplasia, chondrodysplasia punctata, camptomelic dysplasia, congenital lethal hypophosphatasia, perinatal lethal type of osteogenesis imperfecta, short-rib polydactyly syndromes, hypochondroplasia, rhizomelic type of chondrodysplasia punctata, Jansen-type metaphyseal dysplasia, spondyloepiphyseal dysplasia congenita, atelosteogenesis, diastrophic dysplasia, congenital short femur, Langer-type mesomelic dysplasia, Nievergelt-type mesomelic dysplasia, Robinow syndrome, Reinhardt syndrome, acrodysostosis, peripheral dysostosis, Kniest dysplasia, fibrochondrogenesis, Roberts syndrome, acromesomelic dysplasia, micromelia, Morquio syndrome, Kniest syndrome, metatrophic dysplasia, and spondyloepimetaphyseal dysplasia. Further, the CNP variants are useful as an adjunct or alternative to growth hormone for treating idiopathic short stature and other skeletal dysplasias.

In addition, the CNP variants are useful for treating other bone-related conditions and disorders, such as rickets, hypophosphatemic rickets [including X-linked hypophosphatemic rickets (also called vitamin D-resistant rickets) and autosomal dominant hypophosphatemic rickets], and osteomalacia [including tumor-induced osteomalacia (also called oncogenic osteomalacia or oncogenic hypophosphatemic osteomalacia)].

The CNP variants of the disclosure can also be used to treat osteoarthritis. Osteoarthritis is a degenerative disease of the articular cartilage and occurs frequently in the elderly. Osteoarthritis involves destruction of the cartilage and proliferative change in the bone and cartilage resulting from degeneration of articular components, with the change resulting in a secondary arthritis (e.g., synovitis). The extracellular matrix proteins, which are the functional entity of the cartilage, are reduced, and the number of chondrocytes decreases in osteoarthritis (Arth. Rheum. 46(8): 1986-1996 (2002)). By promoting the matrix production, growth and differentiation of chondrocytes, the CNP variants are useful for countering the undesired effects of FGF-2 and increasing matrix synthesis in subjects suffering from arthritis, including osteoarthritis, thereby treating arthritis, including osteoarthritis.

Vascular Smooth Muscle Disorders

CNP and other vasoactive peptides (including ANP, BNP and urodilatin) have vasodilator and diuretic properties and play an important role in cardiovascular homeostasis (J. Cardiovasc. Pharmacol., 117: 1600-06 (1998); Kidney Int., 49: 1732-37 (1996); Am. J. Physiol., 275: H1826-1833 (1998)). CNP is widely distributed in the cardiovascular system, especially in high concentration in vascular endothelial cells (J. Cardiovasc. Pharmacol., 117: 1600-06 (1998)). CNP is a potent relaxant of vascular smooth muscle, particularly in the coronary circulation (Biochem. Biophys. Res. Commun., 205: 765-771 (1994)), and is an inhibitor of smooth muscle cell proliferation (Biochem. Biophys. Res. Commun., 177: 927-931 (1991)). Although the vasodilator effect of CNP is less potent than that of ANP (about 1:100) (Hypertens. Res., 21: 7-13 (1998); Am. J. Physiol., 275: L645-L652 (1998)), CNP mRNA is increased in response to shear stress (FEBS Lett., 373: 108-110 (1995)) and plasma levels of CNP are elevated in inflammatory cardiovascular pathologies (Biochem. Biophys. Res. Commun., 198: 1177-1182 (1994)). CNP has been shown to suppress inflammation through inhibition of macrophage infiltration in injured carotid arteries of rabbits (Circ. Res., 91: 1063-1069 (2002)) and to directly inhibit cardiac fibroblast proliferation through an NPR-B/cGMP-dependent pathway (Endocrinology, 144: 2279-2284 (2003)).

The cardiovascular actions of CNP are mediated via activation of the NPR subtypes, NPR-B and NPR-C (Endocrinology, 130: 229-239 (1992)), the latter accounting for 95% of NPRs expressed in vivo (Science, 293: 1657-1662 (2001)). The CNP/NPR-B pathway leads to elevation of cGMP, a well-established secondary messenger in the cardiovascular system. NPR-C's 37-amino acid portion from the C-terminus has a consensus sequence that interacts with the heterotrimeric G protein G_i (J. Biol. Chem., 274: 17587-17592 (1999)), which has been shown to regulate adenylate cyclase and phospholipase C activity (J. Biol. Chem., 276: 22064-70 (2001); Am. J. Physiol., 278: G974-980 (2000); J. Biol.

Chem., 271: 19324-19329 (1996)). CNP mediates smooth muscle hyperpolarization and relaxation via activation of NPR-C and the opening of a G protein-regulated, inwardly rectifying K^+ channels (Proc. Natl. Acad. Sci. USA, 100: 1426-1431 (2003)). Likewise, CNP has important anti-proliferative effects in cardiac fibroblasts and, through interaction with NPR-C, regulates local blood flow and systemic blood pressure by hyperpolarizing smooth muscle cells (R. Rose and W. Giles, J. Physiol. 586: 353-366 (2008)).

By binding to NPR-B on vascular smooth muscle cells, CNP22 stimulates the production of cGMP, which acts as an intracellular secondary messenger to cause ultimately the relaxation of blood vessels. Based on the hypotensive actions of CNP, the CNP variants of the disclosure are useful for treating hypertension, congestive heart failure, cardiac edema, nephredema, hepatic edema, acute and chronic renal insufficiency, and so on. In addition, activation of cGMP signaling suppresses the growth of vascular smooth muscle cells. Accordingly, the CNP variants of the disclosure can be used to treat conditions or diseases caused by the abnormal growth of vascular smooth muscle cells, including but not limited to restenosis and arteriosclerosis.

The studies described above suggest that CNP may be a potential therapeutic candidate for vascular smooth muscle relaxation and remodeling. Pharmacological effects of CNP concerning certain disorders have been attributed, in part, to vasoprotective effects rather than to vasodilator activity (Am. J. Respir. Crit. Care Med., 170: 1204-1211 (2004)). Therefore, the CNP variants of the present disclosure are useful for treating conditions, e.g., vascular smooth muscle disorders, in which CNP may have a vasoprotective effect, including without limitation inducing smooth muscle relaxation and inhibiting infiltration of macrophages into cardiac tissue. In one embodiment, the CNP variants are used to treat heart failure, including but not limited to acute decompensated heart failure and acute congestive heart failure. In another embodiment, the CNP variants are used to treat asthma, cardiomyopathy, and restenosis of coronary arteries (by increasing smooth muscle cell relaxation and decreasing proliferation of smooth muscle cells).

Pharmaceutical Compositions of CNP Variants

In additional embodiments, the disclosure provides pharmaceutical compositions comprising a CNP variant, and one or more pharmaceutically acceptable excipients, carriers and/or diluents. In certain embodiments, the compositions further comprise one or more other biologically active agents (e.g., inhibitors of proteases, receptor tyrosine kinases, and/or the clearance receptor NPR-C).

In some embodiments, the compositions comprise the desired CNP variant in at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% purity. In certain embodiments, the compositions contain less than about 10%, 5%, 4%, 3%, 2%, 1% or 0.5% of macromolecular contaminants, such as other mammalian (e.g., human) proteins and other CNP variants.

Non-limiting examples of excipients, carriers and diluents include vehicles, liquids, buffers, isotonicity agents, additives, stabilizers, preservatives, solubilizers, surfactants, emulsifiers, wetting agents, adjuvants, and so on. The compositions can contain liquids (e.g., water, ethanol); diluents of various buffer content (e.g., Tris-HCl, phosphate, acetate buffers, citrate buffers), pH and ionic strength; detergents and solubilizing agents (e.g., Polysorbate 20, Polysorbate 80); anti-oxidants (e.g., methionine, ascorbic acid, sodium metabisulfite); preservatives (e.g., Thimerosol, benzyl alcohol, m-cresol); and bulking substances (e.g., lactose, mannitol, sucrose). The use of excipients, diluents and carriers in the formulation of pharmaceutical compositions is known in the art; see, e.g., Remington's Pharmaceutical Sciences, 18th Edition, pages 1435-1712, Mack Publishing Co. (Easton, Pa. (1990)), which is incorporated herein by reference in its entirety.

For example, carriers include without limitation diluents, vehicles and adjuvants, as well as implant carriers, and inert, non-toxic solid or liquid fillers and encapsulating materials that do not react with the active ingredient(s). Non-limiting examples of carriers include phosphate buffered saline, physiological saline, water, and emulsions (e.g., oil/water emulsions). A carrier can be a solvent or dispersing medium containing, e.g., ethanol, a polyol (e.g., glycerol, propylene glycol, liquid polyethylene glycol, and the like), a vegetable oil, and mixtures thereof.

In some embodiments, the compositions are liquid formulations. In certain embodiments, the formulations comprise a CNP variant in a concentration range from about 0.1 mg/ml to about 20 mg/ml, or from about 0.5 mg/ml to about 20 mg/ml, or from about 1 mg/ml to about 20 mg/ml, or from about 0.1 mg/ml to about 10 mg/ml, or from about 0.5 mg/ml to about 10 mg/ml, or from about 1 mg/ml to about 10 mg/ml.

In further embodiments, the compositions comprise a buffer solution or buffering agent to maintain the pH of a CNP-containing solution or suspension within a desired range. Non-limiting examples of buffer solutions include phosphate buffered saline, Tris buffered saline, and Hank's buffered saline. Buffering agents include without limitation sodium acetate, sodium phosphate, and sodium citrate. Mixtures of buffering agents can also be used. In certain embodiments, the buffering agent is acetic acid/acetate or citric acid/citrate. The amount of buffering agent suitable in a composition depends in part on the particular buffer used and the desired pH of the solution or suspension. For example, acetate is a more efficient pH buffer at pH 5 than pH 6, so less acetate may be used in a solution at pH 5 than at pH 6. In some embodiments, the buffering agent has a concentration of about 10 mM±5 mM. In certain embodiments, the pH of a composition is from about pH 3 to about pH 7.5, or from about pH 3.5 to about pH 7, or from about pH 3.5 to about pH 6.5, or from about pH 4 to about pH 6, or from about pH 4 to about pH 5, or is at about pH 5.0±1.0.

In other embodiments, the compositions contain an isotonicity-adjusting agent to render the solution or suspension isotonic and more compatible for injection. Non-limiting examples of isotonicity agents include NaCl, dextrose, glucose, glycerin, sorbitol, xylitol, and ethanol. In certain embodiments, the isotonicity agent is NaCl. In certain embodiments, NaCl is in a concentration of about 160±20 mM, or about 140 mM±20 mM, or about 120±20 mM, or about 100 mM±20 mM, or about 80 mM±20 mM, or about 60 mM±20 mM.

In yet other embodiments, the compositions comprise a preservative. Preservatives include, but are not limited to, m-cresol and benzyl alcohol. In certain embodiments, the preservative is in a concentration of about 0.4%±0.2%, or about 1%±0.5%, or about 1.5%±0.5%, or about 2.0%±0.5%.

In still other embodiments, the compositions contain an anti-adsorbent (e.g., to mitigate adsorption of a CNP variant to glass or plastic). Anti-adsorbents include without limitation benzyl alcohol, Polysorbate 20, and Polysorbate 80. In certain embodiments, the anti-adsorbent is in a concentration from about 0.001% to about 0.5%, or from about 0.01% to about 0.5%, or from about 0.1% to about 1%, or from about 0.5% to about 1%, or from about 0.5% to about 1.5%, or from about 0.5% to about 2%, or from about 1% to about 2%.

In additional embodiments, the compositions comprise a stabilizer. Non-limiting examples of stabilizers include glycerin, glycerol, thioglycerol, methionine, and ascorbic acid and salts thereof. In some embodiments, when the stabilizer is thioglycerol or ascorbic acid or a salt thereof, the stabilizer is in a concentration from about 0.1% to about 1%. In other embodiments, when the stabilizer is methionine, the stabilizer is in a concentration from about 0.01% to about 0.5%, or from about 0.01% to about 0.2%. In still other embodiments, when the stabilizer is glycerin, the stabilizer is in a concentration from about 5% to about 100% (neat).

In further embodiments, the compositions contain an anti-oxidant. Exemplary anti-oxidants include without limitation methionine and ascorbic acid. In certain embodiments, the molar ratio of antioxidant to CNP variant is from about 0.1:1 to about 15:1, or from about 1:1 to about 15:1, or from about 0.5:1 to about 10:1, or from about 1:1 to about 10:1 or from about 3:1 to about 10:1.

Pharmaceutically acceptable salts can be used in the compositions, including without limitation mineral acid salts (e.g., hydrochloride, hydrobromide, phosphate, sulfate), salts of organic acids (e.g., acetate, propionate, malonate, benzoate, mesylate, tosylate), and salts of amines (e.g., isopropylamine, trimethylamine, dicyclohexylamine, diethanolamine). A thorough discussion of pharmaceutically acceptable salts is found in *Remington's Pharmaceutical Sciences,* 18th Edition, Mack Publishing Company, (Easton, Pa. (1990)).

The pharmaceutical compositions can be administered in various forms, such as tablets, capsules, granules, powders, solutions, suspensions, emulsions, ointments, and transdermal patches. The dosage forms of the compositions can be tailored to the desired mode of administration of the compositions. For oral administration, the compositions can take the form of, e.g., a tablet or capsule (including softgel capsule), or can be, e.g., an aqueous or nonaqueous solution, suspension or syrup. Tablets and capsules for oral administration can include one or more commonly used excipieints, diluents and carriers, such as mannitol, lactose, glucose, sucrose, starch, corn starch, sodium saccharin, talc, cellulose, magnesium carbonate, and lubricating agents (e.g., magnesium stearate, sodium stearyl fumarate). If desired, flavoring, coloring and/or sweetening agents can be added to the solid and liquid formulations. Other optional ingredients for oral formulations include without limitation preservatives, suspending agents, and thickening agents. Oral formulations can also have an enteric coating to protect the CNP variant from the acidic environment of the stomach. Methods of preparing solid and liquid dosage forms are known, or will be apparent, to those skilled in this art (see, e.g., Remington's Pharmaceutical Sciences, referenced above).

Formulations for parenteral administration can be prepared, e.g., as liquid solutions or suspensions, as solid forms suitable for solubilization or suspension in a liquid medium prior to injection, or as emulsions. For example, sterile injectable solutions and suspensions can be formulated according to techniques known in the art using suitable diluents, carriers, solvents (e.g., buffered aqueous solution, Ringer's solution, isotonic sodium chloride solution), dispersing agents, wetting agents, emulsifying agents, suspending agents, and the like. In addition, sterile fixed oils, fatty esters, polyols and/or other inactive ingredients can be used. As further examples, formulations for parenteral administration include aqueous sterile injectable solutions, which can contain anti-oxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient; and aqueous and nonaqueous sterile suspensions, which can contain suspending agents and thickening agents.

Compositions comprising a CNP variant can also be lyophilized formulations. In certain embodiments, the lyophilized formulations comprise a buffer and bulking agent, and optionally an antioxidant. Exemplary buffers include without limitation acetate buffers and citrate buffers. Exemplary bulking agents include without limitation mannitol, sucrose, dexran, lactose, trehalose, and povidone (PVP K24). In certain embodiments, mannitol is in an amount from about 3% to about 10%, or from about 4% to about 8%, or from about 4% to about 6%. In certain embodiments, sucrose is in an amount from about 6% to about 20%, or from about 6% to about 15%, or from about 8% to about 12%. Exemplary anti-oxidants include, but are not limited to, methionine and ascorbic acid.

The disclosure also provides kits containing, e.g., bottles, vials, ampoules, tubes, cartridges and/or syringes that comprise a liquid (e.g., sterile injectable) formulation or a solid (e.g., lyophilized) formulation. The kits can also contain pharmaceutically acceptable vehicles or carriers (e.g., solvents, solutions and/or buffers) for reconstituting a solid (e.g., lyophilized) formulation into a solution or suspension for administration (e.g., by injection), including without limitation reconstituting a lyophilized formulation in a syringe for injection or for diluting concentrate to a lower concentration. Furthermore, extemporaneous injection solutions and suspensions can be prepared from, e.g., sterile powder, granules, or tablets comprising a CNP-containing composition. The kits can also include dispensing devices, such as aerosol or injection dispensing devices, pen injectors, autoinjectors, needleless injectors, syringes, and/or needles.

As a non-limiting example, a kit can include syringes having a single chamber or dual chambers. For single-chamber syringes, the single chamber can contain a liquid CNP formulation ready for injection, or a solid (e.g., lyophilized) CNP formulation or a liquid formulation of a CNP variant in a relatively small amount of a suitable solvent system (e.g., glycerin) that can be reconstituted into a solution or suspension for injection. For dual-chamber syringes, one chamber can contain a pharmaceutically acceptable vehicle or carrier (e.g., solvent system, solution or buffer), and the other chamber can contain a solid (e.g., lyophilized) CNP formulation or a liquid formulation of a CNP variant in a relatively small amount of a suitable solvent system (e.g., glycerin) which can be reconstituted into a solution or suspension, using the vehicle or carrier from the first chamber, for injection.

As a further example, a kit can include one or more pen injector or autoinjector devices, and dual-chamber cartridges. One chamber of a cartridge can contain a pharmaceutically acceptable vehicle or carrier (e.g., solvent system, solution or buffer), and the other chamber can contain a solid (e.g., lyophilized) CNP formulation or a liquid formulation of a CNP variant in a relatively small amount of a suitable solvent system (e.g., glycerin) which can be reconstituted into a solution or suspension, using the vehicle or carrier from the first chamber, for injection. A cartridge can comprise an amount of the CNP variant that is sufficient for dosing over a desired time period (e.g., 1 day, 2 days, 3 days, 1 week, 2 weeks, 3 weeks, 4 weeks, etc.). The pen injector or autoinjector can be adjusted to administer a desired amount of the CNP formulation from a cartridge.

In addition, pharmaceutical compositions comprising a CNP variant can be formulated as a slow release, controlled release or sustained release system for maintaining a relatively constant level of dosage over a desired time period, such as 1 week, 2 weeks, 3 weeks, 1 month, 2 months, or 3 months. Slow release, controlled release and sustained release formulations can be prepared using, e.g., biodegrad-

able polymeric systems {which can comprise, e.g., hydrophilic polymers [e.g., polylactide, polyglycolide, poly(lactide-glycolide)]}, and can take the form of, e.g., microparticles, microspheres or liposomes, as is known in the art.

Dosages and Frequency of Dosing

As used herein, the term "therapeutically effective amount" of an active agent (e.g., a CNP variant) refers to an amount that provides therapeutic benefit to a patient. The amount may vary from one individual to another and may depend upon a number of factors, including the overall physical condition of the patient. A therapeutically effective amount of a CNP variant can be readily ascertained by one skilled in the art, using publicly available materials and procedures. For example, the amount of a CNP variant used for therapy should give an acceptable rate of growth based on growth charts for children ages 0-17 years with achondroplasia (214 females and 189 males), which list height for age, head circumference, and segmental growth (Horton W A et al., Standard growth curves for achondroplasia, J. Pediatr., 93: 435-8 (1978)). CDC charts can be used to assess weight for age and weight for height or BMI for age. Secondary outcomes with courses that are more chronic in nature can also be measured.

Having a longer serum half-life than wild-type CNP22, the CNP variants can potentially be administered less frequently than CNP22. The dosing frequency for a particular individual may vary depending upon various factors, including the disorder being treated and the condition and response of the individual to the therapy. In certain embodiments, a pharmaceutical composition containing a CNP variant is administered to a subject about one time per day, one time per two days, one time per three days, or one time per week. In one embodiment, for treatment of bone-related disorders (e.g., skeletal dysplasias, including achondroplasia), a daily or weekly dose of a CNP variant is administered to patients until and/or through adulthood.

The CNP variants described herein can be administered to patients at therapeutically effective doses to treat, ameliorate or prevent bone-related disorders (e.g., skeletal dysplasias, including achondroplasia) and conditions (e.g., vascular smooth muscle disorders) in which CNP can provide a vasoprotective effect. The safety and therapeutic efficacy of the CNP variants can be determined by standard pharmacological procedures in cell cultures or experimental animals, such as, for example, by determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50}. Active agents exhibiting a large therapeutic index are normally preferred.

Data obtained from cell culture assays and animal studies can be used to formulate a range of dosage for use in humans. The dosage normally lies within a range of circulating concentrations that include the ED_{50}, with little or minimal toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized. The therapeutically effective dose can be determined from cell culture assays and animal studies.

In certain embodiments, the CNP variants described herein are administered at a dose in the range from about 5 or 10 nmol/kg to about 300 nmol/kg, or from about 20 nmol/kg to about 200 nmol/kg. In some embodiments, the CNP variants are administered at a dose of about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 125, 130, 140, 150, 160, 170, 175, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 350, 400, 450, 500, 750, 1000, 1250, 1500, 1750 or 2000 nmol/kg or other dose deemed appropriate by the treating physician. In other embodiments, the CNP variants are administered at a dose of about 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950 or 1000 ug/kg, or about 1, 1.25, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5 or 10 mg/kg, or other dose deemed appropriate by the treating physician. The doses of CNP variants described herein can be administered according to the dosing frequency/frequency of administration described herein, including without limitation daily, 2 or 3 times per week, weekly, every 2 weeks, every 3 weeks, monthly, etc.

The frequency of dosing/administration of a CNP variant for a particular subject may vary depending upon various factors, including the disorder being treated and the condition and response of the suhject to the therapy. The CNP variant can be administered in a single dose or in multiple doses per dosing. In certain embodiments, the CNP variant is administered, in a single dose or in multiple doses, daily, every other day, every 3 days, 2 times per week, 3 times per week, weekly, bi-weekly, every 3 weeks, monthly, every 6 weeks, every 2 months, every 3 months, or as deemed appropriate by the treating physician.

In some embodiments, a CNP variant is administered so as to allow for periods of growth (e.g., chondrogenesis), followed by a recovery period (e.g., osteogenesis). For example, the CNP variant may be administered intravenously, subcutaneously or by another mode daily or multiple times per week for a period of time, followed by a period of no treatment, then the cycle is repeated. In some embodiments, the initial period of treatment (e.g., administration of the CNP variant daily or multiple times per week) is for 3 days, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks or 12 weeks. In a related embodiment, the period of no treatment lasts for 3 days, 1 week, 2 weeks, 3 weeks or 4 weeks. In certain embodiments, the dosing regimen of the CNP variant is daily for 3 days followed by 3 days off; or daily or multiple times per week for 1 week followed by 3 days or 1 week off; or daily or multiple times per week for 2 weeks followed by 1 or 2 weeks off; or daily or multiple times per week for 3 weeks followed by 1, 2 or 3 weeks off; or daily or multiple times per week for 4, 5, 6, 7, 8, 9, 10, 11 or 12 weeks followed by 1, 2, 3 or 4 weeks off.

Modes of Administration

The CNP variants, or pharmaceutical compositions comprising them, can be administered to subjects in various ways such as, e.g., by injection subcutaneously, intravenously, intra-arterially, intraperitoneally, intramuscularly, intradermally, or intrathecally. In an embodiment, the CNP variants are administered by a single subcutaneous, intravenous, intra-arterial, intraperitoneal, intramuscular, intradermal or intrathecal injection once a day.

The CNP variants can also be administered by direct injection at or near the site of disease. Further, the CNP variants can be administered by implantation of a depot at the target site of action (e.g., an abnormal or dysplasic bone). Alternatively, the CNP variants can be administered sublingually under the tongue (e.g., sublingual tablet) or by inhalation into the lungs (e.g., inhaler or aerosol spray), by delivery into the nasal cavity (e.g., intranasal spray), by delivery into the eye (e.g., eye drop), or by transdermal delivery (e.g., by means of a patch on the skin). The CNP variants may also be administered orally in the form of microspheres, microcapsules, liposomes (uncharged or charged (e.g., cationic)), polymeric microparticles (e.g., polyamides, polylactide, polyglycolide, poly(lactide-glycolide)), microemulsions, and the like.

A further method of administration is by osmotic pump (e.g., an Alzet pump) or mini-pump (e.g., an Alzet mini-osmotic pump), which allows for controlled, continuous and/or slow-release delivery of the CNP variant or pharmaceutical composition over a pre-determined period. The osmotic pump or mini-pump can be implanted subcutaneously, or near the target site (e.g., the long bones of limbs, the epiphyses, etc.).

As explained above, the CNP variants can be used to treat conditions or diseases caused by the abnormal growth of vascular smooth muscle cells, including but not limited to restenosis and arteriosclerosis. For local delivery of a CNP variant to the diseased bodily vessel (e.g., blood vessel), the CNP variant can be delivered by means of a medical device (e.g., a stent) implanted at the diseased site. In one embodiment, the CNP variant is impregnated in a polymeric matrix or polymeric coating disposed over a stent. In another embodiment, the CNP variant is contained in reservoirs or channels formed in the body of a stent and covered by a porous polymeric membrane or layer through which the CNP variant can diffuse. The polymeric matrix, coating, membrane or layer can comprise at least one biodegradable (e.g., hydrophilic) polymer, as is known in the art. In a further embodiment, the CNP variant can be contained in micropores in the body of a stent. The CNP variant can be delivered from a stent by burst release, pulse release, controlled release or sustained release, or a combination thereof. For example, the stent can locally deliver the CNP variant to the diseased site in a burst release followed by a sustained release. Sustained release can be over a period up to about 2 weeks, 1 month, 2 months, 3 months, 6 months or 1 year.

It will be apparent to one skilled in the art that the CNP variants or compositions thereof can also be administered by other modes. Determination of the most effective mode of administration of the CNP variants or compositions thereof is within the skill of the skilled artisan.

The CNP variants can be administered as pharmaceutical formulations suitable for, e.g., oral (including buccal and sub-lingual), rectal, nasal, topical, pulmonary, vaginal or parenteral (including intramuscular, intraarterial, intrathecal, subcutaneous and intravenous) administration, or in a form suitable for administration by inhalation or insufflation. Depending on the intended mode of administration, the pharmaceutical formulations can be in the form of solid, semi-solid or liquid dosage forms, such as tablets, suppositories, pills, capsules, powders, liquids, suspensions, emulsions, creams, ointments, lotions, and the like. The formulations can be provided in unit dosage form suitable for single administration of a precise dosage. The formulations comprise an effective amount of a CNP variant, and one or more pharmaceutically acceptable excipients, carriers and/or diluents, and optionally one or more other biologically active agents.

Combination Therapy

In one embodiment, a CNP variant can be used in combination with one or more other active agents useful for treating, ameliorating or preventing CNP-responsive conditions or disorders such as, e.g., bone-related disorders (e.g., skeletal dysplasias) and vascular smooth muscle disorders. The other active agent(s) can enhance the effects of the CNP variant and/or exert other pharmacological effects in addition to those of the CNP variant. Non-limiting examples of active agents that can be used in combination with the CNP variants described herein are other natriuretic peptides (e.g., BNP) and inhibitors (e.g., antagonists) of peptidases and proteases (e.g., NEP and furin), NPR-C and tyrosine kinases (e.g., FGFR-3). By preventing NEP cleavage of the CNP variant, an NEP inhibitor can prolong the half-life of the variant. Examples of NEP inhibitors include, without limitation, thiorphan and candoxatril. Co-use of an NPR-C inhibitor can also prolong the half-life of the CNP variant via inhibition of the variant's clearance by NPR-C. A non-limiting example of an NPR-C inhibitor is the fragment FGIPMDRIGRNPR (SEQ ID NO: 82), which would be released at the target site (e.g., bone growth plate) upon proteolytic cleavage of the FGIPMDRIGRNPR-CNP22 chimera (Analog CZ) (SEQ ID NO: 82) or similar chimeras comprising variants of CNP22 (e.g., those containing amino acid substitution(s), addition(s), and/or deletion(s) relative to CNP22). Co-use of a tyrosine kinase inhibitor can accentuate the effects of a CNP therapy by inhibiting the tyrosine kinase receptor FGFR-3, a negative regulator of chondrocyte and bone growth. Non-limiting examples of tyrosine kinase inhibitors include those disclosed in U.S. Pat. Nos. 6,329,375 and 6,344,459.

To achieve the appropriate therapeutic outcome in the combination therapies, one would generally administer to the subject the CNP composition and other therapeutic(s) in a combined amount effective to produce the desired therapeutic outcome (e.g., restored bone growth). This process may involve administering the CNP composition and other therapeutic agent(s) at the same time. Simultaneous administration can be achieved by administering a single composition or pharmacological protein formulation that includes both the CNP variant and other therapeutic agent(s). Alternatively, the other therapeutic agent(s) can be taken separately at about the same time as a pharmacological formulation (e.g., tablet, injection or drink) of the CNP variant. The CNP variant can also be formulated into a foodstuff such as brownies, pancakes, or cake, suitable for ingestion.

In other alternatives, administration of the CNP variant can precede or follow administration of the other therapeutic agent(s) by intervals ranging from minutes to hours. In embodiments where the other therapeutic agent(s) and the CNP composition are administered separately, one would generally ensure that the CNP variant and the other therapeutic agent(s) are administered within an appropriate time of one another so that both the CNP variant and the other therapeutic agent(s) can exert, synergistically or additively, a beneficial effect on the patient. For example, one can administer the CNP composition within about 0.5-6 hours (before or after) of the other therapeutic agent(s). In one embodiment, the CNP composition is administered within about 1 hour (before or after) of the other therapeutic agent(s).

Identifying and Monitoring Patient Populations

Protocols can be established to identify subjects suitable for CNP therapy and to determine whether a given patient is responsive to CNP therapy. For example, for treatment of bone-related disorders, indicators of growth can be measured, such as long bone growth measurements in utero and neonatal and measurements of bone growth biomarkers such as CNP, cGMP, Collagen II, osteocalcin, and Proliferating Cell Nuclear Antigen (PCNA).

One CNP signaling marker is cGMP (guanosine 3',5' cyclic monophosphate). The level of this intracellular signaling molecule increases after CNP binds to and activates its cognate receptor NPR-B. Elevated levels of cGMP can be measured from cell culture extracts (in vitro) after CNP exposure, conditioned media from bone ex-plant studies (ex vivo) after CNP exposure, and in the plasma (in vivo) within minutes of CNP administration subcutaneously, intravenously, or via other routes of administration known in the art.

Cartilage and bone-specific analytes (or cartilage- and bone-associated markers) can also be measured to assess CNP efficacy. For example, fragments of cleaved collagen type II are a cartilage-specific marker for cartilage turnover.

Type II collagen is the major organic constituent of cartilage and fragments of type II collagen (cleaved collagen) are released into circulation, and subsequently secreted into the urine, following cartilage turnover. Cartilage turnover precedes new bone formation.

A bone-specific biomarker for bone formation which can be measured is N-terminal propeptides of type I procollagen (PINP). The synthesis of type I collagen is an important step in bone formation, as type I collagen is the major organic component in bone matrix. During collagen synthesis, propeptides are released from the procollagen molecule and can be detected in serum. In addition, fragments of collagen type I can be measured as a marker for bone resorption.

Other potential biomarkers for cartilage and bone formation and growth include aggrecan chondroitin sulfate (cartilage-specific marker for cartilage turnover), propeptides of type TT collagen (cartilage-specific marker for cartilage formation), alkaline phosphatase (bone-specific) and osteocalcin (bone-specific marker for bone formation). Cartilage- and bone-associated biomarkers can be measured, e.g., in serum from efficacy/pharmacodynamic in vivo studies and from the conditioned media of ex vivo studies, using commercially available kits.

In one embodiment, the level of at least one bone- or cartilage-associated biomarker is assayed or measured in a subject that has been administered a CNP variant in order to monitor the effects of the CNP variant on bone and cartilage formation and growth in vivo. For example, an increase in the level of at least one bone- or cartilage-associated biomarker may indicate that administration of a CNP variant has a positive effect on bone growth and is a useful treatment for skeletal dysplasias and other bone- or cartilage-related diseases or disorders associated with decreased CNP activity. Exemplary bone- or cartilage-associated biomarkers include, but are not limited to, CNP (e.g, endogenous levels of CNP), cGMP, propeptides of collagen type II and fragments thereof, collagen type II and fragments thereof, osteocalcin, proliferating cell nuclear antigen (PCNA), propeptides of type I procollagen (PINP) and fragments thereof, collagen type I and fragments thereof, aggrecan chondroitin sulfate, and alkaline phosphatase.

In an embodiment, biomarkers are measured by obtaining a biological sample from a subject who will be administered, is being administered or has been administered a CNP variant. Biomarkers can be measured using techniques known in the art, including, but not limited to, Western Blot, enzyme linked immunosorbant assay (ELISA), and enzymatic activity assay. The biological sample can be blood, serum, urine, or other biological fluids.

Additional aspects and details of the disclosure will be apparent from the following examples, which are intended to be illustrative rather than limiting.

F. Examples

Example 1

Synthesis of CNP Variants

CNP variants were prepared using the methods described herein. Substitutions with natural or unnatural amino acids or peptidomimetics were made, as indicated in Tables 1-3 (shown in Example 3), at the respective amino acid residues in the wild-type sequence of CNP22. In certain variants, additional amino acids were added to the N-terminal and/or C-terminal ends of the whole or a portion of the wild-type CNP22 sequence (see Table 3).

Also prepared were CNP variants in which a PEG (or PEO) moiety was conjugated to the N-terminus of CNP22 or variants thereof (see Table 4, shown in Example 3). PEGylation reagents can be obtained from the commercial sources shown in Table 5.

TABLE 5

Vendor	Product Name	Name	MW (Da)	PEGylation Reagent
NOF	Sunbright ME-200CS	mPEG20K	20,000	CH3(CH2CH2O)450-(CH2)5COO—NHS
NOF	Sunbright ME-050CS	mPEG5K	5,000	CH3(CH2CH2O)110-(CH2)5COO—NHS
Pierce	(Methyl-PEG12)3-PEG4-NHS Ester	(mPEG12)3-PEG4	2,400	[CH3(CH2CH2O)12]3-(CH2CH2O)4-NHCO(CH2)3-COO—NHS
NOF	Sunbright ME-020HS	mPEG2K	2,000	CH3(CH2CH2O)45-(CH2)5COO—NHS
NOF	Sunbright ME-020CS	mPEG2K	2,000	CH3(CH2CH2O)45-(CH2)5COO—NHS
NOF	Sunbright ME-010HS	mPEG1K	1,000	CH3(CH2CH2O)23-CO(CH2)2COO—NHS
Pierce	Methyl PEG24-NHS Ester	MS(PEG)24	1,200	CH3(CH2CH2O)24-(CH2)2COO—NHS
Pierce	EZ-Link NHS-PEG12-Biotin	PEO12-Biotin	940	Biotin-(CH2CH2O)12-(CH2)2COO—NHS
Pierce	Methyl PEG12-NHS Ester	MS(PEG)12	690	CH3(CH2CH2O)12-(CH2)2COO—NHS
Pierce	EZ-Link NHS-PEG4-Biotin	PEO4-biotin	590	Biotin-(CH2CH2O)4-(CH2)2COO—NHS
Pierce	Mono(lactosylamido) mono(succinimidyl)suberate	LSS	590	
Pierce	EZ-link NHS-LC-LC-Biotin	LC-LC-Biotin	570	
Pierce	EZ-link NHS-LC-Biotin	LC-Biotin (LC = long chain)	450	
Pierce	EZ-link NHS-Biotin	Biotin	340	

The PEG (also called PEO) polymers purchased from Pierce Biotechnology (Rockford, Ill.) are monodispersed—i.e., they contain a single discrete polymer of a particular molecular weight. By contrast, the PEG polymers purchased from NOF (Nippon Oil and Fat) are polydispersed—i.e., they

contain a mixture of polymers having a distribution of molecular weights.

To PEGylate CNP22 or variants thereof, reaction and purification conditions are optimized for each PEG-CNP conjugate. According to a general PEGylation procedure, reaction mixtures contain about 1 mM CNP22 or a variant thereof, and about 1 to 5 mM NHS-activated PEG in potassium phosphate buffer, pH between about 5.0 and 6.5. To mono-PEGylate selectively at the peptide N-terminus and minimize PEGylation at an internal site (e.g., Lys4 of CNP22), the PEGylation reaction can be conducted under more acidic conditions (e.g., at a pH between about 5.5 and 6.5) to protonate selectively and hence deactivate the more basic primary amino group on the lysine side chain. After about 1 to 3 hours of incubation at room temperature, the PEGylation reaction is quenched by addition of aqueous glycine buffer. Reaction products are then separated hy reverse-phase HPLC, optimized for each PEG-CNP conjugate. Fractionation samples are speedvacced to dryness, and reconstituted/formulated in 1 mM HCl. Identification and purity of each PEG-CNP product are determined by liquid chromatography-mass spectrometry (LC/MS).

Example 2A

Recombinant Production of CNP Variants

CNP variants can be produced using recombinant technology. In certain embodiments, the CNP variants are produced as fusion proteins comprising a cleavable peptide, carrier protein or tag. Exemplary methods for recombinantly producing CNP fusion proteins are disclosed below.

Materials and Methods

Cloning of CNP Fusion Proteins into Expression Vectors

CNP DNA fragments were amplified using polymerase chain reaction (PCR) and the amplified PCR fragments were digested with Nde I and BamHI and cloned into pET21a vector (Novagen, Gibbstown, N.J.). CNP fusion protein DNA was synthesized by DNA2.0 and cloned into different expression vectors (Table 6).

TABLE 6

Construct	Vector	Product	Chemical cleavage	Final product	Expression *E. coli* strain
pJexpress-TAF-CNP	pJexpress401	TAF-CNP inclusion bodies	Formic acid (Asp-Pro)	Pro-CNP	BL21; BL21(DE3)
pJexpress-KSI-CNP(M/N)	pJexpress404	KSI-CNP(M/N) inclusion bodies	CNBr (Met-X)	CNP(M/N)	BL21
pET-31b-KSI-CNP	pET-31b	KSI-CNP inclusion bodies	Formic acid (Asp-Pro)	Pro-CNP	BL21(DE3)
pET-32a-Trx-CNP	pET-32a	Trx-CNP fusion protein (soluble)	Formic acid (Asp-Pro)	Pro-CNP	BL21(DE3)
pMAL-CNP	pMAL-c2X	MBP-CNP fusion protein (soluble)	Formic acid (Asp-Pro)	Pro-CNP	BL21(DE3)

CNP: GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC [Gly-CNP37 (SEQ ID NO: 75);
TAF: human transcription factor TAF12;
KSI: ketosteroid isomerase;
MBP: maltose-binding protein;
Trx: thioredoxin

Expression of CNP Fusion Proteins in *E. coli*

CNP fusion protein expression plasmids were transformed into *E. coli* BL21 or BL21(DE3). Transformed cells were plated on LB plates containing 100 ug/ml carbeniciline or 50 ug/ml kanamycin and incubated overnight at 37° C. One single colony was picked and cultured in 4 ml LB medium containing 100 ug/ml of carbeniciline or 50 ug/ml kanamycin at 37° C. with shaking. When an OD_{600} of bacterial culture reached 0.6, 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) was added to the cell media and the media was incubated at 37° C. for 3 hours with shaking. For cell harvest, bacterial cells were centrifuged at 4000 rpm for 10 minutes and the cell pellets were stored at −80° C. Cell pellets were lysed with B-PER II Bacterial Extraction Reagent (PIERCE, 0.4 ml per 4 ml of bacterial culture) and Benzonase Nuclease (Novagen, 0.025 U/ml) at room temperature for 10 minutes. Bacterial crude extract was reserved and centrifuged to obtain supernatant. Supernatant and crude extract were assayed for CNP fusion protein expression and solubility by SDS-PAGE and Western Blot.

Detection of CNP Fusion Protein Expression with SDS-PAGE and Western Blot

Ten uL of cell lysates or soluble supernatants was run on sodium dodecyl sulfate-polyacrylimide gel electrophoresis (SDS-PAGE) (Invitrogen, Carlsbad, Calif., NuPAGE 4-12% Bis-tris Gel, MES SDS buffer). The gel was stained using 20 ml Imperial Protein Stain (Thermo Fisher, Rockford, Ill.) at room temperature for 1 hour and de-stained with water. For Western blot, the protein was transferred to membrane with Gel blot (Invitrogen). The membrane was blocked in TBS buffer with 5% milk at room temperature for 1 hour. Rabbit anti-CNP22 antibody (1:2500 dilution) (Bachem, Torrance, Calif.) was added to the membrane, which was then incubated at room temperature with shaking for 2 hours, and then the membrane was washed 3 times with TBS buffer.

Alkaline phosphate (AP) conjugated anti-rabbit IgG (1:5000 dilution) was added to the membrane, which was then incubated at room temperature with shaking for 1 hour, and then the membrane was washed 3 times with TBS buffer. Ten ml WESTERN BLUE®Stabilized Substrate (Promega, Madison, Wis.) was added to the membrane, which was then incubated at room temperature with shaking for 1 to 5 min, and then the membrane was washed with TBS buffer to remove excess stain.

Expression of TAF-CNP Fusion Protein in *E. coli* BL21

Cells (*E. coli* strain BL21) expressing TAF-CNP fusion protein were obtained from glycerol stock stored at −80° C. and were grown in 4 ml LB medium containing 50 ug/ml of kanamycin at 37° C. overnight with shaking (250 rpm). Four ml of overnight grown cell culture was transferred to 200 ml

LB medium containing 50 ug/ml of kanamycin and was grown at 37° C. with shaking (250 rpm). When the OD_{600} reached 0.6, IPTG was then added to a final concentration of 1 mM to induce protein expression at 37° C. with shaking (250 rpm) for 3 hours. Cells were then spun down at 3000 rpm for 10 minutes and the resulting cell pellet was frozen at −80° C.

Purification of TAF-CNP Inclusion Bodies and Formic Acid Cleavage

The cell pellet (from 200 ml culture) was resuspended in 25 ml of B-PER II buffer (PIERCE), the pellet was sonicated for 10 minutes (50%, 1 second, pause 2 seconds) on ice, centrifuged at 12000 rpm for 20 minutes at 4° C., and then the pellet was resuspended in 25 ml 20× diluted B-PER II buffer. This was repeated until the supernatant became clear (3-5 times). One mL of resuspended TAF-CNP inclusion bodies was transferred to a 1.5 ml tube and centrifuged at 14000 rpm for 15 min. The supernatant was discarded and the pellet was dissolved with 10 ul of 88% formic acid, and then 490 ul of Millipore filtered water was added immediately. The pellet was mixed well by vortex and incubated at 55° C. for 20 to 24 hours (70° C./6 hours are alternative conditions). The products of the formic acid cleavage were assayed by SDS-PAGE and LC/MS (C4RP).

LC/MS Sample Preparation

Inclusion bodies were isolated from about 8 mL of culture (about 1.5 OD) and the pellet was solubilized in 10 uL nt formate. Resolubilized pellet was immediately diluted to 2% or 10% final formate concentration (0.5 mL) and incubated at 55° C. for 21 hours (pH 2) (cloudiness was more evident in the 2% formate sample the next day). Both samples were centrifuged at 15000 rpm for 2 minutes. Twenty uL of supernatant was injected into an LC/MS (C4 RP) apparatus.

Results

CNP Fusion Proteins were Expressed in *E. coli*

All CNP fusion proteins were expressed in *E. coli* induced with 1 mM IPTG at 37° C. for 3 hours (FIG. **1**). The constructs pJexpress-TAF-CNP, pJexpress-KSI-CNP(M/N) and pET-31b-KSI-CNP were expressed as inclusion bodies, while the constructs pET-32a-Trx-CNP and pMAL-CNP were expressed as soluble fusion proteins. Western blot using anti-CNP22 antibody confirmed the expression of the CNP fusion proteins (FIG. **1**).

CNP was Produced from TAF-CNP Inclusion Bodies by Formic Acid Cleavage

TAF-CNP inclusion bodies were partially purified and treated with 2% formic acid at 55° C. for 20 to 24 hours (70° C./6 hours are alternative conditions). The majority of TAF-CNP was cleaved and one extra band having similar size as Gly-CNP37 peptide appeared on SDS-PAGE (FIG. **2**). The cleaved sample was further analyzed by LC/MS (C4 RP). The LC/MS results showed that CNP was released in soluble form from TAF-CNP inclusion bodies after formic acid cleavage. LC/MS analysis indicated that formic acid cleavage of the CNP fusion proteins resulted in formation of cyclized Pro-Gly-CNP37 (MW=4102). Calculation of protein amounts based on analysis suggested that about 60 ug of formic acid-generated CNP was produced from 8 mL of very low OD culture. From a small scale (e.g., about 8 mL) of low OD (e.g., 1.2 OD) cell culture, approximately 8 ug/ml CNP was produced, while fermentation of a larger scale (e.g., about 8 L) of higher OD (e.g., 380D) cell culture can produce approximately 1 mg/ml CNP.

Conclusion

Five expression constructs were generated to express CNP fusion proteins. Expression of all five constructs produced soluble (Trx and MBP) or insoluble (TAF and KSI) CNP fusion proteins. Approximately 1 mg/ml soluble CNP can be produced from TAF-CNP inclusion bodies by a simple formic acid cleavage procedure.

Example 2B

Production of Additional CNP Variants in *E. coli*

Recombinant production of CNP variants was carried out as described in Example 2A. In this example, additional CNP constructs were generated with a QuikChange II XL site-directed mutagenesis kit (Stratagene) or were synthesized by DNA2.0. Additional CNP constructs and expression vectors are listed in Table 7.

TABLE 7

Construct	Vector	Product	Chemical cleavage	Final product	Expression *E. coli* strain
pJexpress-TAF(C/A)-Pro-CNP38	pJexpress401	TAF(C/A)-Pro-CNP38 inclusion bodies	Formic acid (Asp-Pro)	Pro-CNP38	BL21(DE3)
pJexpress-TAF(4D/4E)-Pro-CNP38	pJexpress401	TAF(4D/4E)-Pro-CNP38 inclusion bodies	Formic acid (Asp-Pro)	Pro-CNP38	BL21(DE3)
pJexpress-TAF(C/A&4D/4E)-Pro-CNP38	pJexpress401	TAF(C/A&4D/4E)-Pro-CNP38 inclusion bodies	Formic acid (Asp-Pro)	Pro-CNP38	BL21(DE3)
pJexpress-TAF(C/A&10D/10E)-Pro-CNP38	pJexpress401	TAF(C/A&10D/10E)-Pro-CNP38 inclusion bodies	Formic acid (Asp-Pro)	Pro-CNP38	BL21(DE3)
pJexpress-TAF-NL-(C/A & 6D/6E)-Pro-CNP38	pJexpress401	TAF-NL-(C/A & 6D/6E)-Pro-CNP38 inclusion bodies	Formic acid (Asp-Pro)	Pro-CNP38	BL21(DE3)
pJexpress-BMP-Pro-CNP38	pJexpress401	BMP-Pro-CNP38 inclusion bodies	Formic acid (Asp-Pro)	Pro-CNP38	BL21; BL21(DE3)

TABLE 7-continued

Construct	Vector	Product	Chemical cleavage	Final product	Expression *E. coli* strain
pJexpress-TAF-Pro-CNP37	pJexpress401	TAF-Pro-CNP37 inclusion bodies	Formic acid (Asp-Pro)	Pro-CNP37	BL21; BL21(DE3)
pJexpress-BMP-Pro-CNP37	pJexpress401	BMP-Pro-CNP37 inclusion bodies	Formic acid (Asp-Pro)	Pro-CNP37	BL21; BL21(DE3)
pJexpress-TAF Pro-HSA-CNP	pJexpress401	TAF-Pro-HSA-CNP inclusion bodies	Formic acid (Asp-Pro)	Pro-HSA-CNP	BL21
pJexpress-TAF	pJexpress401	TAF inclusion bodies	N/A	N/A	BL21(DE3)
pJexpress-BMP	pJexpress401	BMP inclusion bodies	N/A	N/A	BL21(DE3)
pJexpress-TAF(C/A)	pJexpress401	TAF(C/A) inclusion bodies	N/A	N/A	BL21(DE3)
pJexpress-TAF(4D/4E)	pJexpress401	TAF(4D/4E) inclusion bodies	N/A	N/A	BL21(DE3)
pJexpress-TAF (C/A&4D/4E)	pJexpress401	TAF(C/A&4D/4E) inclusion bodies	N/A	N/A	BL21(DE3)
pJexpress-TAF (C/A&10D/10E)	pJexpress401	TAF(C/A&10D/10E) inclusion bodies	N/A	N/A	BL21(DE3)
pJexpress-TAF-NL-(C/A & 6D/6E)	pJexpress401	TAF-NL-(C/A&6D/6E) inclusion bodies	N/A	N/A	BL21(DE3)
pJexpress-TAF-Pro-CNP53	pJexpress401	TAF-Pro-CNP53 inclusion bodies	Formic acid (Asp-Pro)	Pro-CNP53	BL21(DE3)
pJexpress-TAF-CNP34	pJexpress401	TAF-CNP34 inclusion bodies	Formic acid (Asp-Pro)	CNP34	BL21(DE3)

CNP38: GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC [Gly-CNP37 (SEQ ID NO: 75)];

Pro-CNP38: PGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC [Pro-Gly-CNP37] (SEQ ID NO: 145);

CNP37: QEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (SEQ ID NO: 60);

HSA-CNP: GHKSEVAHRFKGANKKGLSKGCFGLKLDRIGSMSGLGC [HSA-CNP27 (SEQ ID NO: 144)];

Pro-CNP53: PDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIG-SMSGLGC (SEQ ID NO: 185);

CNP34: PNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (SEQ ID NO: 163);

TAF-Pro-CNP38: MVLTKKKLQDLVREVCPNEQLDEDVEEMLLQIADDFIESVVTAA-CQLARHRKSSTLEVKDVQLHLERQWNM-WIMGSSHHHHHHSSGLVPRGSHT-GDDDDKHMDPGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (SEQ ID NO: 196);
TAF: human transcription factor TAF12 histone fold domain (HFD) and linker from pET-15b vector;

TAF-NL: TAF12 HFD without a linker;

TAF12 HFD: VLTKKKLQDLVREVCPNEQLDEDVEEMLLQIADDFIESVVTAACQLA-RHRKSSTLEVKDVQLHLERQWNMWI (SEQ ID NO: 197);
pET-15b linker: MGSSHHHHHHSSGLVPRGSHTGDDDDKHMD (SEQ ID NO: 195);

TAF(C/A): cysteine in TAF is changed to alanine;

TAF(D/E): aspartic acid in TAF is changed to glutamic acid (number indicates which amino acid residue is changed);
TAF(C/A & D/E): cysteine and aspartic acid in TAF are changed to alanine and glutamic acid, respectively;
BMP: bone morphogenetic protein 2 with seven C/A (cysteine to alanine) mutations;

KSI: ketosteroid isomerase;

MBP: maltose-binding protein;

TRX: thioredoxin

Results

All CNP fusion proteins were expressed in *E. coli* induced with 1 mM IPTG at 37° C. for 3 hours. Constructs pJexpress-BMP-Pro-CNP38, pJexpress-TAF-Pro-CNP37, pJexpress-BMP-Pro-CNP37, pJexpress-Pro-HSA-CNP, pJexpress-TAF and pJexpress-BMP were expressed as inclusion bodies. A Western Blot with anti-CNP antibody was used to confirm the expression (FIG. **3**).

Pro-Gly-CNP37 ("Pro-CNP38") was Produced from TAF-Pro-CNP38 Inclusion Bodies by Formic Acid Cleavage

Formic acid is used as a denaturant for inclusion body proteins and can specifically cleave the peptide bond between Asp and Pro under optimized conditions. TAF-Pro-CNP inclusion bodies were partially purified as described above and treated with 50% formic acid at 25° C., 37° C., 42° C. and 55° C. for 24 hours. Most of the TAF-Pro-CNP38 was cleaved and one extra band with a similar size as the Gly-CNP37 ("CNP38") peptide showed on SDS-PAGE from the 37° C., 42° C. and 55° C. cleavages (FIG. **4**A). The cleavage reactions at 37° C. and 55° C. were neutralized with 10 M NaOH and centrifuged at 14,000 rpm for 15 minutes. The un-cleaved TAF-Pro-CNP38, TAF and other inclusion bodies precipitated in the pellets. The supernatants contained soluble Pro-CNP38 and were further analyzed by LC/MS. The LC/MS

result showed the supernatants contained a mixture of non-specific cleaved peptides generated by excess acidic hydrolysis.

When TAF-Pro-CNP inclusion bodies were treated with 2% and 10% formic acid at 55° C. for 20 hours, the majority of TAF-Pro-CNP38 was cleaved and one extra band having a similar size as Gly-CNP37 was observed on SDS-PAGE (FIG. **4**B). The cleaved sample was further analyzed by LC/MS. The LC/MS analysis showed that correct Pro-CNP38 was released in soluble form from TAF-Pro-CNP38 inclusion bodies after formic acid cleavage. The yields of Pro-CNP38 from 2% and 10% formic acid cleavages were similar (FIG. **4**C).

Formic Acid Cleavage and Neutralization for Pro-Gly-CNP37 ("Pro-CNP38") Production and Purification

Formic acid can dissolve and cleave TAF-Pro-CNP38, BMP-Pro-CNP38 and other inclusion bodies. The pH neutralization results in precipitation of insoluble contaminating proteins/peptides (un-cleaved TAF-Pro-CNP38 and BMP-Pro-CNP38, TAF, BMP and others). The soluble Pro-CNP38 stays in the supernatant after centrifugation. TAF-Pro-CNP38 and BMP-Pro-CNP38 were cleaved in 2% formic acid at 55° C. or 70° C. for 24 hours. The cleavage reactions were neutralized with 1:1 ratio of 0.5 M Tris buffer and centrifuged at 14,000 rpm for 15 minutes. Results showed that the supernatants contained almost pure peptides and there was no observed recovery loss of Pro-CNP38 upon neutralization. This is a simple and efficient step for Pro-CNP38 purification.

Analysis of Varying Conditions for Formic Acid Cleavage of TAF-Pro-CNP38 Inclusion Bodies for Pro-Gly-CNP37 ("Pro-CNP38") Production

Formic acid can specifically cleave the peptide bond between Asp and Pro under optimized conditions. Non-specific cleavage of peptide bonds between Asp and any other amino acids or even non-specific cleavage of any peptide bond may occur if the formic acid cleavage conditions are not optimized. TAF-Pro-CNP38 inclusion bodies were cleaved with 2% formic acid at 42° C., 55° C. or 70° C. for 6, 24 or 48 hours. FIG. **5**A shows that TAF-Pro-CNP38 was cleaved completely at 70° C. for 24 hours or at 55° C. for 48 hours. The 70° C. cleavage could be completed within 17 hours (FIG. **5**B). Although the 70° C./24 h cleavage gave the highest yield, the non-specific cleavage products (for example, the peptide with molecular weight of 3142 generated from Pro-CNP38 by cleaving between peptide bond Asp and Arg) increased dramatically (FIG. **5**C).

The yield and purity of Pro-CNP38 production were improved when TAF-Pro-CNP38 inclusion bodies were purified or treated with B-PER II buffer before formic acid cleavage. Because B-PER II buffer contains detergent octylthioglucoside and is relatively expensive, other commonly used detergents or buffers were tested for large-scale Pro-CNP38 production. TAF-Pro-CNP38 inclusion bodies were re-suspended in different detergents (Octylsucrose; Triton x-100; Tween-20; NP-40; CA-630) or buffers (B-PER II; B-PER II 1/20 dilution; B-PER; B-PER phosphate buffer; 25 mM tris, 150 mM NaCl, pH 7.9; 25 mM tris, pH 7.5; filtered water; PBS) and incubated at room temperature (RT) for 24 hours. All detergents were 1% in 25 mM tris buffer, pII 7.5. After incubation in detergent or buffer, TAF-Pro-CNP38 inclusion bodies were cleaved by 2% formic acid at 55° C. for 22 hours. Results showed that BPER II continued to exhibit good yield, and positive results were also obtained with CA630 and Triton X-100.

Pro-Gly-CNP37 ("Pro-CNP38") Proteolytic Cleavage Products

One unidentified protease, possibly a membrane associated protease, cleaved the Pro-CNP38 peptide (produced from BL21 strain, MW 4102) into two peptides during Pro-CNP38 purification, resulting in peptides PGQEHPNAR (MW 1004) (SEQ ID NO: 198) and KYKGANKKGLSKGCFGLKLDRIGSMSGLGC (MW 3115) (SEQ ID NO: 199). Not to be bound by theory, one possible reason why detergents may improve the yield and purity of Pro-CNP38 is that detergents can remove most, but possibly not all, of the unidentified protease. High temperature, basic pH and EDTA were tested to identify if these agents could inhibit the protease cleavage. TAF-Pro-CNP38 inclusion bodies were incubated at RT or 120° C. for 2 hrs and centrifuged at 14000 rpm for 15 min. The pellets were re-suspended in 2% formic acid and incubated at 55° C. or 70° C. for 18 hours. A 1:1 ratio of 0.5 M Tris was added to neutralize the cleavage. The neutralized samples were centrifuged at 14000 rpm for 5 min and the supernatants were left at RT for 6 hrs or 22 hrs with or without 10 mM EDTA, pH 10. The proteolytic cleavage was assayed by LC/MS (Table 8).

TABLE 8

LC/MS result of Pro-CNP38 proteolytic cleavage

Sample		A210 Peak Area	Protein Conc. (mg/mL)		Percent MW4102	Percent MW3115
1	H 55 C.	942	0.04	*	91.8	8.2
2	H 70 0	2878	0.11	*	79.2	20.7
3	H 70 6H	2675	0.10	*	80.6	19.4
4	H 70 24	2741	0.11	*	79.2	20.7
5	H 70 EDTA	2385	0.09	*	80.8	19.2
6	H 70 pH 10	1917	0.07	*	81.2	18.8
7	55 C.	1291	0.05		61.1	38.9
8	70 C. 0	4533	0.18		96.8	3.2
9	70 C. 6H	4120	0.16		97.5	2.5
10	70 C. 24	4108	0.16		96.5	3.5
11	70 C. EDTA	4336	0.17		96.9	3.1
12	70 C. pH 10	3425	0.13		97.5	2.4

All samples (8-12) cleaved at 70° C. showed limited proteolytic cleavage (less than 4% cleavage of Pro-CNP38). Almost 40% of Pro-CNP38 was cleaved by protease when cleavage was carried out at 55° C. (sample 7). Basic pH and EDTA did not influence the non-specific proteolytic cleavage. High temperature (120° C. for 2 hrs) non-specifically cleaved Pro-CNP38.

It should be noted that the BL21(DE3) strain from Stratagene does not have the unidentified protease.

Production of Pro-Gly-CNP37 ("Pro-CNP38") and Other CNP Variants from Different Constructs:

Pro-CNP38 can be produced in large scale in *E. coli* by means of overexpression of a TAF-Pro-CNP38 fusion protein as inclusion bodies followed by formic acid cleavage of the fusion protein. Following the methods described herein, other TAF-CNP fusion proteins (TAF-CNP34 and TAF-Pro-CNP53) were expressed as inclusion bodies and then cleaved with formic acid to generate the CNP variants CNP34 and Pro-CNP53. FIG. **6** depicts the expression of TAF-CNP34, FIG. **7** the expression of TAF-Pro-CNP53, FIG. **8** the products of formic acid cleavage of TAF-CNP34 and TAF-Pro-CNP53, FIG. **9** the peak for CNP-34 in an LC/MS chromatogram, and FIG. **10** the peak for Pro-CNP53 in an LC/MS chromatogram.

Use of formic acid may result in unspecific cleavage(s) at peptide bond(s) other than the targeted Asp-Pro bond. To

improve the purity and overall titer of the desired formic acid cleavage product, different residues of aspartic acid in TAF12 or fragments thereof were changed to glutamic acid. Moreover, one or more cysteine residues in TAF12 or fragments thereof were changed to alanine to prevent unspecific disulfide bond formation. All TAF-Pro-CNP38 fusion proteins having such mutations in TAF12 were expressed as inclusion bodies and cleaved with formic acid to produce Pro-CNP38. FIG. **7** shows the expression of TAF-NL-(C/A & 6D/6E)-Pro-CNP38 and TAF(C/A & 10D/10E)-Pro-CNP38, FIG. **11** the expression of TAF(C/A & 4D/4E)-Pro-CNP38 and TAF(4D/4E)-Pro-CNP38, FIG. **12** the products of formic acid cleavage of TAF(4D/4E)-Pro-CNP38 and TAF(C/A & 4D/4E)-Pro-CNP38, and FIG. **13** the products of formic acid cleavage of TAF-NL-(C/A & 6D/6E)-Pro-CNP38 and TAF(C/A & 10D/10E)-Pro-CNP38. Table 9 summarizes the purity (prior to purification) and titer of Pro-CNP38 obtained from the various TAF-Pro-CNP38 constructs.

TABLE 9

Construct	Purity	Titer (ug/mL)
pJexpress-TAF-Pro-CNP38	32%	44
pJexpress-TAF(C/A)-Pro-CNP38	41%	50
pJexpress-TAF(4D/4E)-Pro-CNP38	36%	52
pJexpress-TAF(C/A & 4D/4E)-Pro-CNP38	42%	58
pJexpress-TAF(C/A & 10D/10E)-Pro-CNP38*	32%	26
pJexpress-TAF-NL-(C/A & 6D/6E)-Pro-CNP38	50%	55

*Cells with pJexpress-TAF(C/A&10D/10E)-Pro-CNP38 growed more slowly and the final cell density (OD_{600}) was lower compared to other TAF-Pro-CNP38 constructs.

Large-scale Production of Pro-Gly-CNP37 ("Pro-CNP38") by Fermentation and Formic Acid Cleavage

BL21(DE3) cells comprising the pJexpress-TAF-CNP construct were grown in a 10 liter fermenter at 37° C. for about 16-17 hours until OD_{600} reached 64. The cells were then grown/cultured in the presence of 1 mM IPTG at about 35-37° C., to induce expression of the TAF-Pro-CNP38 fusion protein, for about 7-8 hours until OD_{600} reached 160. The fermentation produced a titer of 9 g/L TAF-Pro-CNP38. FIG. **14** displays a Western blot of the TAF-Pro-CNP38 fusion protein produced in the fermentation.

A cell pellet recovered from 750 mL cell culture from the 10 L fermentation was resuspended in phosphate-buffered saline, pH 7.4 (PBS) and lysed by three passes through a pressure homogenizer (10,000 bar). The resulting lysate was centrifuged at 6,500 g for 10 minutes and the supernatant was discarded. The pellet fraction containing insoluble TAF-Pro-CNP38 fusion protein inclusion bodies was resuspended in 500 mL PBS with a rotostator. The suspension was centrifuged at 6,500 g for 10 minutes and the supernatant was discarded. The resulting inclusion body pellet was resuspended in 500 mL water with a rotostator and incubated at 55° C. for 30 minutes. 250 mL 6% formic acid was added to the warmed inclusion body suspension for a final concentration of 2% formic acid and incubated at 55° C. for 20-24 hours. 50 mL of 400 mM Na_2HPO_4 was added after 20-24 hours to begin the neutralization of the formic acid cleavage reaction, and the resulting mixture was titrated with 50% w/v NaOH to pH 6.9-7.4 and left at room temperature for 30 minutes. Upon neutralization, a heavy precipitate formed and was removed by centrifugation at 6,500 g for 10 minutes. The supernatant was retained and contained on average 1.3 g/L culture of 80% pure Pro-CNP38. The majority of the *E. coli* and TAF-related proteins and peptides remained in the pellet.

The soluble Pro-CNP38 resulting from the formic acid cleavage and neutralized supernatant was 80% pure and contained a mixture of linear and cyclized Pro-CNP38 peptides along with other product-related impurities. The pH neutral supernatant containing Pro-CNP38 was sterile-filtered. Further purification by anion-exchange chromatography using a Fractogel TMAE Hi-CAP column (EMD Biosciences) to remove DNA, endotoxins, and peptide contaminants, which should bind to the column, was performed at pH 7-7.4. The flow-through fraction contained partially cyclized Pro-CNP38. Cupric sulfate was added to the flow-through fraction to a final concentration of 10 uM and incubated at room temperature for 1 hour. The addition of Cu^{++} at neutral pH catalyzed the oxidation of free cysteine sulfhydryl groups on the peptide to form an intramolecular disulfide bond, resulting in 100% cyclized Pro-CNP38 and no detectable linear peptide. The conductivity of the solution was adjusted to <15 mS/cm by the addition of water. Cation-exchange chromatography using an SP-Sepharose column (GE Healthcare) and a sodium phosphate buffer (pH 7) was then performed to remove any remaining DNA and endotoxins in the flow-through and further purify Pro-CNP38 to about 95-96% purity with <0.5% non-product related impurities. FIG. **15** is an SDS-PAGE of eluate fractions from the SP-Sepharose column; the highest concentrations of Pro-CNP38 were found in fractions 22 to 30. Reverse-phase HPLC/MS analysis of non-pooled fraction 24 indicated the presence of 90% Pro-CNP38, 5% Pro-CNP38 with an oxidized methionine residue, 3% Pro-CNP38 cleaved at the Gly-Cys bond to form CNP-17, and 1.6% Pro-CNP38 cleaved at the Asp-Arg bond in the cyclic domain. The yield of pure Pro-CNP38 peptide upon final purification was 0.9 g/L cell culture (36% total recovery).

Pro-CNP38 collected from five separate purifications was pooled for formulation. The pooled product contained 93.5% Pro-CNP38, 3.3% Pro-CNP38 with an oxidized methionine residue, 1.3% deamidated Pro-CNP38, and 1% Pro-CNP38 cleaved at the Gly-Cys bond to form CNP-17. Samples were diluted with 50 mM sodium phosphate (pH 7) to a conductivity of 10 mS/cm and loaded onto a CM-Sepharose column (GE-Healthcare) for concentration and buffer exchange. The weak cation-exchange property of the CM-Sepharose resin allows peptides to dissociate from the column with weak acid solutions rather than typical salt gradients. Acid concentrations required for dissociation of Pro-CNP38 from the CM-Sepharose column depended upon column loading. At 50 mg Pro-CNP38 per mL of resin, 10 mM HCl was sufficient for Pro-CNP38 elution. At 9 mg Pro-CNP38 per mL of resin, 50 mM HCl was required for elution. When the loading was 9 mg Pro-CNP38 per mL of resin, Pro-CNP38 eluted in less than one column volume, which significantly concentrated the peptide. The eluate fraction contained 20.3 mg/mL of 95% pure Pro-CNP38, along with 3% Pro-CNP38 with an oxidized methionine residue, 1% deamidated Pro-CNP38, and <1% Pro-CNP38 cleaved at the Gly-Cys bond to form CNP-17. The concentrated solution of Pro-CNP38 in weak acid is suitable for dilution into appropriate buffers for either liquid or lyophilized formulations.

Example 3

Cleavage of CNP Variants by Neutral Endopeptidase In Vitro

To determine the effects of amino acid substitutions, amino acid extensions, backbone modifications, side chain modifications and PEGylation on the susceptibility of CNP variants to neutral endopeptidase (NEP) cleavage, peptide cleavage assays were carried out using an in vitro assay that monitored disappearance of the non-cleaved CNP variant.

Recombinant human NEP (1 ug/mL final concentration) was added to 100 uM CNP variant diluted in 0.1 M Tris, pH 7. The reaction mixture was incubated at 37° C. for various periods of time, and the reaction was quenched with EDTA (10 mM final) followed by heat denaturation. The reaction mixture was reduced and then the reaction products were analyzed using HPLC and mass spectroscopy. The half-life of the CNP variant was calculated based on the disappearance of intact CNP variant over time. The results for digested CNP variants were compared to a parallel wtCNP22 digestion and normalized to the results for 100 uM CNP22 digested by 1 mg/mL NEP ($t_{1/2}$=80 min).

Table 1 lists the half-lives, based on the in vitro NEP cleavage assay, of various CNP variants having backbone or side chain modifications. Removal of three of the six NEP cleavage sites in Analog L nevertheless resulted in a substantially shorter half-life. Of tested CNP variants, the greatest resistance to NEP cleavage was exhibited by Analog N, which contains the D-enantiomer of all 22 amino acids of CNP22, and by Analog M, which has an N-methylated amide bond at both Leu9 and Leu11. However, both Analogs N and M failed to stimulate production of cGMP (see below).

Comparing the half-lives of Analogs A, B, E, F, G and H to one another, half-lives were determined to be about 1.5- to about 2.5-fold longer for Analogs E and G compared to those for Analogs A, B, F and H. All of these six analogs showed resistance or improved resistance to cleavage at the Cys6-Phe7 bond relative to wtCNP22 (data not shown). The rank order of analog resistance to NEP at 1 ug/ml, based on half-life, is Analog G (3-Cl-Phe)≥Analog E (D-Phe)>Analog H ("beta-2 Phe"), Analog B (N-Me-Phe), and Analog F (t-Bu-Gly)=wtCNP22>Analog A (Cys-CH_2—NH). Analogs E and G have about 1.5 times longer half-life in comparison to wtCNP22. Besides resistance to cleavage of the Cys6-Phe7 bond, Analogs B, E, F, G and H also exhibited resistance to cleavage of the Gly8-Leu9 bond in the presence of 1 ug/mL NEP (data not shown). These results indicate that CNP variants having backbone or side chain modifications between Cys6 and Gly8 can be resistant to NEP cleavage of the Cys6-Phe7 bond and/or Gly8-Leu9 bond, but do not necessarily have improved overall resistance to NEP or a longer half-life than CNP22. The results seem to be contrary to reports in the literature that NEP first cleaves at the Cys6-Phe7 bond of CNP22 and then elsewhere.

TABLE 1

Analog	Backbone and Side Chain Modifications Natriuretic Peptide	cGMP Response rel. to 1 uM CNP22[1] 10 nM	 1 uM	NEP Cleavage ($t_{1/2}$, min)
	CNP22 (SEQ ID NO: 1)	46 ± 10	100 ± 13	80[2]
N	D-CNP22 (all D-amino acids) (SEQ ID NO: 115)	2	1	>>160
A	CNP22, C6—CH2—NH (reduced carbonyl) (SEQ ID NO: 56)	6	66	55
B	CNP22, N-methyl-F7 (methylated amide) (SEQ ID NO: 57)	2	38	80
BD	CNP22, N-methyl-L9 (SEQ ID NO: 116)	2	8	ND
BN	CNP22, N-methyl-L11 (SEQ ID NO: 117)	10	51	ND
BE	CNP22, N-methyl-L20 (SEQ ID NO: 118)	2	5	ND
M	CNP22, N-methyl-L9, N-methyl-L11 (SEQ ID NO: 94)	1	11	>>160
K	CNP22, N-methyl-L9, N-methyl-L20 (SEQ ID NO: 92)	1	1	80
L	CNP22, N-methyl-L9, N-methyl-L11, N-methyl-L20 (SEQ ID NO: 93)	18	10	30
J	CNP22, C6—CH2—NH, N-methyl-L9, N-methyl-L20 (SEQ ID NO: 91)	ND	ND	50
E	CNP22, D-F7 (D-Phe) (SEQ ID NO: 136)	2	6	130
H	CNP22, Beta-2-F7 (3-amino-2-phenylpropionyl) (SEQ ID NO: 57)	2	2	80
G	CNP22, 3-chloro-F7 (SEQ ID NO: 137)	17	93	135
F	CNP22, t-butyl-G8 (SEQ ID NO: 58)	2	18	80
V	CNP22, K4G, 3,4-dichloro-F7 (SEQ ID NO: 119)	ND	ND	68
X	CNP22, K4G, 3-methyl-F7 (SEQ ID NO: 120)	ND	ND	68
	ANP	10	23	ND

[1]Stimulation of cGMP production in NIH3T3 cells by natriuretic peptide relative to cGMP production in the presence of 1 uM CNP22

[2]CNP22 NEP resistance $t_{1/2}$ averaged 80 min. Due to variations in NEP catalytic activity between experiments, all CNP22 $t_{1/2}$ digestions were normalized to 80 min and the difference coefficient was used to calculate analog $t_{1/2}$ in each experiment to obtain an adjusted $t_{1/2}$.

ND = Not Determined

Table 2 lists the half-lives, based on the in vitro NEP cleavage assay, of various CNP variants having substitutions with natural and/or unnatural amino acids. Of tested variants, the greatest resistance to NEP cleavage was shown by Analog BK, which has K4R and G15S substitutions, and Analog BJ, which has K4R and G15N substitutions.

TABLE 2

Analog	Specificity Mutations Natriuretic Peptide	cGMP Response relative to 1 uM CNP22[1] 10 nM	1uM	NEP Cleavage ($t_{1/2}$, min)
	CNP22 (SEQ ID NO: 1)	46 ± 10	100 ± 13	80
AH	CNP22, K4R (SEQ ID NO: 35)	59	121	80
BP	CNP22, K4R, G5S (SEQ ID NO: 121)	45	ND	ND
BO	CNP22, K4R, G5R (SEQ ID NO: 122)	18	80	ND
P	CNP22, K4G (SEQ ID NO: 123)	ND	ND	68
Z	CNP22, K4R, F7Y (SEQ ID NO: 95)	2	18	ND
AB	CNP22, K4R, G8S (SEQ ID NO: 97)	26 ± 26	86 ± 17	ND
AA	CNP22, K4R, G8V (SEQ ID NO: 96)	3	25	ND
AC	CNP22, K4R, G8T (SEQ ID NO: 98)	11 ± 2	66 ± 16	80
AD	CNP22, K4R, L9T (SEQ ID NO: 99)	4	68	ND
BH	CNP22, K4R, K10R (SEQ ID NO: 112)	12	80	ND
BF	CNP22, K4R, K10Cit (SEQ ID NO: 110)	6	33	ND
BG	CNP22, K4R, K10Q (SEQ ID NO: 111)	9	45	ND
BY	CNP22, K4R, K10S (SEQ ID NO: 124)	16	53	ND
BK	CNP22, K4R, G15S (SEQ ID NO: 114)	13 ± 1	71 ± 11	≥160
BJ	CNP22, K4R, G15N (SEQ ID NO: 113)	4	41	150
AE	CNP22, K4R, G15R (SEQ ID NO: 100)	0.3	0.3	ND
AF	CNP22, K4R, G15Cit (SEQ ID NO: 101)	1.4	2	ND
BZ	CNP22, K4R, S16Q (SEQ ID NO: 125)	42	116	ND
BX	CNP22, K4R, M17N (SEQ ID NO: 126)	40 ± 2	103 ± 17	ND
AG	CNP22, K4R, M17V (SEQ ID NO: 102)	10	65	ND
BQ	CNP22, K4R, G19S (SEQ ID NO: 127)	21	63	ND
BR	CNP22, K4R, G19R (SEQ ID NO: 128)	22 ± 6	84 ± 10	ND
AJ	CNP22, K4R, L20V (SEQ ID NO: 103)	0.2	8	ND
AK	CNP22, K4R, L20t-butyl-Ala (SEQ ID NO: 104)	1	21	ND
AT	CNP22, G1E, K4E (SEQ ID NO: 105)	11	54	60
BS	CNP22, K4R, L20R (SEQ ID NO: 129)	11	8	ND
BT	CNP22, K4R, G21S (SEQ ID NO: 130)	7	39	ND
BU	CNP22, K4R, G21T (SEQ ID NO: 131)	6	21	ND
BW	CNP22, K4R, G21R (SEQ ID NO: 132)	20	21	ND
	ANP	10	23	ND

[1]Stimulation of cGMP production in NIH3T3 cells by natriuretic peptide relative to cGMP production in the presence of 1 uM CNP22
[2]CNP22 NEP resistance $t_{1/2}$ averaged 80 min. Due to variations in NEP catalytic activity between experiments, all CNP22 $t_{1/2}$ digestions were normalized to 80 min and the difference coefficient was used to calculate analog $t_{1/2}$ in each experiment to obtain an adjusted $t_{1/2}$.
ND = Not Determined

Table 3 lists the half-lives, based on the in vitro NEP cleavage assay, of CNP variants having N-terminal and/or C-terminal modifications, including amino acid extensions. Of the analogs tested, Analogs AZ, CC, CF, BL, CS, CK and CL, Pro-Gly-CNP37 and HSA-CNP27 were most resistant to NEP degradation.

TABLE 3

	N- and C-Terminal Modifications Natriuretic Peptide	cGMP Response rel. to 1 uM CNP22[1] 10 nM	1 uM	NEP Cleavage ($t_{1/2}$, min)
	CNP22	46 ± 10	100 ± 13	80
BC	Pentanoic acid (N-term.)-CNP22, G1E (SEQ ID NO: 109)	ND	ND	ND
BB	Heptanoic acid (N-term.)-CNP22, G1E (SEQ ID NO: 108)	32 ± 4	84 ± 19	45-65
AV	Pentanoic acid (N-term.)-CNP22, G1E, K4E (SEQ ID NO: 106)	ND	ND	120
AW	Heptanoic acid (N-term.)-CNP22, G1E, K4E (SEQ ID NO: 107)	ND	ND	<20
AX	CNP17 (delta N-term) (SEQ ID NO: 2)	18	69	ND
	R-CNP22 (SEQ ID NO: 40)	ND	ND	ND
AZ	R-CNP22, K4R (SEQ ID NO: 41)	54 ± 11	106 ± 15	≥160
	ER-CNP22 (SEQ ID NO: 38)	ND	ND	ND

TABLE 3-continued

	N- and C-Terminal Modifications Natriuretic Peptide	cGMP Response rel. to 1 uM CNP22[1] 10 nM	1 uM	NEP Cleavage ($t_{1/2}$, min)
BA	ER-CNP22, K4R (SEQ ID NO: 39)	38 ± 10	113 ± 10	90
	GANRR-CNP22 (SEQ ID NO: 65)	ND	ND	ND
AY	GANRR-CNP22, K4R (SEQ ID NO: 36)	59 ± 9	105 ± 20	65
	GANQQ-CNP22 (SEQ ID NO: 64)	ND	ND	ND
CH	GANQQ-CNP22, K4R (SEQ ID NO: 69)	44 ± 8	95 ± 11	ND
	GANPR-CNP22 (SEQ ID NO: 66)	ND	ND	ND
CI	GANPR-CNP22, K4R (SEQ ID NO: 37)	50 ± 1	105 ± 12	ND
	GANSS-CNP22 (SEQ ID NO: 67)	ND	ND	ND
CG	GANSS-CNP22, K4R (SEQ ID NO: 70)	27 ± 1	88 ± 1	95
CA	AAWARLLQEHPNA-CNP22 (SEQ ID NO: 61)	24	76	ND
CB	AAWARLLQEHPNAR-CNP22 (SEQ ID NO: 62)	36	84	ND
CC	DLRVDTKSRAAWAR-CNP22 (SEQ ID NO: 63)	34	101	>160
CF	GQPREPQVYTLPPS-CNP22 (IgG1(Fc) fragment) (SEQ ID NO: 79)	23 ± 9	72 ± 19	>160
	PNARKYKGANKK-CNP22 (CNP34)	ND	ND	ND
BL	QEHPNARKYKGANKK-CNP22 (CNP37) (SEQ ID NO: 60)	43 ± 15	97 ± 27	>>160
	PQEHPNARKYKGANKK-CNP22 (Pro-CNP37)	ND	ND	ND
CE	GERAFKAWAVARLSQ-CNP22 (HSA fragment) (SEQ ID NO: 81)	15	87	ND
CY	GQHKDDNPNLPRGANPR-CNP22 (HSA fragment) (SEQ ID NO: 80)	ND	ND	ND
CQ	GHHSHEQHPHGANQQ-CNP22 (HRGP fragment) (SEQ ID NO: 76)	16	95	ND
CX	GHHSHEQHPHGANPR-CNP22 (HRGP fragment) (SEQ ID NO: 78)	ND	ND	ND
CS	GQEHPNARKYKGANPK-CNP22 (modified CNP37) (SEQ ID NO: 129)	19	61	>>160
CT	GQEHPNARKYKGANQK-CNP22 (modified CNP37) (SEQ ID NO: 130)	60	121	ND
CU	GQEHPNARKYKGANQQ-CNP22 (modified CNP37) (SEQ ID NO: 131)	9	57	ND
DB	GQEHPNARKYKGANKK-CNP22 (Gly-CNP37) (SEQ ID NO: 75)	50 ± 14	98 ± 17	>>160
	PGQEHPNARKYKGANKK-CNP22 (Pro-Gly-CNP37)	49 ± 6	103 ± 17	>>160
CW	GQEHPNARKYKGANKP-CNP22 (modified CNP37) (SEQ ID NO: 74)	ND	ND	ND
CR	GAHHPHEHDTHGANQQ-CNP22 (HRGP fragment) (SEQ ID NO: 128)	14 ± 5	77 ± 12	ND
CZ	FGIPMDRIGRNPR-CNP22 (osteocrin "NPR-C inhibitor") (SEQ ID NO: 82)	ND	ND	ND
DA	GKRTGQYKLGSKTGPGPK-CNP22 (FGF2 "heparin-binding domain" fragment) (SEQ ID NO: 83)	ND	ND	ND
CK	GQPREPQVYTGANQQ-CNP22, K4R (IgG1(Fc) fragment) (SEQ ID NO: 84)	2	32	≥160
CL	GVPQVSTSTGANQQ-CNP22, K4R (HSA fragment) (SEQ ID NO: 85)	3	35	>160

TABLE 3-continued

	N- and C-Terminal Modifications	cGMP Response rel. to 1 uM CNP22[1]		NEP Cleavage
	Natriuretic Peptide	10 nM	1 uM	($t_{1/2}$, min)
	GHKSEVAHRFKGANKK-CNP22 (HSA-CNP27) (SEQ ID NO: 144)	51 ± 9	109 ± 15	>>160
	PGHKSEVAHRFKGANKK-CNP22 (Pro-HSA-CNP27)	32	107	ND
CN	GQTHSSGTQSGANQQ-CNP22, K4R (fibrinogen) (SEQ ID NO: 87)	12	115	ND
CM	GQPSSSSQSTGANQQ-CNP22, K4R (fibronectin) (SEQ ID NO: 86)	ND	ND	ND
CO	GSTGQWHSESGANQQ-CNP22, K4R (fibrinogen) (SEQ ID NO: 88)	2	33	ND
CP	GSSSSSSSSSGANQQ-CNP22, K4R (zinc finger) (SEQ ID NO: 89)	ND	ND	ND
CD	SPKMVQGSG-CNP17-KVLRRH ("BNP tails") (SEQ ID NO: 68)	25	102	ND
CJ	RSSCFGGRIDRIGAC ("C-ANP4-23", ANP-derived) (SEQ ID NO: 133)	ND	ND	ND
	CNP22, K4R, K10R, N.-term.--N-term. dimer/ disuccinimidyl glutarate (SEQ ID NO: 134)	19	44	ND
	CNP22, K4R, K10R, N-term.--N.-term. dimer/Bis-PEO5 (SEQ ID NO: 135)	19	41	ND
BM	CNP53 (SEQ ID NO: 4)	61	101	>>160
	ANP	10	23	ND

[1]Stimulation of cGMP production in N1H3T3 cells by natriuretic peptide relative to cGMP production in the presence of 1 uM CNP22
[2]CNP22 NEP resistance $t_{1/2}$ averaged 80 min. Due to variations in NEP catalytic activity between experiments, all CNP22 $t_{1/2}$ digestions were normalized to 80 min and the difference coefficient was used to calculate analog $t_{1/2}$ in each experiment to obtain an adjusted $t_{1/2}$.
ND = Not Determined

Table 4 lists the half-lives, based on the in vitro NEP cleavage assay, of CNP variants conjugated to PEG (or PEO) polymers at the N-terminus. All the PEGylated CNP variants tested and shown in Table 4 displayed resistance or enhanced resistance to NEP cleavage except for PEO12-GANPR-CNP22(K4R), which had the same half-life as wtCNP22. N-terminal PEGylation of CNP22 having a K4G substitution does not seem to confer substantial improvement in NEP resistance. For example, PEG2K-CNP22(K4G) was only slightly more resistant to NEP cleavage than CNP22 (data not shown), whereas PEG2K-CNP22 had a much longer half-life in vitro than CNP22.

TABLE 4

N-Terminal PEGylation		cGMP Response rel. to 1 uM CNP22[1]		NEP Cleavage
Natriuretic Peptide	PEG	10 nM	1uM	($t_{1/2}$, min)
CNP22		46 ± 10	100 ± 13	80
CNP22	PEG20K	0	15	>>160
CNP22	PEG5K	8 ± 1	20 ± 7	>>160
CNP22	PEG2K	6 ± 2	32 ± 4	>>160
CNP22	PEO4-$(PEO12)_3$ (branched)	17 ± 1	52 ± 6	>>160
CNP22	PEO24 (1.2 kDa)	8 ± 5	46 ± 10	>>160
CNP22	PEG1K	15 ± 3	68 ± 17	>160
CNP22	PEO12 (0.6 kDa)	12 ± 7	57 ± 18	160
CNP22	(PEO12)-Biotin	19	81	140
CNP22, K4G	(PEO12)-Biotin	10	27	100
CNP22, K4R	PEO24	15	56	ND
CNP22, K4R	PEO12	13	44	ND
CNP-17	PEG2K	5	50	>160
R-CNP22, K4R (SEQ ID NO: 41)	PEO24	15 ± 2	75 ± 12	ND
R-CNP22, K4R (SEQ ID NO: 41)	PEO12	23 ± 2	93 ± 19	≥160
ER-CNP22, K4R (SEQ ID NO: 39)	PEO24	6 ± 2	60 ± 10	ND
ER-CNP22, K4R (SEQ ID NO: 39)	PEO12	20 ± 1	92 ± 25	ND

TABLE 4-continued

N-Terminal PEGylation		cGMP Response rel. to 1 uM CNP22[1]		NEP Cleavage
Natriuretic Peptide	PEG	10 nM	1uM	($t_{1/2}$, min)
GANRR-CNP22, K4R (SEQ ID NO: 36)	PEG2K	15 ± 2	45 ± 18	ND
GANRR-CNP22, K4R (SEQ ID NO: 36)	PEO24	28 ± 9	82 ± 18	>>160
GANRR-CNP22, K4R (SEQ ID NO: 36)	PEG1K	15 ± 0.4	56 ± 23	>160
GANRR-CNP22, K4R (SEQ ID NO: 36)	PEO12	40 ± 2	99 ± 13	>160
GANQQ-CNP22, K4R (SEQ ID NO: 69)	PEO24	16 ± 13	73 ± 30	ND
GANQQ-CNP22, K4R (SEQ ID NO: 69)	PEO12	30	78	ND
GANPR-CNP22, K4R (SEQ ID NO: 37)	PEO24	ND	ND	ND
GANPR-CNP22, K4R (SEQ ID NO: 37)	PEO12	ND	ND	80
GANSS-CNP22, K4R (SEQ ID NO: 70)	PEO24	8 ± 5	46 ± 21	ND
GANSS-CNP22, K4R (SEQ ID NO: 70)	PEO12	8 ± 0.3	52 ± 13	ND

[1]Stimulation of cGMP production in NIH3T3 cells by natriuretic peptide relative to cGMP production in the presence of 1 uM CNP22
[2]CNP22 NEP resistance $t_{1/2}$ averaged 80 min. Due to variations in NEP catalytic activity between experiments, all CNP22 $t_{1/2}$ digestions were normalized to 80 min and the difference coefficient was used to calculate analog $t_{1/2}$ in each experiment to obtain an adjusted $t_{1/2}$.
ND = Not Determined

FIG. **16** shows the NEP resistance profile of five N-terminal PEGylated conjugates of CNP22. The CNP22 peptides conjugated to PEG (or PEO) polymers of increasing mass exhibited increasing resistance to NEP degradation. In particular, PEO24-CNP22, PEG2K-CNP22 and PEG5K-CNP22 were resistant to NEP degradation over the assay period of 160 minutes.

FIG. **17** displays the NEP resistance profile of CNP variants CNP37 (Analog BL), CNP53 and GANRR-CNP22 (K4R) (SEQ ID NO: 36) having an N-terminal amino acid extension. As can be clearly seen, both CNP37 and CNP53 were resistant to NEP degradation in this in vitro assay, whereas GANRR-CNP22(K4R) (SEQ ID NO: 36) had the same lability to NEP hydrolysis as CNP22.

FIG. **18** depicts the NEP resistance profile of CNP17 and GANRR-CNP22(K4R) (SEQ ID NO: 36) conjugated to a PEG (or PEO) moiety at the N-terminus. PEGylation of GANRR-CNP22(K4R) (SEQ ID NO: 36) greatly improved the NEP resistance of this CNP variant, with PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) being completely resistant to NEP cleavage over the assay period of 160 minutes. Increasing the mass of the PEO moiety from about 0.6 kDa (PEO12) to about 1.2 kDa (PEO24) improved the NEP resistance of PEGylated GANRR-CNP22(K4R) (SEQ ID NO: 36). PEGylation of GANRR-CNP22(K4R) (SEQ ID NO: 36) to a monodispersed PEO24 moiety rather than a polydispersed PEG1K moiety also improved NEP resistance. Finally, although both PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) and PEG2K-CNP17 have a similar total mass (keeping in mind that PEG2K is polydispersed), the former displayed substantially better NEP resistance.

NEP resistance assays were also performed on wtCNP22 and CNP variants G-CNP37, GHKSEVAHRFK-wtCNP27 ("CNP27-HSA", SEQ ID NO: 144) and PEO12-GANRR-CNP22(K4R) ("CNP27-PEO12") (SEQ ID NO: 36). FIG. **19** shows that G-CNP37 and CNP27-HSA were completely resistant to NEP cleavage, and CNP27-PEO12 exhibited much greater stability to NEP degradation compared to wtCNP22.

Example 4

CNP Variant Stimulation of cGMP Production in NIH3T3 Cells

To determine the functional activity of CNP variants, the production of cGMP was measured in NIH3T3 cells exposed to the CNP variants. Murine NIH3T3 cells express endogenously the CNP signaling receptor, NPR-B, which shares 98% protein sequence identity with human NPR-B. NIH3T3 cells were cultured in high glucose Dulbecco's Modified Eagle Medium supplemented with 10% fetal calf serum and antibiotics at 37° C. with 5% CO). Twenty four to 48 hours prior to signaling, cells were passaged to 12-well plates with a density of $2-5\times10^5$ cells per well at the time of the assay. CNP variants were resuspended in 1 mM HCl to a stock concentration of 1 mg/mL (455 uM for wtCNP22) and subsequently diluted to a 30 uM working stock solution with phosphate-buffered saline (PBS). Ten-fold serial dilutions were prepared in phosphate-buffered saline. Culture medium was removed from the cells and replaced with 0.4 mL PBS/Dulbecco's modified Eagle medium (50/50, v/v) containing 0.75 mM isobutylmethylxanine. Plates were incubated at 37° C., 5% CO_2 for 15 minutes before addition of 0.2 mL CNP variant in PBS and continued incubation at 37° C. for 15 minutes. Reactions were stopped by the addition of 0.2 mL lysis buffer supplied with the CatchPoint cGMP assay kit (Molecular Devices), and cGMP production was determined with the CatchPoint cGMP Assay (Molecular Devices). All stimulation experiments were performed in duplicate.

Tables 1-4 summarize the ability of CNP variants having backbone or side chain modifications, amino acid substitutions, N-terminal amino acid extensions, and/or N-terminal PEGylation, respectively, to stimulate cGMP production in NIH3T3 cells. In all four tables, the values for cGMP production in NIH3T3 cells exposed to 10 nM or 1 uM CNP variant are normalized to cell number and cGMP production in the presence of 1 uM wtCNP22.

Regarding the results in Table 1, only Analog G having 3-Cl-Phe at position 7 displayed substantially the same NPR-B stimulation activity at 1 uM as wtCNP22. With respect to Table 2, various CNP variants with amino acid substitutions, including Analogs AH, BO, AB, BH, BZ, BX and BR, showed substantially similar NPR-B stimulation activity as wtCNP22.

Considering the results in Table 3, many CNP variants having N-terminal and/or C-terminal modifications, including amino acid extensions, exhibited comparable NPR-B stimulation activity as wtCNP22. The functional CNP variants include Analog BB, which is CNP22(G1E) attached to heptanoic acid at the N-terminus, and Analog CD, which is the cyclic domain of CNP22 ("CNP17" retaining the Cys6 to Cys22 sequence) conjugated to the N-terminal and C-terminal "tails" of BNP. FIG. **20** illustrates that GANRR-CNP22

(K4R) (SEQ ID NO: 36), CNP37 (Analog BL) and CNP53 all had similar NPR-B stimulation activity as wtCNP22 in the in vitro assay.

Of note from Table 3 is that among the CNP variants assayed for both CNP functionality and NEP resistance, Analog AZ (R-CNP22(K4R)), Analog CC, Analog CF, Analog BL (CNP37), Analog DB (Gly-CNP37) and GHKSEVAHRFK-CNP27 (HSA-CNP27) (SEQ ID NO: 144) all had substantially similar NPR-B stimulation activity as CNP22 while being substantially more resistant to NEP cleavage than CNP22.

With regard to the results in Table 4, nine N-terminal PEGylated CNP variants at 1 uM stimulated cGMP production to at least about 70% of the level achieved by wtCNP22. Several noteworthy aspects appear in Table 4. First, N-terminal PEGylation of GANRR-CNP22(K4R) (SEQ ID NO: 36) with a monodispersed PEO polymer (PEO12 is about 0.6 kDa, PEO24 about 1.2 kDa) resulted in better NPR-B functionality than that with a polydispersed PEG polymer (PEG1K has a polymer number average molecular weight (M_n) of around 1 kDa, PEG2K around 2 kDa) (see also FIG. **21**). Second, N-terminal PEGylation of wtCNP22 with a polydispersed PEG polymer of increasing M_n (PEG1K, PEG2K, PEG5K and PEG20K) or with a monodispersed PEO polymer of greater mass (PEO12 and PEO24) correspondingly decreased the NPR-B activation ability of the CNP variants (see also FIG. **22**). Third, PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36), having the N-terminal GANRR (SEQ ID NO: 8) extension, stimulated greater cGMP production than PEO24-CNP22 and PEO24-CNP22 (K4R). Also of note is that among the N-terminal PEGylated CNP variants assayed for both CNP functionality and NEP resistance, PEO12-R-CNP22(K4R), PEO12-GANRR-CNP22(K4R) (SEQ ID NO: 36) and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) all had substantially similar NPR-B stimulation activity as CNP22 while being much more resistant to NEP degradation than CNP22.

Among the CNP variants listed in Tables 1-4 and assayed for both CNP functionality and NEP resistance, Analogs G, BK, AZ, CC, CF, BL and DB, Pro-Gly-CNP37, HSA-CNP27 (GHKSEVAHRFK-CNP27) (SEQ ID NO: 144), PEG1K-CNP22, (PEO12)-biotin-CNP22, PEO12-R-CNP22(K4R), PEO12-GANRR-CNP22(K4R) (SEQ ID NO: 36), and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) all had substantially similar NPR-B stimulation activity as wtCNP22 while being substantially more resistant to NEP cleavage than wtCNP22.

cGMP production assays were also carried out on wtCNP22 and CNP variants G-CNP37, GHKSEVAHRFK-wtCNP27 ("CNP27-HSA", SEQ ID NO: 144), wtCNP29 and PEO12-GANRR-CNP22(K4R) ("CNP27-PEO12") (SEQ ID NO: 36). FIG. **23** shows that CNP22 and all the CNP variants assayed induced production of similar cGMP levels at either low or high dose of CNP.

Example 5

Binding Specificity for NPR-A, NPR-B and NPR-C

Signaling Competition Assay

To determine the binding specificity of CNP variants for the clearance receptor NPR-C, a signaling competition assay is carried out. Expression plasmids for human NPR-B or NPR-C (each purchased from OriGene) are transiently transfected and receptors are expressed in HEK293T cells. Forty hours after transfection, NPR-B, NPR-C and native HEK293T cells are harvested, counted and plated at a ratio of 1:1 (NPR-B cells:competing cells (either NPR-C or native HEK293T cells)) in 12-well or 96-well plates. Twenty hours after plating, cells are processed for the NPR-B/cGMP stimulation assay described in Example 4. If present, the natriuretic clearance receptor, NPR-C, is expected to bind and internalize CNP, thereby reducing the overall CNP concentration available for signaling through NPR-B, resulting in decreased cGMP production and a shift in the dose-response curve to the right. A rightward shift in the dose-response curve has been verified for wtCNP22. CNP variants having reduced affinity for NPR-C are not expected to induce a shift, or are expected to induce a smaller shift, in the dose-response curve to the right. This signaling competition assay is similar to that previously described by Cunningham (U.S. Pat. No. 5,846,932; B. Cunningham, EMBO J. 13(11): 2508-2515 (1994); H. Jin et al., J. Clin. Invest. 98(4): 969-976 (1996)).

The cGMP stimulation activity of wtCNP-22, Pro-Gly-wtCNP37 and ANP through NPR-B and NPR-A, and their selectivity for NPR-B vs. NPR-C and for NPR-A vs. NPR-C, were evaluated in signaling competition assays. NPR-A, NPR-B and NPR-C individually were transiently tranfected into HEK293T cells. Thirty hours after transfection, the cells were plated into 96-well plates: (A) 20,000 NPR-B cells+20,000 mock transfected cells; (B) 20,000 NPR-B cells+20,000 NPR-C cells; (C) 20,000 NPR-A cells+20,000 mock transfected cells; and (D) 20,000 NPR-A cells+20,000 NPR-C cells. Twenty hours after plating, the culture media was removed and replaced with serum-free media:PBS (1:1)+ 0.75 uM IBMX for 15 minutes. For cGMP signaling through NPR-B, dose series for ANP, CNP-22 and Pro-Gly-CNP37 were added and incubated at 37° C. for 12 minutes before the assay was stopped by cell lysis. For cGMP signaling through NPR-A, dose series for CNP-22 and Pro-Gly-CNP37 were incubated at 37° C. for 12 minutes, while dose series for ANP were incubated at 37° C. for 6 minutes (because NPR-A appeared to be a "faster" guanylyl cyclase than NPR-B, the incubation time for ANP was shortened in order not to max out (use up all cellular GTP) too soon when signaling with ANP). FIGS. **24**A and B show that CNP-22 and Pro-Gly-CNP37 ("Pro-CNP38") stimulated cGMP production through NPR-B with similar dose-response curves, and to a much greater extent than through NPR-A, and exhibited a similar profile for NPR-B vs. NPR-C selectivity in the signaling competition assays.

Determination of Binding Affinities (K_i) for NPR-A, NPR-B and NPR-C

The binding affinities (K_i) of CNP variants for NPR-A, NPR-B and NPR-C are determined in a heterologous competition binding assay (U.S. Pat. No. 5,846,932; B. Cunningham, EMBO J. 13(11): 2508-2515 (1994); H. Jin et al., J. Clin. Invest. 98(4): 969-976 (1996)). Membranes from HEK293 cells, or another suitably transfectable cell line (e.g., HeLa cells), expressing human NPR-A, NPR-B or NPR-C are prepared for radio-labeled ligand binding assays. Membrane preparations are diluted in an appropriate buffer and varying concentrations of wtCNP22 or CNP variant (competitor) are added with I^{125}-labeled wtCNP22 (Bachem). Samples are incubated at room temperature to allow for ligand/receptor equilibration and bound peptide is separated from free peptide by filtration through PVDF filter membranes. Filters are washed before the addition of scintillant and counting by a scintillation counter. Binding is measured in duplicate for each concentration of competitor peptide. CNP variant affinity (K_i, equilibrium dissociation constant) and B_{max}, (receptor number) are calculated by non-linear regression analysis and/or the Cheng-Prusoff equation.

CNP variants exhibiting reduced affinity to NPR-C are expected to have reduced susceptibility to clearance by NPR-C and thus a longer plasma or serum half-life. Increased half-life of the CNP variants in circulation would increase the availability of the variants for therapeutic activity.

Example 6

Effect of CNP Variants on the Growth of Rat Chondrosarcoma (RCS) Cells and cGMP Production in RCS Cells

To assess the ability of CNP variants to stimulate bone growth, skeletal dysplasia is simulated in cell culture by treating rat chondrosarcoma (RCS) cells with fibroblast growth factor 2 (FGF-2), which activates fibroblast growth factor receptor 3 (FGFR-3) and induces growth arrest (Krejci et al., J. Cell Sci., 118(21):5089-5100 (2005)).

Optimal CNP treatment parameters are determined by varying CNP concentration (0.05, 0.1, 0.2 and 0.5 uM), and treatment duration and interval (continuous; 2.5 min, 10 min, 30 min, 1 hr, 2 hr, 4 hr and 8 hr once a day; 2.5 min, 10 min, 30 min, 1 hr, 2 hr and 4 hr twice a day). After 72 hours, cells are counted using an automated cell counter, and the amount of extracellular matrix is estimated using alcian hlue staining.

RCS cells are then treated with a CNP variant using the optimal conditions determined from the growth experiments with wtCNP22. The concentration of cGMP is measured by competitive ELISA for untreated RCS cells, RCS cells treated with CNP, and RCS cells treated with the CNP variant. Cell growth and matrix synthesis resulting from treatment with the CNP variant are also monitored and compared to those resulting from CNP treatment.

To assess the effect of CNP variants in a human cell culture system, primary re-differentiated human chondrocytes in alignate beads are treated with wtCNP22 and CNP variants, and cGMP concentration is determined by competitive ELISA as a measure of effective CNP signaling.

The methods described herein can be employed to assess the ability of CNP variants to stimulate cGMP production in and growth of rat chondrosarcoma cells in vitro.

Example 7

Dose Response Study in Rat Chondrosarcoma Cells

The tyrosine kinase receptor fibroblast growth factor receptor 3 (FGFR-3), a negative regulator of chondrocyte growth, is contitutively on in achondroplasia subjects. Stimulation of the FGFR-3 receptor with FGF-2 causes growth arrest by prolonged activation of Erk MAPK, and causes decreased matrix synthesis and loss of matrix, as well as a change in cell shape. Continuous exposure of rat chondrosarcoma (RCS) cells to fibroblast growth factor 2 (FGF-2) simulates achondroplasia in cell culture hy activating FGFR-3 and inducing growth arrest (Krejci et al., J. Cell Sci., 118(21): 5089-5100 (2005)). To determine the dose of CNP variant and frequency of dosing that stimulate sufficient growth of bone cells, a dose response study was performed using the RCS cell assay as described in Example 6.

RCS cells were seeded at 10×10^3 cells per well in 24-well plates, grown for 24 hr, treated for 72 hr, and then counted. RCS cells were continuously exposed to FGF-2 (5 ng/mL) to simulate a constitutively active FGFR-3, which induced cell growth arrest (see bar #5 in FIG. **25**). Wild-type CNP22 (0.2 uM) was cultured continuously (72 hr), 1 hr daily or 2 hr daily. All stimulants were changed daily Continuous exposure of RCS cells to 0.2 uM CNP22 in the presence of 5.0 ng/mL FGF-2 partially reversed FGF2-induced growth arrest, leading to the growth of approximately 200×10^3 cells per well (bar #6 in FIG. **25**), compared to approximately 100×10^3 cells per well in the absence of CNP22 (bar #5 in FIG. **25**).

Both 1 hr exposure to CNP22 (0.2 uM) once a day and 2 hr exposure to CNP22 (0.2 uM) once a day achieved about 84% of the effect of continuons CNP22 (0.2 nM) exposnre on chondrocyte growth (bars #7 and 8 in FIG. **25**). These results demonstrate that continuous exposure of growth-arrested chondrocytes to CNP22 is not required for reversal of cell growth arrest. Additionally, dose response studies demonstrate that lower doses of CNP22 are capable of reversing growth arrest (FIG. **26**A).

Furthermore, histological and cell morphological analysis of the extracellular matrix showed that CNP22 treatment antagonized FGF2-mediated loss of chondrosarcoma extracellular matrix and increased matrix synthesis. Exposure to FGF-2 decreased matrix synthesis and increased degradation, while addition of CNP22 to the FGF-2 cell culture increased matrix synthesis and partially inhibited FGF-2, as assessed by ^{35}S-sulfate and ^{3}H-Pro incorporation into, or decrease from, matrix (FIGS. **27**A-D). Analysis of aggrecan and fibronectin production (mRNA and protein) in RCS cells cultured with FGF-2 and CNP22 shows that FGF-2 decreased aggrecan level and increased fibronectin level, which was inhibited by addition of CNP22 (FIGS. **28**A-C). FGF-2 induces and activates matrix-processing molecules predominantly via Erk, and addition of CNP22 shows some effect on this activation.

Additional high-throughput assays for measuring growth arrest, such as crystal violet staining, are useful for measuring the effects of CNP22 and variants thereof on RCS cells.

Similar dose response studies can be conducted with the CNP variants described herein to determine their effective dose for reversing FGF2-induced growth arrest of RCS cells.

Example 8A

Ex Vivo Stimulation of Growth of Tibia and Femur from Mice with Mild Achondroplasia

Mouse tibial organ culture model has been used to demonstrate the efficacy of wild-type CNP22 in stimulating longitudinal bone growth. Treatment of wild-type tibiae with CNP22 at 10^{-8}, 10^{-7} or 10^{-6} M for 6 days increased longitudinal growth by 31%, 40% and 42%, respectively. Histological evaluation also showed expansion of the hypertrophic zone, e.g., an increase in the number and size of hypertrophic chondrocytes in the growth plate (Agoston et al., BMC Dev. Biol. 7:18 (2007)). Similar findings were observed in tibiae isolated from FGFR3^{Ach} mice (Yasoda et al., Nat. Med. 10: 80-86 (2004)).

To determine the efficacy of CNP variants in stimulating longitudinal bone growth, CNP variants were tested in a mouse organ culture model of endochondral bone growth in wild-type mice and transgenic mice having a G380R mutation in the human FGFR-3 gene (FGFR3$^{wt/Ach}$ heterozygote) which represent a mouse model of mild achondroplasia. In brief, the pharmacological activity of wild-type CNP22 and CNP variants was compared in an organ culture model of embryonic or neonatal mouse tibiae, isolated from wild-type and FGFR3$^{wt/Ach}$ littermates. Overall bone growth and histological changes within the growth plate were assessed. Conditioned culture medium is also assessed for biomarkers of intracellular signaling (cGMP), cartilage metabolism (type II collagen, other collagens, aggrecan chondroitin sulfate), bone metabolism (bone alkaline phosphatase, osteocalcin, type I collagen [C-telopeptide, N-telopeptide]), and inflammation (interleukin-1, interleukin 6, interleukin-11).

Effective CNP variants are identified by their ability, e.g., to stimulate production of cGMP, and bone growth as measured by increased longitudinal bone length and expansion of the cells in the hypertrophic zone of the growth plate.

Measurement of Bone Growth

The efficacy of wtCNP22, CNP37 and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) in stimulating longitudinal femoral growth was evaluated in the mouse organ culture model. For these experiments femora were isolated from 2-3 day old wild-type mice and cultured in alphaMEM supple-

mented with 0.2% BSA, 0.5 mM L-glutamine, 40 units penicillin/mL and 40 ug streptomycin/mL, for 8 days in the presence of vehicle, CNP22 or CNP variants. The treatment commenced at day 0 and was repeated every two days thereafter, as the medium was changed. Bones were measured prior to treatment and every two days thereafter, using a dissection microscope fitted with a 1 cm eye-piece reticule. Conditioned medium was used for biomarker analysis. At day 8 bones were fixed in 4% paraformaldehyde for 24 hr, decalcified in 5% formic acid for 24 hrs, dehydrated and embedded in paraffin. Bones were sectioned at 5 um (microns), which were then deparaffinized, rehydrated, and stained with Alcian Blue for 30 min (pH 2.5; MasterTech). Alcian Blue stains cartilage blue. Stained sections were visualized and photographed by brightfield microscopy. The thickness of the hypertrophic region of the growth plate cartilage was determined by image analysis.

FIG. **29** illustrates the effect of wtCNP22, CNP37 and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) on longitudinal growth of 3-day old wild-type mouse femurs treated with the CNP peptides every two days. The results were normalized to measurements prior to treatment (day 0). The studies were performed in triplicate (vehicle) or quadruplicate (CNP peptides). As shown in FIG. **29**, CNP37 and PEO24-GANRR-CNP22(K4R) (SEQ lD NO: 36), as well as CNP22, were effective in stimulating longitudinal femoral growth, with the N-terminal PEGylated CNP variant being the most effective.

The growth of wild-type and FGFR3^{ach} mouse femur and tibia in response to CNP22, CNP37 and PEO24-GANRR-CNP22(K4R) ("CNP27-PEO24") (SEQ ID NO: 36) was also assessed. Culture of either wild-type or achondroplastic (FGFR3^{ach}) mouse tibia showed that CNP37 and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) both increased the longitudinal growth of the tibia compared to vehicle or CNP22 (FIGS. **30** and **31**). CNP22, CNP37 and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) also stimulated the growth of wild-type mouse femur (FIG. **32**). Moreover, each of CNP22, PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) and CNP37 increased the longitudinal growth of FGFR3^{ach} mouse femur compared to vehicle (FIG. **33**).

Furthermore, ex vivo distribution of CNP37 in the growth plate of FGFR3^{ach} mouse tibia was evaluated. Bone samples were prepared as described above. Paraffin sections were cut and heat fixed for 1 hour at 60° C. Antigen retrieval with 1% hyaluronidase at 37° C. (30 minutes) was followed by a 1 hour serum block (10% Normal Goat Serum). CNP22 antibody (1:500 dilution; Peninsula Laboratories Inc., San Carlos, Calif.) was applied overnight at 4° C. For immunodetection, Vectastain ELITE ABC kit (Vector, Burlingame, Calif.) was used according to the manufacturer's recommendations. Specific bound peroxidase was visualized by incubation with DAB substrate kit (Vector) and the reaction was developed for 3 minutes. Slides were then dehydrated and mounted and photographed using a brightfield microscope. Staining for CNP in the growth plate of FGFR3^{ach} mouse tibia showed that CNP immunoreactivity was increased in the regions of articular and hypertrophic chondrocytes (FIG. **34**), indicating that CNP37 was delivered to the chondrocytes.

In addition to the distribution of CNP variants in the bone growth plate, the ex vivo effects of CNP37, CNP22 and vehicle on cells in the FGFR3^{ach} and wild-type growth plate, e.g., hypertrophic cell size and cellularity of proliferating zone, were also evaluated. Bone samples were prepared, including Alcian Blue staining, as described above. Images of the entire proximal growth plate were taken at 4× magnitude. The growth plate is divided into three zones, starting from the epiphyseal side of cartilage: the resting zone (individual small chondrocytes), proliferating zone (columns of stacked chondrocytes parallel to the long axis of the bone), and hypertrophic zone (large chondrocytes and thin septa between the chondrocytes). In these regions, measurements were made by ImageJ software, including the number of proliferating chondrocytes per column and the density of hypertrophic chondrocytes. A test square (4×4 mm^2) at five different regions of the hypertrophic zone was used to determine the density of hypertrophic chondrocytes. The cell size of hypertrophic chondrocytes was calculated by 1 over determined cell density. Cellularity of proliferating columns was increased by CNP37 and CNP22 in both wild-type and FGFR3^{ach} mice (FIGS. **35**B and C). Chondrocyte hypertrophy in FGFR3^{ach} mice was also increased as a result of culture with CNP22 or CNP37 (FIGS. **36**B and C).

Ex vivo studies of cultures of mouse bones indicated that CNP37 was delivered to the growth plate and was able to increase chondrocyte cellularity and hypertrophy, which are associated with growth plate expansion and longitudinal bone growth. To assess the biodistribution of CNP37 in the bone growth plate in vivo and the in vivo effects of CNP37 on the growth plate (including total growth plate thickness, hypertrophic zone thickness, and cellularity of proliferating zone), bone samples were obtained from FGFR3^{ach} mice treated with vehicle or CNP37 as described above. For biodistribution and in vivo effects studies, tibias were fixed and stored in 70% ethanol. For immunohistochemistry, the samples were decalcified in 5% formic acid for 2 days, dehydrated and embedded in paraffin. Bones were sectioned at 5 um (microns), which were then deparaffinized, rehydrated, and used for CNP immunohistochemistry as described above. For cellular image analysis, bones were sectioned at 5 (microns), and then deparaffinized, rehydrated, and stained with Alcian Blue for 30 minutes (pH 2.5; MasterTech) and Hematoxylin & Eosin for 30 seconds. Stained sections were visualized and photographed by brightfield microscopy. Thicknesses of the growth plate and the proliferating and hypertrophic zones were measured using ImageJ software.

In vivo biodistribution studies demonstrated that, similar to the ex vivo studies, CNP immunoreactivity was increased in the regions of articular and hypertrophic chondrocytes in the tibia growth plate of FGFR3^{ach} mice treated with CNP37, indicating that CNP37 was delivered in vivo to the growth plate of FGFR3^{ach} mouse tibia (FIG. **37**). Furthermore, CNP37 treatment significantly increased the total growth plate thickness, proliferating zone thickness and hypertrophic zone thickness of FGFR3^{ach} mouse tibia in vivo (FIGS. **38**A-C).

These results demonstrate that CNP variants of the disclosure penetrate into the growth plate of wild-type and achondroplastic animals, increase the number and size of chondrocytes, increase the thickness of the proliferating zone and the hypertrophic zone of the growth plate, and increase longitudinal bone growth in treated wild-type and achondroplasic animals. Therefore, the CNP variants are useful for stimulating bone growth in achondroplastic subjects.

Measurement of Biomarkers

In addition to measurement of bone growth in response to CNP variants, assay of the levels of biomarkers for cartilage and bone formation and growth induced in response to CNP variants is useful for evaluating the effect of CNP variants on bone growth.

Femurs and tibias were isolated from wild-type and FGFR3^{ach} mice as described above. Bones were cultured with CNP22 or a variant thereof for eight days with the replacement of media every two days. On the eighth day, media was

collected and analyzed for the biomarkers cGMP (cyclic guanosine 3', 5' cyclic monophosphate) and fragments of cleaved collagen type II, a cartilage-specific marker for cartilage turnover. Both markers were measured using commercially available enzyme-linked immunosorbent assays (ELISA) for cGMP (Cayman Chemical Co., Ann Arbor, Mich.) and cleaved collagen type II (Cartilaps) (Immunodiagnostic Systems, Fountain Hills, Ariz.), following the manufacturer's protocol.

Levels of cGMP and collagen type II fragments were measured from cell culture extracts after exposure to CNP22, CNP37 or PEO24-GANRR-CNP22(K4R) ("CNP27-PEO24") (SEQ ID NO: 36). FIGS. **39-42** show a large increase ($p<0.01$) in the levels of cGMP in the media after exposure of ex-planted wild-type and $FGFR3^{ach}$ mouse femurs and tibias to CNP22, CNP37 or PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36). In addition, exposure of wild-type and $FGFR3^{ach}$ mouse femurs to CNP22, CNP37 or PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) increased the levels of cleaved collagen type II, with the treated $FGFR3^{ach}$ mouse femurs showing significantly increased ($p<0.05$) levels of collagen type II fragments (FIG. **43**). Elevated levels of collagen type II fragments indicate a turnover of the cartilage matrix, and cartilage turnover typically precedes new bone formation in growing bones.

Example 8B

Ex Vivo Stimulation of Growth of Femur from Mice with Severe Achondroplasia

The effect of a CNP variant on the growth of bones from mice with severe achondroplasia was evaluated ex vivo. Used in the study were transgenic mice expressing a human FGFR-3gene having a Y367C mutation ($FGFR3^{Y367C}$) [S. Pannier et al., Biochim. Biophys. Acta, 1792(2): 140-147 (2009)], which represent a mouse model of severe achondroplasia. Femurs were isolated at embryonic day 16.5 and cultured for 6 days in the presence of 1 uM Pro-Gly-CNP37. Bone lengths were measured at Day 1 and Day 7. The bones were then paraffin-embedded, sectioned and stained with hematoxylin and eosin to assess histological changes and cellular morphology. Treatment of bone explants isolated from $FGFR3^{Y367C}$ mice with Pro-Gly-CNP37 ("ProCNP38") resulted in increase in bone growth and expansion in the growth plate (FIG. **44**). Femurs from $FGFR3^{Y367C}$ mice treated with vehicle for 6 days showed an 18% deficiency in growth length-wise compared to wild-type femurs treated with vehicle. Treatment of femurs from $FGFR3^{Y367C}$ mice with 1 uM Pro-Gly-CNP37 for 6 days reduced the growth deficiency to just 11%, i.e., reduced the growth defect by around 40%.

Example 9

Serum/Plasma Stability of CNP Variants In Vitro

In preparation for pharmacokinetics (PK) studies, the stability of CNP variants in serum and/or plasma is evaluated.

Briefly, the analyte is isolated by the removal of serum or plasma proteins by either a 2% trichloroacetic acid precipitation or a 1:3 serum:acetonitrile precipitation. The precipitation mixture is vortexed at 14,000 rpm for five minutes, and a portion of the supernatant is removed and diluted with water prior to transfer to a silanized autosampler vial for analysis. Serum extracts are then analyzed by reverse-phase high performance liquid chromatography (RP-HPLC) with electrospray ionization mass spectrometry (ESI-MS). A single mass (m/z), shown to be specific for the CNP variant, is monitored for quantitation purposes.

Initially, analytical stability and recovery is determined. Analytical (RP-HPLC and ESI-MS) parameters are optimized through the analysis of matrix standards (serum extracts fortified with analyte post-precipitation). After optimization, analytical recovery is determined by spiking serum samples at known concentrations and comparison of the analyte response to that of matrix standards prepared at similar concentrations. Analyte stability in serum extracts is also determined to assure no significant losses occur after serum precipitation and prior to actual analysis. To test the effect of freezing on serum stability, a two-cycle freeze/thaw study is also performed. In this study a serum sample is spiked with CNP variant and analyzed prior to freezing overnight at −20° C. The sample is then thawed at room temperature and re-analyzed. The process is repeated for a second freeze/thaw cycle.

Serum stability of CNP variant is determined by spiking of serum/plasma samples with CNP variant at a concentration of 10 ug/mL. The sample is placed in a 37° C. water bath for a period of three hours. At 30 minute intervals duplicate aliquots of serum are removed and analyzed. If rapid losses of analyte are evident (>50% in 30 minutes), the study may be repeated with 10 minute timepoints.

In an exemplary method for determining the stability of CNP variants in murine plasma, a mixture of CNP variant (10 uL of a stock solution of about 2.5-5.0 mg/mL), heparinized murine plasma (50 uL, Bioreclamation, CD-1 Lith Hep 62231), and 5 M NaCl (10 uL) is incubated at 37° C. and 5% CO_2 for 0-5 hr, and then quenched with 10× protease inhibitor cocktail (15 uL, Sigma P2714). For extraction, 150 uL of MeOH/0.1% FA is added to 85 uL of the reaction mixture, and the resulting mixture is vortexed for 1 min and then centrifuged at 15° C. for 15 min. 75 uL of the supernatant is added to 300 uL of aqueous 0.1% FA. A small portion of the resulting mixture is subjected to analysis by LC/MS.

Example 10

Pharmacokinetics and cGMP Production in Rats and Mice

Studies were conducted in normal rats to evaluate the pharmacokinetics (PK) profile of CNP22 and certain CNP variants and the time courses of plasma cGMP concentration after single intravenous (i.v.) or subcutaneous (s.c.) administration of the CNP peptides. Plasma CNP immunoreactivity was determined by using a competitive radioimmunoassay (RIA) with an anti-CNP rabbit polyclonal antibody. Plasma cGMP concentration was determined by RIA using a commercially available kit (YAMASA cyclic GMP Assay kit, YAMASA Corporation).

Normal male rats, 7-8 weeks of age, were used. Recombinant wild-type CNP22, CNP37 and PEO24-GANRR-CNP22 (K4R) (SEQ ID NO: 36) were evaluated. A dosage of 20 nmol/kg of each CNP peptide as a solution in 5% mannitol was intravenously injected once into the tail, or a dosage of 50 nmol/kg of each CNP peptide as a solution in 0.03 mol/L acetic acid buffer solution, pH 4.0, containing 1% (w/v) benzyl alcohol and 10% (w/v) sucrose, was subcutaneously injected once into the back.

Plasma CNP immunoreactivity was determined by the competitive RIA using anti-CNP rabbit polyclonal antibody. Standard and QC samples were prepared. Fifty uL of the standard, QC and assay samples were added, respectively, to

test tubes containing 50 uL of RIA buffer. Diluted anti-CNP rabbit polyclonal antibody (100 uL) was added to the tubes. All tubes were kept at 4° C. overnight. ^{125}I-[Tyr^{0}]-CNP22 solution (100 uL) and rabbit IgG solution (100 uL) were added and left at approximately 4° C. overnight. One milliliter of anti-rabbit IgG goat serum containing 10% polyethylene glycol was added, vortexed and left at approximately 4° C. for at least 1 hour, and then the insoluble fraction was precipitated by centrifugation. After aspiration of the supernatant, the amount of radiation (gamma line) in the sediment was measured by a gamma-counter. Each sample was measured in duplicate, and the mean was adopted as the value determined.

Plasma cGMP concentrations in the sample at 5, 30, 60 and 90 minutes after i.v. dosing, or at 5, 30, 60, 120 and 180 minutes after s.c. dosing, were determined by the competitive RIA using anti-cGMP monoclonal antibody. Standard samples were prepared. 100 uL of the assay samples (standard solutions for the calibration curve or the diluted plasma samples for cGMP determination) were transferred to test tubes. Then 100 uL of anti-cGMP monoclonal antibody solution and 100 uL of ^{125}I-labeled succinyl cGMP tyrosine methyl ester solution were added to the tubes, respectively. All tubes were kept at 4° C. overnight. After the addition of 500 uL dextran charcoal solution, the tubes were vortexed and then placed on ice for 10 minutes. The reaction mixture was centrifuged and 500 uL of the supernatant was transferred from each sample to a new test tube. The amount of radiation (gamma line) in the supernatant was measured by a gamma-counter. Each sample was measured in duplicate, and the mean was adopted as the value determined.

Plasma CNP immunoreactivity was employed for pharmacokinetics (PK) analysis. PK analysis was performed using WINNONLIN® Professional (Pharsight Corporation). The PK profiles of CNP22, CNP37 and PEO24-GANRR-CNP22 (K4R) (SEQ ID NO: 36) after i.v. administration were calculated using PK parameters such as concentration at 0 hour (C_0: extrapolation, pmol/mL), total body clearance (CL_{tot}: mL/min/kg), distribution volume at steady state (V_{dss}: mL/kg), area under the plasma concentration-time curve (AUC: pmol·min/mL), mean residence time (MRT: min), and half-life ($T_{1/2}$: min). The PK profiles of the CNP peptides after s.c. administration were calculated using PK parameters such as maximum plasma concentration (C_{max}: pmol/mL), time to reach C_{max} (T_{max}: min), area under the plasma concentration-time curve (AUC: pmol·min/mL), mean residence time (MRT: min), and half-life ($T_{1/2}$: min).

In plasma spike recovery experiments, the RIA detected CNP22, CNP37 and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) similarly (data not shown).

Procedures similar to those described above are employed to study in mice the PK profiles of CNP22 and variants thereof and their ability to stimulate cGMP production.

The PK profiles of CNP22, CNP37 and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) after i.v. administration in three rats are illustrated in FIG. **45**. As shown by FIG. **45**, CNP37 and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) had a much longer half-life and a much greater bioavailability than CNP22. The half-life, $T_{1/2}$ (min), was 1.42 (±0.45) for CNP22, 22.3 (±1.5) for PEO24-GANRR-CNP22 (K4R) (SEQ ID NO: 36), and 49.5 (±28.0) for CNP37. The area under the curve, AUC (pmol·min/mL), was 320 (±54) for CNP22, 1559 (±568) for CNP37, and 2084 (±424) for PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36).

The PK profiles of the three CNP peptides after s.c. administration in three rats are depicted in FIG. **46**. Compared to CNP22, PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) had a much longer half-life (78.1 min (±16.4) vs. 10.0 (±5.0)) and a much greater bioavailability (60% (±6%) vs. 19% (±9%)).

The time courses of plasma cGMP concentrations after i.v. administration of the three CNP peptides in three rats are displayed in FIG. **47**. FIG. **47** clearly demonstrates that i.v. administration of CNP37 and PEO24-GANRR-CNP22 (K4R) (SEQ ID NO: 36) resulted in much higher plasma levels of cGMP at 30, 60 and 90 minutes than i.v. administration of CNP22.

The time profiles of plasma cGMP concentrations after s.c. administration of the three CNP peptides in three rats are shown in FIG. **48**. Subcutaneous administration of PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) and CNP37 also resulted in substantially higher plasma concentrations of cGMP than s.c. administration of CNP22, with the difference relative to CNP22 increasing over time for PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36), but decreasing over time for CNP37.

The rat studies indicate that compared to wtCNP22, the CNP variants CNP37 and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) had a substantially longer half-life in vivo, had a substantially greater bioavailability in vivo, and stimulated substantially higher levels of cGMP production in vivo for an extended period of time. The resistance of CNP37 and PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) to NEP degradation correlates to a longer plasma half-life in vivo, which in turn correlates to prolonged NPR-B/cGMP signaling in vivo. These results show that compared to CNP22, the CNP variants of the disclosure, administered by i.v. or s.c. injection (e.g., once daily), can be more effective in treating CNP-responsive conditions or disorders, such as bone-related disorders and vascular smooth muscle disorders.

Example 11

Pharmacokinetics Study in Mice

To determine CNP variants having increased NEP resistance, for efficacy study in FGFR3^{ach} mice (see Example 13), a pharmacokinetics (PK) study is carried out that compares the pharmacokinetics properties of CNP variants to wild-type CNP22. The FGFR3^{ach} mouse is a mutant mouse model of mild achondroplasia, containing a single transgene on a background of FVB mice.

Wild-type CNP22 or variant thereof is administered as a single intravenous (i.v.) dose in 6-week old wild-type FVB mice. Exemplary PK studies were conducted using wtCNP22. Six-week old FVB/N mice were intravenously administered wtCNP22 in a single dose at 100 nmol/kg. Mean plasma levels of CNP22 were calculated, and the estimated half-life of CNP22 was determined to be from 0.76 min to 1.03 min.

CNP variants displaying greater resistance to NEP degradation are expected to exhibit increased serum concentrations over time and a longer half-life in vivo.

Example 12

Efficacy of CNP Variants in Wild-Type Mice

The in vivo effects of CNP variants on bone growth were assessed in wild-type mice. Three week old FVB wild-type male mice received daily subcutaneous (s.c.) injection of either vehicle, G-CNP37 (200 nmol/kg) or PEO12-GANRR-

CNP22(K4R) ("CNP27-PEO12") (SEQ ID NO: 36) (200 nmol/kg) for 5 weeks. Body weight was measured at least once weekly. Tail length was measured at least once weekly using digital caliper readings, and body length (naso-anal length), bone length (tibia, femur, ulna and humerus), skull length (anterior to posterior cranial segment) and lumbar vertebrae 5 (LV5) length were measured after 5 weeks of treatment using a caliper. X-rays were taken at baseline and after 5 weeks of treatment.

Five weeks of treatment of wild-type mice with G-CNP37 resulted in significant body weight gain, with increased body weight being observed beginning at Day 9 ($p<0.05$) (FIG. **49**). Treatment with G-CNP37 also resulted in significantly increased tail length, beginning at the second week after treatment ($p<0.01$) (FIG. **50**).

Table 10 shows the percentage change in tail length, body (naso-anal) length, skull (anterior to posterior cranial segment) length, bone (femur, tibia, humerus and ulna) lengths, and lumbar vertebrae 5 (LV5) length in wild-type mice injected s.c. once daily with 200 nmol/kg of either G-CNP37 or PEO12-GANRR-CNP22(K4R) ("CNP27-PEO12") (SEQ ID NO: 36) for 5 weeks, relative to a value of 100% for wild-type mice treated with vehicle only.

TABLE 10

	Tail	Naso-Anal	Anterior to Posterior (Skull)	Femur	Tibia	Humerus	Ulna	LV5
G-CNP37	119%**	114%**	104%*	109%**	105%**	104%**	101%	108%*
CNP27-PEO12	101%	104%**	101%	102%*	100%	102%*	96%*	103%

**$p < 0.01$,

*$p < 0.05$

Treatment with G-CNP37 resulted in significantly increased tail length, body (naso-anal) length, skull length, proximal bone (femur and humerus) length, distal bone (tibia) length, and vertebral (lumbar vertebrae 5) length compared to treatment with vehicle.

Lower doses of Pro-Gly-CNP37 dosed daily s.c. at 5 nmol/kg, 20 nmol/kg or 70 nmol/kg for five weeks resulted in a dose-dependent increase in tail length, body (naso-anal) length, and bone lengths compared to vehicle. Table 11 shows the percentage change in tail length, body (naso-anal) length, and bone (femur, tibia, humerus and ulna) lengths in wild-type mice injected s.c. once daily with 5 nmol/kg, 20 nmol/kg or 70 nmol/kg Pro-Gly-CNP37 for 5 weeks, relative to a value of 100% for wild-type mice treated with vehicle only.

TABLE 11

	Tail	Naso-Anal	Femur	Tibia	Humerus	Ulna
5 nmol/kg	107.6%**	102.7%**	103.3%**	101.7%**	101.1%	100.9%*
20 nmol/kg	107.8%**	107.6%**	107.1%**	103.4%**	102.3%**	102.5%**
70 nmol/kg	113.6%**	112.5%**	109.3%**	105.9%**	103.6%**	104.1%**

**$p < 0.01$,

*$p < 0.05$

In another study, various dosing regimens of Pro-Gly-CNP37 were administered for nine weeks followed by one week of recovery. Wild-type FVB mice were dosed s.c. with:

(1) vehicle daily for nine weeks, followed by one week of recovery;

(2) 20 nmol/kg Pro-Gly-CNP37 daily for one week, followed by three doses per week for eight weeks and one week of recovery;

(3) 20 nmol/kg Pro-Gly-CNP37 on alternating weeks; or

(4) 5 nmol/kg Pro-Gly-CNP37 daily for nine weeks followed by one week of recovery.

Increased growth in tail length, body length, and bone lengths was observed in all treatment groups (Groups 2, 3 and 4) at the end of the study. Table 12 shows the percentage change in tail length, body (naso-anal) length, and bone (femur, tibia, humerus and ulna) lengths in wild-type mice administered Pro-Gly-CNP37 ("Pro-CNP38") under the dose regimens described above, relative to a value of 100% for wt mice treated with vehicle only.

TABLE 12

	Total Pro-CNP38 received (nmol/kg)	Tail	Naso-Anal	Femur	Tibia	Humerus	Ulna
Group 2	620	104.8%**	106.0%**	105.3%**	100.8%	102.5%	101.7%
Group 3	700	106.7%**	106.5%**	104.1%**	102.5%**	102.6%*	102.8%*
Group 4	350	104.9%**	105.1%**	107.3%**	102.7%**	102.3%**	103.6%**

**p < 0.01,
*p < 0.05

While all dose regimens of Pro-Gly-CNP37 increased axial and appendicular growth parameters measured, daily dosing of Pro-Gly-CNP37 promoted appendicular growth (femur, tibia, humerus, and ulna) at a lower total dose level (Group 4) compared to regimens with less frequent dosing (Groups 2 and 3).

Example 13

Efficacy in Mouse Model of Mild Achondroplasia

The efficacy of CNP variants in enhancing growth and correcting achondroplasia was tested in a mouse model of mild achondroplasia, using a strain of transgenic mice expressing a human FGFR-3 gene having a G380R mutation ($FGFR3^{ach}$) (Wang et al., Proc. Natl. Acad. Sci. USA, 96(8): 4455-4460 (1999); Naski et al., Development USA, 125: 4977-4988 (1998); U.S. Pat. Nos. 6,265,632 and 6,136,040).

At 3 weeks of age, $FGFR3^{ach}$ mice and their wild-type littermates were anesthetized to have lateral whole-body X-ray images taken by Faxitron, and randomized by body weight into the following treatment groups (n=8/group): (1) wild-type/vehicle, (2) $FGFR3^{ach}$/vehicle, (3) $FGFR3^{ach}$/CNP37, and (4) $FGFR3^{ach}$/PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36). The mice received once daily subcutaneous (s.c.) administration of designated test article (vehicle or 200 nmol/kg CNP variant) for 5 weeks. Satellite groups (n=3) were used to confirm exposure of each test article following a single subcutaneous administration on Day 1. Wild-type male FVB mice that received daily s.c. injection of vehicle for 5 weeks were used as a control for normal growth.

X-ray measurements at baseline and at the end of the study were performed to determine change in the head length, area of the skull, and the external auditory meatus (EAM, the ear canal running from the outer ear to the middle ear). Body weight and tail length were measured at least once weekly, using a digital caliper to measure tail length, and body (naso-anal) length was measured after 5 weeks of treatment. Bone (tibia, femur, ulna and humerus) lengths were measured using a digital caliper at necropsy.

On Day 37, all mice were sacrificed by terminal anesthesia and whole-animal photographs and X-ray images by Faxitron were taken. Left and right tibia, femur, humerus, and ulna were collected and measured using a digital caliper. The left portions of each bone were processed for histology, and the right portions were snap-frozen for archival. Samples obtained from the bones were used to evaluate the effects of CNP variants on endochondral bone growth.

Treatment of $FGFR3^{ach}$ mice with CNP37 by once daily s.c. injection for 5 weeks resulted in significantly increased body length (FIG. **51**), tail length (FIG. **52**), distal bone (ulna and tibia) lengths (FIGS. **53**A and B), and proximal bone (humerus and femur) lengths (FIGS. **54**A and B). Moreover, treatment with CNP37 increased the head length (FIG. **56**), area of the external auditory meatus (FIG. **57**), and spine length (FIG. **58**) via extension of the vertebral bodies. Additionally, treatment with CNP37 corrected rhizomelia (disproportion of the length of the proximal limbs) of $FGFR3^{ach}$ mice, i.e., restored proportional growth of proximal bones, as assessed by the femur:tibia ratio (FIG. **55**). Table 13 summarizes the percentage change in tail length, body (naso-anal) length, and bone (femur, tibia, humerus and ulna) lengths in $FGFR3^{ach}$ mice injected s.c. once daily with 200 nmol/kg of either CNP37 or PEO24-GANRR-CNP22(K4R) ("CNP27-PEO24") (SEQ ID NO: 36) for 5 weeks, relative to a value of 100% for $FGFR3^{ach}$ mice treated with vehicle only.

TABLE 13

	Tail	Naso-Anal	Femur	Tibia	Humerus	Ulna
CNP37	115%**	109%**	112%**	107%**	105%*	106%**
CNP27-PEO24	100%	99%	100%	102%	100%	101%

**p < 0.01,
*p < 0.05

The results of these studies show that CNP37 can stimulate spinal and long bone growth, help to correct rhizomelia by preferentially increasing the length of the femur over the tibia, and help to restore craniofacial proportions in $FGFR3^{ach}$ mice. These results indicate that CNP37 and potentially other CNP variants may be effective in correcting the symptoms of achondroplasia and treating subjects having defects in bone growth or in need of increased bone growth.

Example 14

Measurement of Biomarkers and Evaluation of Immunogenicity in Wild-Type and Achondroplastic Mice

Measurement of Biomarkers After CNP Administration

The levels of bone growth biomarkers were measured in wild-type and achondroplastic ($FGFR3^{ach}$) mice.

Transgenic FVB $FGFR3^{ach}$ mice (a mouse model of mild achondroplasia) were treated daily for 5 weeks by subcutaneous injection with either vehicle (30 mM acetic acid/acetate buffer, 1% benzyl alcohol, 10% sucrose, pH 4.0), CNP22, CNP37 or PEO24-GANRR-CNP22(K4R) ("CNP27-PEO24") (SEQ ID NO: 36) at 200 nmol/kg for each CNP compound, as described above. Plasma and serum were collected during the study. Harvested plasma was stored with 10× protease inhibitor at −80° C. until analysis. cGMP levels were measured 15 minutes post-injection on Day 36 from K2-ETDA plasma collection tubes. Fragments of cleaved collagen type II (cartilage-associated biomarkers), osteocalcin (bone-associated biomarker), and IgG (relating to immunogenicity) were measured from terminal bleed serum at the

end of the study (Day 37). cGMP was measured using a commercially available ELISA kit (Cayman Chemical Co., Cat. No. 581021.1), cleaved collagen type II (Cartilaps) was measured using a commercially available kit from Immunodiagnostic Systems (Cat. No. 3CAL4000), and osteocalcin was measured using a commercially available kit from Biomedical Technologies Inc. (Stoughton, Mass.).

FIG. **59** shows an increase in cGMP plasma levels in FGFR3^{ach} mice treated with CNP37 or PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) compared to vehicle. FIGS. **60** and **61** show that administration of CNP37 resulted in the greatest elevation of serum levels of cleaved collagen type II and osteocalcin. These results indicate that administration of the CNP peptides, in particular CNP37, to FGFR3^{ach} mice led to increased levels of bone growth markers, suggesting increased bone formation and growth in CNP peptide-treated FGFR3^{ach} mice.

For evaluation of biomarkers in wild-type mice, wild-type FVB mice were treated daily for 5 weeks by subcutaneous injection with either vehicle (30 mM acetic acid/acetate buffer, 1% benzyl alcohol, 10% sucrose, pH 4.0), 200 nmol/kg G-CNP37, 200 nmol/kg PEO12-GANRR-CNP22(K4R) ("CNP27-PEO12") (SEQ ID NO: 36), or 20 nmol/kg or 70 nmol/kg Pro-Gly-CNP37. Plasma and serum were collected during the study. Harvested plasma was stored with 10× protease inhibitor at −80° C. until analysis. cGMP levels were measured 15 minutes post-injection on Day 36 from K2-ETDA plasma collection tubes. The levels of cleaved collagen type II, bone-specific alkaline phosphatase, and IgG (relating to immunogenicity) were measured from terminal bleed serum at the end of the study (Day 37). cGMP and cleaved collagen type II (Cartilaps) were measured as described above. Bone-specific alkaline phosphatase was measured using a commercially available kit (Cusabio, Cat. No. CSB-E11914m).

Administration of G-CNP37 significantly increased ($p<0.05$) the level of cGMP (FIG. **62**) and particularly the level of fragments of cleaved collagen type II (FIG. **63**) in wild-type mice compared to vehicle. The significantly higher level of collagen type II fragments resulting from administration of G-CNP37 indicates turnover of the cartilage matrix, suggesting that G-CNP37 stimulated new bone formation in growing bones in wild-type mice.

Both doses of Pro-Gly-CNP37 ("Pro-CNP38"), 20 and 70 nmol/kg, significantly increased ($p<0.05$) plasma cGMP level 15 minutes after administration compared to wild-type mice treated with vehicle (FIG. **64**). Administration of the higher dose (70 nmol/kg) of Pro-Gly-CNP37 also significantly increased ($p<0.05$) the level of cleaved collagen type II compared to vehicle-treated mice (FIG. **65**), suggesting that the higher dose of Pro-Gly-CNP37 stimulated cartilage matrix turnover prior to new bone formation in wild-type mice. Furthermore, administration of the higher dose (70 nmol/kg) of Pro-Gly-CNP37 increased ($p<0.05$) the level of bone-specific alkaline phosphatase compared to vehicle-treated mice (FIG. **66**), suggesting that the higher dose of Pro-Gly-CNP37 increased bone remodeling in wild-type mice.

Evaluation of Immunogenicity of CNP Variants

Because the CNP variants are peptide derivatives, it is possible that administration of the peptides may lead to an immunogenic response in vivo. To assess whether an immune response occurred after successive administrations of CNP variant, measurement of serum antibody levels was performed.

An IgG assay was performed to assess whether an IgG immune response was triggered by 5 week exposure of achondroplastic FGFR3^{ach} mice to CNP22, CNP37 or PEO24-GANRR-CNP22(K4R) ("CNP27-PEO24") (SEQ ID NO: 36). IgG is the most predominant immunoglobulin in mouse and human serum and is produced as part of the secondary immune response to an antigen. The IgG response to administration of the CNP peptides was determined as follows. 96-well plates were coated with 100 ng/mL CNP22, CNP37 or PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) in BupH PBS buffer (Pierce/Thermo, Cat. No. 28372, Rockford, Ill.). After overnight incubation, plates were blocked with Casein-PBS blocking buffer (Pierce/Thermo, Cat. No. 37528) for two hours at room temperature with shaking at 300 rpm. After washing with wash buffer (1×PBS with 0.05% Tween), diluted serum samples from a terminal bleed (diluted 1:25) were added to the plate. Positive and negative controls were also loaded onto the plate. Positive control was 1:25 diluted serum (pooled from 6 individual FVB mice) with anti-CNP22 antibody added (Bachem rabbit anti-CNP22 IgG, Cat. No. T-4222) at 1:1000 dilution. Negative control was the diluted pooled serum. After two hours of incubation, the plates were washed and secondary antibody diluted in blocking buffer was added to the wells. For the mouse serum samples, anti-mouse IgG Fcγ (peroxidase-conjugated affinipure goat anti-mouse IgG, Fcγ fragment, Cat. No. 115-035-071, Jackson Immunoresearch, West Grove, Pa.) was added at a 1:10,000 dilution. For the positive and negative controls, anti-rabbit IgG-HRP (Santa Cruz Biotechnology, Cat. No. SC-2004, Santa Cruz, Calif.) was added to blocking buffer. After two hours of incubation at room temperature with shaking at 300 rpm, plates were washed with wash buffer. 100 uL of TMB (One-step TMB, Pierce/Thermo, Cat. No. 34022) was added to all wells. Plates were incubated at room temperature for 15 minutes with shaking at 300 rpm. Colorimetric reactions were stopped by the addition of 100 uL of 2 N H_2SO_4. Plates were read at 450 nm (Spectramax, Molecular Devices, Sunnyvale, Calif.) and data was analyzed using SoftMax Pro software (Molecular Devices).

Serum samples from FGFR3^{ach} mice treated with CNP22 or PEO24-GANRR-CNP22(K4R) (SEQ ID NO: 36) indicated no positive IgG immune response in any of the mice. Only one out of nine CNP37-treated FGFR3^{ach} mice showed a slightly positive IgG response.

The immunogenic response of wild-type mice administered G-CNP37, PEO12-GANRR-CNP22(K4R) (SEQ ID NO: 36) or Pro-Gly-CNP37, as measured by an increase in serum IgG level, was also assessed by examining terminal bleed serum samples as described above. None of the wild-type mice treated with PEO12-GANRR-CNP22(K4R) (SEQ ID NO: 36) or Pro-Gly-CNP37 (20 or 70 nmol/kg) showed a positive IgG immune response, and only one out of six wild-type mice administered G-CNP37 showed a slightly positive IgG response.

Measurement of Biomarkers Under Different CNP Dosing Regimens

Wild-type FVB mice were treated daily for 9 weeks by subcutaneous (s.c.) injection of vehicle (30 mM acetic acid/acetate buffer, 1% benzyl alcohol, 10% sucrose, pH 4.0) or Pro-Gly-CNP37 ("Pro-CNP38") under different dosing regimens. Group 1 included vehicle-treated mice injected s.c. daily for 9 weeks followed by 1 week of no treatment. Group 2 included mice treated with Pro-Gly-CNP37 (20 nmol/kg) once daily for 1 week, followed by three doses per week for 8 weeks, followed by 1 week of no treatment. Group 3 contained mice treated with Pro-Gly-CNP37 (20 nmol/kg) once daily in alternate weeks (weeks 1, 3, 5, 7 and 9), with 1 week of no treatment following each week of treatment. Group 4 contained mice treated with Pro-Gly-CNP37 (5 nmol/kg)

once daily for 9 weeks followed by 1 week of no treatment. Finally, Group 5 included mice treated with Pro-Gly-CNP37 (5 nmol/kg) once daily for 5 weeks without any week of no treatment. Terminal bleed serum was collected from the mice, and cleaved collagen type II, total alkaline phosphatase and total antibody levels were measured therefrom. Cleaved collagen type II levels were measured as described above. Total alkaline phosphatase levels, predominantly from the liver and bones, were measured with the assistance of a veterinary diagnostic laboratory testing facility (Antech).

A total antibody assay was developed to evaluate potential immune response. The platform used for the total antibody assay was an electrochemiluminescent assay (ECLA). The ECLA platform utilizes a biotin-labeled drug (here, biotin-Pro-Gly-CNP37) and a ruthenium-labeled drug (here, Ru—Pro-Gly-CNP37). The biotin-labeled drug binds to a streptavidin-coated plate that contains electrodes, and the ruthenium-labeled drug functions as the detection component of the assay, as ruthenium can be electrochemically stimulated. A drug-specific antibody (here, a CNP-specific antibody) binds to the biotin-labeled drug and the ruthenium-labeled drug and "bridges" the two labeled drugs. Among the advantages of the ECLA platform, any isotype of antibody (IgG, IgM, etc.) can be detected, and the ECLA assay is species-independent.

The total antibody assay was performed as follows. Pro-Gly-CNP37 was labeled with biotin in a 4:1 challenge ratio, and Pro-Gly-CNP37 was also labeled separately with ruthenium in a 10:1 challenge ratio. Both separate labeling reactions were quenched by the addition of glycine, and the samples from both reactions were buffer-exchanged to PBS. Low and high QCs were prepared by using commercially available anti-CNP22 antibody (Bachem rabbit anti-CNP22 IgG, Cat. No. T-4222) added at low and high concentrations to 5% diluted FVB mouse serum. Mouse serum samples were diluted to 5% using assay diluent (5% BSA in PBS, MSD Cat. No. R93AA-1). A working solution was prepared by adding biotin-labeled Pro-Gly-CNP37 to assay diluent, then adding ruthenium-labeled Pro-Gly-CNP37 to assay diluent, and then combining the two solutions together. Twenty-five uL of low and high QCs were loaded onto the plate as controls. Then 25 uL of sample was added to a non-binding 96-well plate, followed by the addition of 50 uL of working solution to all wells. Samples and QCs were incubated with the working solution at room temperature for 2 hours with shaking at 350 rpm. In the meantime, an MSD streptavidin plate (MSD Cat. No. L13SA-1) was blocked with blocking buffer (MSD Cat. No. R93AA-1) at room temperature for 2 hours with shaking at 350 rpm. At the end of the two hour incubation, the MSD streptavidin plate was washed, and then 50 uL of sample or QC was transferred to the MSD plate. The plate was then incubated at room temperature for 1 hour with shaking at 350 rpm. At the end of the 1 hour incubation, the MSD plate was washed and 150 uL of 2× read buffer (MSD Cat. No. R92TC-2) was added to the plate. The plate was read using an MSD PR400 machine.

All five groups of mice exhibited substantially comparable levels of cleaved collagen type II (FIG. **67**). Treatment of the mice in Group 5 with 5 nmol/kg Pro-Gly-CNP37 once daily for 5 weeks significantly increased ($p<0.001$) total alkaline phosphatase level (FIG. **68**), which is indicative of bone remodeling. Serum samples from each of the four groups of mice treated with Pro-Gly-CNP37 did not show a positive antibody response in the total antibody assay.

Not to be bound by any theory, there are possible explanations as to why the four groups of CNP-treated mice did not exhibit statistically significant difference in cleaved collagen type II level compared to vehicle-treated mice, and why only the CNP-treated mice in Group 5 exhibited a statistically significant increase in total alkaline phosphatase level compared to vehicle-treated mice. It is possible that because the vehicle-treated mice in the study were also growing, cleaved collagen type II and alkaline phosphatase, which are biomarkers of growth, were also produced in the vehicle-treated mice. In addition, there was a one week period of no treatment for the mice in each of Groups 1 to 4, which possibly diluted out any changes in the cleaved collagen type II and total alkaline phosphatase levels between the CNP-treated mice in Groups 1-3 and the vehicle-treated mice. There was no period of no treatment for the CNP-treated mice in Group 5, and those mice exhibited significantly ($p<0.001$) greater total alkaline phosphatase level compared to vehicle-treated mice.

Example 15

Dose Responses of CNP Variants in Mice

The effects of varying doses of CNP variants were assessed in wild type FVB mice.

For the dose study, in two separate studies (S1 and S2), groups of 10 mice were administered Pro-Gly-wtCNP37 ("Pro-CNP38") at 20 and 70 nmol/kg subcutaneously once daily for 36 injections. Tail length and body weight were measured over the course of treatment. Animals were sacrificed at the end of the experiment and bone length assessed.

Tail length of vehicle treated animals was approximately 8 cm at day 36, whereas animals treated with 20 nmol/kg Pro-CNP38 exhibited tail length of approximately 8.75 cm and 70 nmol/kg Pro-CNP38 increased tail length to approximately 9.5 cm. Administration of Pro-CNP38 in either dose induces a significant ($p<0.05$) relative increase in total body length compared to control treated animals, demonstrating approximately a 130% increase in growth velocity at 20 nmol/kg and approximately a 160% increase in growth velocity at 70 nmol/kg Pro-CNP38 (FIG. **69**).

Treatment with Pro-CNP 38 also significantly increased bone length in most of the long bones assessed as well as total naso-anal (body length) of the animal. Table 14 is representative of the relative % increase in bone length in treated animals in comparison to vehicle treated animals.

TABLE 14

Pro-CNP38	Tail	Naso-Anal	LV4-6	Femur	Tibia	Humerus	Ulna
20 nmol/kg	8%*	7%*	10%*	7%*	4%*	3%*	3%
70 nmol/kg	16%*	13%*	13%*	10%*	7%*	4%*	5%*

Relative % increase,
*$p < 0.05$ ANNOVA (Dunnett's) v vehicle

Bone mineral density (BMD) and bone mineral content (BMC) were also evaluated after administration of Pro-CNP38 at different doses. Results (FIG. **70**) showed that administration of Pro-CNP38 at 70 nmol/kg significantly decreased bone mineral density (FIG. **70**A) and increased bone mineral content (FIG. **70**B), suggesting there is a delay in bone mineralization in treated animals, but the mineralization process itself is not adversely affected by treatment with CNP.

There were no significant changes in organ weight between Pro-CNP38 or vehicle treated animals.

Bioanalytical Studies of Dose Response in Mice

Bioanalytical studies were carried out to measure markers of CNP activity after in vivo administration of varying doses

of Pro-CNP38. Plasma cGMP levels, serum levels of collagen type II and serum levels of alkaline phosphatase from in vivo samples were analyzed. Wild type mice were administered 20 and 70 nmol/kg Gly-wtCNP37 ("CNP38"), 20 and 70 nmol/kg Pro-Gly-wtCNP37 ("Pro-CNP38") and 70 and 200 nmol/kg of GHKSEVAHRFK-wtCNP27 ("HSA-CNP27") (SEQ ID NO: 144) subcutaneously once daily for 36 days. Plasma was collected 15 minutes after the last injection on day 36 and mice were sacrified 24 hr later. At sacrifice, terminal bleed serum was collected and used for biomarker analysis as described previously.

FIG. **71** shows that 20 nmol/kg CNP38 and 70 nmol/kg HSA-CNP27 signficantly increased plasma cGMP ($p<0.01$), raising plasma cGMP levels to approximately 300 pmol and 400 pmol, respectively. Adminsitration of 70 nmol/kg CNP38 increased cGMP to approximately 500 pmol ($p<0.01$), while administration of 70 nmol/kg Pro-CNP38 increased cGMP to approximately 575 pmol ($p<0.001$). Administration of 200 nmol/kg HSA-CNP27 increased cGMP to approximately 675 pmol ($p<0.001$).

CNP variants also significantly increased serum levels of cleaved collagen type II (FIG. **72**). CNP38 at 20 nmol/kg increased collagen to approximately 9 pg/ml ($p<0.05$), CNP38 at 70 nmol/kg increased collagen to approximately 8 pg/ml ($p<0.05$), Pro-CNP38 at 20 nmol/kg increased collagen to approximately 12 pg/ml ($p<0.05$), Pro-CNP38 at 70 nmol/kg increased collagen to approximately 16 pg/ml ($p<0.05$), HSA-CNP27 at 70 nmol/kg increased collagen to approximately 10 pg/ml, and HSA-CNP27 at 200 nmol/kg increased collagen to approximately 10 pg/ml ($p<0.05$).

Serum alkaline phosphatase (AP) levels also increased after administration of CNP variants (FIG. **73**). CNP38 at 20 nmol/kg increased AP to approximately 130 IU/L, CNP38 at 70 nmol/kg increased AP to approximately 160 IU/L ($p<0.001$), Pro-CNP38 at 20 nmol/kg increased AP to approximately 155 IU/L ($p<0.001$), Pro-CNP38 at 70 nmol/kg increased AP to approximately 180 IU/L ($p<0.001$), HSA-CNP27 at 70 nmol/kg increased AP to approximately 120 IU/L, and HSA-CNP27 at 200 nmol/kg increased AP to approximately 140 IU/L ($p<0.01$). Table 15 illustrates the percent of total AP that is bone specific.

TABLE 15

	Total AP IU/L	Bone-specific AP IU/L	% of Total Alk Phos
Vehicle	109.8	24.7	22.5
CNP38 (20 nmol/kg)	135.9	47.9	35.2
CNP38 (70 nmol/kg)	166.3	63.5	38.2
Pro-CNP38 (20 nmol/kg)	159.9	65.9	41.2
Pro-CNP38 (70 nmol/kg)	183.6	78.4	42.7
HSA-CNP27 (20 nmol/kg)	121.4	51.7	42.6
HSA-CNP27 (70 nmol/kg)	136.1	76.5	56.2

Analysis of anti-CNP antibodies showed that only HSA-CNP27 elicited an IgG antibody response in mice.

The results above illustrate that administration of CNP variants increases the concentration of collagen type II and alkaline phosphatase in serum, indicating that CNP increases factors relevant for increased bone growth, and suggest that administration of CNP variants at doses as low as 20 nmol/kg are effective in increasing bone growth in vivo.

cGMP Response After Different Dose Regimens

Bioanalytical analysis was also assessed at different times after administration of Pro-Gly-wtCNP37 ("Pro-CNP38") to wild type CD-1 mice, 8-10 weeks old, (n=3 per treatment group). Pro-CNP38 was given in a single subcutaneous dose of 200 nmol/kg and levels of cGMP measured at 15 minutes, 3 hours, 1 day, 2 days and 3 days post injection in the plasma, epiphysis, cortical bone (marrow removed), lung and brain. Blood was collected on K2EDTA. Tibial and femoral epiphysis and cortical bone, ear pinna, brains, kidneys and lungs were harvested, placed in boiling water for 5 minutes, then frozen to −70° C. Both plasma and tissue were assayed for cGMP (Cayman Chemical cyclic GMP ELISA kit).

Results showed that cGMP levels increased at 15 minutes post injection in plasma (approximately 1300 pmol/ml) and epiphysis (approximately 2.5 pmol/ml/mg). By 3 hours, plasma levels had decreased approximately to control levels, while levels in the epiphysis were approximately 3 fold lower than cGMP levels at 15 minutes post injection, but higher than control levels. Levels of cortical bone increased to approximately 0.5 pmol/ml/mg at 15 minutes and remained at this level at 3 hours. Levels of cGMP at all timepoints were back to control levels by 1 day after injection. Little to no cGMP was detected in lung or brain at any timepoint.

cGMP levels were also measured in mice administered multiple injections of Pro-CNP38. Groups of mice (n=3) were given Pro-CNP38 as follows: 20 nmol/kg single dose, subcutaneously; 200 nmol/kg single dose, subcutaneously; 20 nmol/kg subcutaneously on days 0 and 1; 200 nmol/kg subcutaneously on days 0 and 1; 20 nmol/kg subcutaneously on days 0 and 3; 200 nmol/kg subcutaneously on days 0 and 3. Mice were sacrificed 15 minutes after the final dose of Pro-CNP38 and plasma levels of cGMP analyzed. There does not appear to be a modulation of the plasma cGMP signal due to the different dose regimens. cGMP responses in the cartilage are also investigated.

Evaluation of Potential Desensitization of NPR-B Receptor

Histological analysis shows that CNP, when administered daily at 200 nmol/kg, accumulates in the growth plate of animals, based on increased CNP immunoreactivity. It is possible that this accumulation, or daily stimulation of the CNP receptor, in the growth plate could desensitize the CNP receptor.

To determine if multiple dosing desensitizes the NPR-B receptor in vitro, normal human articular chondrocytes were cultured with Pro-Gly-wtCNP37 ("Pro-CNP38") for varying times and cGMP secretion measured.

Primary normal human chondrocytes isolated from articular cartilage were cultured as recommended by the supplier (Lonza). At 60-80% confluence the chondrocytes were treated with 1 uM Pro-CNP38 twice, with increasing amounts of time between treatments (first treatment at time 0, then at 15 min, 30 min, 60 min, 2 hrs, 3 hrs, 4 hrs, 6 hrs after the initial treatment). In the following experiment the treatments were either applied twice, with increasing amounts of time between treatment (first treatment at time 0, then at 6, 16, 24, 48 hrs after initial treatment) or only once, in parallel to the second treatment (only at 6, 16, 24 and 48 hrs-naïve responses). Treatments were either applied for 15 min only (acute treatment), or for the duration of the experiment (chronic treatment when CNP was left in the medium). Cell lysates and conditioned medium were collected and analyzed for total cGMP secretion (Molecular Devices ELISA).

In a short term experiment, cells were stimulated with Gly-wtCNP37 ("CNP38") 1 uM two times for 15 minutes (acute treatment), or two times with CNP38 throughout culture (chronic treatment). The periods between treatments were 15 min, 30 min, 60 min, 2 hrs, 3 hrs, 4 hrs, and 6 hrs. Peak cGMP stimulation was obtained by treating cells once only, at the last time point of the experiment, (6 hours; >0.1 pmol/well in the acute experiment and 0.2 pmol/well in the chronic experiment). In the acute experiment, when the cells

are treated twice, the response decreases to 0.1 pmol/well if the cells are treated at time 0 and then again at 15 minutes. In the acute experiment, when the cells are treated twice, the response decreases to approximately 0.5 pmol/well if the cells are treated at time 0 and again at 30 min, 60 min, 2, 3, and 4 hours. In the chronic experiment, when the cells are treated twice, the response decreases to approximately 0.16 pmol/well if the cells are treated at time 0 and at 15 minutes. In the chronic experiment, when the cells are treated twice, the response decreases to approximately 0.6 pmol/well if the cells are treated at time 0 and again at 30 min, 60 min, or 2 hours. In the chronic experiment, when the cells are treated twice, the response decreases to <0.5 pmol/well if the cells are treated at time 0 and again at 2, 4 or 6 hours.

Long term studies were also carried out. For acute treatment, 1 uM CNP38 was added to cell culture as described above. For chronic treatment, 1 uM CNP38 was added to cell culture throughout the experiment duration as described above. Results indicate that the NPR-B receptor can desensitize after repeated, daily CNP administration in vitro, and 48 hours between doses is sufficient to recover to 60% of the maximial NPR-B response to CNP38, if the CNP is removed after the first dose, and to <40% if the CNP is incubated throughout the experiment (FIG. **74**).

To evaluate whether treatment with a CNP variant desensitizes the NPR-B receptor in vivo, experiments were conducted with wild-type mice. CD-1 male mice 8-10 weeks of age were injected subcutaneously with vehicle control or Pro-Gly-wtCNP37 ("Pro-CNP38") at 200 nmol/kg on the appropriate days. The mice were either injected with Pro-CNP38 daily for up to 8 days, or were injected with Pro-CNP38 on the first day, the first and second days, or the first and third days of the study. Fifteen minutes following the final injection, the mice (n=3 per treatment group) were deeply anesthetized and ex-sanguinated via thoracotomy and aortic cannulation. Circulating blood was flushed from the body with PBS via an aortic cannula. The kidneys and right tibias, right femurs and left femurs were collected, boiled in water for 5 minutes and/or snap frozen in liquid nitrogen and stored on dry ice or in a −70° C. freezer until cGMP assay. For estimation of cGMP production in cartilage, distal femurs and/or proximal tibias were dissected, weighed and pulverized using Covaris Cryoprep CP02. Powdered samples were homogenized using a Covaris E210 sonicator in 5% perchloric acid, and neutralized in 60% KOH. Samples were then centrifuged at 10,000 rpm at 4° C. for 5 minutes, and the supernatant was used for the cGMP assay (Cyclic GMP Enzyme Immunoassay Kit, Cayman, Mich.). Additionally, left tibias were collected, fixed in 10% normal buffered formalin, and saved for further immunohistochemical analysis.

FIG. **75**A shows that repeated daily treatment of wild-type mice with 200 nmol/kg of Pro-CNP38 for 1, 4, 6, 7 and 8 days did not result in desensitization of the cGMP response. To the contrary, potentiation of the cGMP response was observed after 4 days of daily treatment. Further daily treatment resulted in a plateau of the cGMP reponse, up to 8 days. The results indicate that daily treatment of wild-type mice with 200 nmol/kg of Pro-CNP38 for up to 8 days do not desensitize the cGMP response.

The kinetics of the cGMP response in distal femoral cartilage after treatment of the wild-type mice with Pro-CNP38 was also investigated. Treatment of the mice once a day for two days potentiated the cGMP response to Pro-CNP38, compared with the cGMP response to a single treatment (FIG. **75**B). When the mice were treated on the first and third days, but not on the first and second days, the cGMP response after the second treatment (on the third day) was substantially similar to the cGMP response observed after a single treatment on day one (FIG. **75**B). The results suggest that dosing on consecutive days is beneficial for potentiating the cGMP response to Pro-CNP38 in this mouse study.

Activation of NPR-B in Different Tissues

To evaluate potential activation of NPR-B in different tissues by a CNP variant, wild-type male CD-1 mice were injected with 200 nmol/kg of Gly-CNP37, and the cGMP response was measured in certain tissues at different time points. Two mice were used for each treatment group. Each mouse received one subcutaneous injection of Gly-CNP37 or vehicle control. Fifteen, 30 or 60 minutes or 3 hours following the injection, the mice were deeply anesthetized and ex-sanguinated via thoracotomy and aortic cannulation. Circulating blood was removed by a PBS flush with an aortic cannula. Heart, liver, lung, kidney, ear pinna, aorta and brain were collected. All tissues were boiled in water for 5 minutes, finely dissected (including flushing of marrow from femoral cortices with PBS), weighed, cooled in liquid nitrogen, and pulverized in a BioPulverizer. The resulting powdered samples were homogenized with a Polytron in 6% pre-chilled perchloric acid and neutralized with 60% KOH. Samples were then centrifuged at 10,000 rpm and 4° C. for 5 minutes, and the supernatant was used for the assay of cGMP (Cyclic GMP Enzyme Immunoassay Kit, Cayman Chemical Company, Ann Arbor, Mich.). The results were normalized for tissue weight.

Secretion of cGMP in response to treatment with Gly-CNP37 ("CNP" in FIG. **76**) was detectable in distal femurs (cartilage and bone), femoral cortices (bone), ear pinna (cartilage), and kidney (FIGS. **76**A-D). Maximal cGMP responses in those tissues were observed 15 min after treatment. Liver, heart, lung and brain tissues did not exhibit appreciable cGMP secretion in response to Gly-CNP37 relative to vehicle control at the studied time points (FIGS. **76**E-H). The results indicate that treatment with 200 nmol/kg of Gly-CNP37 stimulated cGMP secretion in cartilage, bone and renal tissues.

Example 16

Dose Responses of CNP Variants in Monkeys

The effects of the CNP variant Pro-Gly-CNP37 on bone growth and the levels of bone growth-related biomarkers are evaluated in cynomolgus monkeys. Eight normal juvenile cynomolgus monkeys (about 2.5 years of age at the start of the on-going study) are subcutaneously injected daily with 10 or 36 μg/kg/day of Pro-Gly-CNP37 (n=4 per dose group). Four such monkeys are administered vehicle as control. The total length of treatment is 6 months. Various measures of growth plate expansion and bone growth are made by digital X-ray and magnetic resonance imaging, and by measurement of limb and body lengths externally. Blood and urine samples are collected periodically for clinical pathology and measurement of levels of Pro-Gly-CNP37 and biomarkers. Following termination of the study, gross pathology is performed and tissue samples are evaluated histologically for assessment of efficacy and safety.

Data obtained thus far in the on-going study show that both doses of Pro-Gly-CNP37 have increased growth plate width by digital X-ray (FIG. **77**), increased right and left tibia lengths by digital X-ray (FIGS. **78**A and B), increased leg length by external measurement (FIG. **79**), increased arm length by external measurement (FIG. **80**), increased body length by external measurement (FIG. **81**), and increased the serum level of alkaline phosphatase, a biomarker for bone

formation (FIG. **82**). The data demonstrate that Pro-Gly-CNP37 can stimulate bone growth in normal juvenile cynomolgus monkeys at hemodynamically acceptable doses.

Example 17

Effects of CNP Variants on Cardiovascular System in Mice

Natriuretic peptides such as CNP have been reported to affect the cardiovascular system. Wang et al. (Eur J Heart Fail. 9:548-57. 2007) describe that CNP has been shown to have a cardioprotective effect in preventing myocardial ischaemia/reperfusion injury and improving cardiac remodelling after myocardial infarction in rats. Wang demonstrated that mice overexpressing CNP have reduced incidence of cardiac hypertrophy caused by myocardial infarction. Additionally, CNP has been shown to cause endothelium-independent vasodilation (M. Honing et al., Hypertension, 37:1179-1183 (2001)) and therefore may transiently decrease blood pressure in vivo.

To assess the effects of CNP variants on the cardiovascular system, the blood pressure and heart rate in anesthetized wild-type FVB mice is studied following subcutaneous injection of the variants.

After a pilot study to define a broad dose range of cardiovascular activity, a dose-response study is conducted to examine the effects of three different dose levels of each CNP variant. Three male FVB mice aged 8 weeks comprise each treatment group. Doses are administered subcutaneously to anesthetized mice, and systolic, diastolic and mean arterial pressure (MAP), as well as heart rate, are monitored via implanted intraarterial pressure transducers.

Example 18

Formulation of CNP Variants

CNP preformulation studies were carried out to assess the stability of CNP variant Gly-wtCNP37 ("CNP38") at different pH's (pH 3, 4, 5, 6, 7 and 8) and temperatures (5° C., 25° C., and 40° C.) over time. CNP38 exhibited greater stability at pH 4-6 than at the other pH's in the studies. CNP38 was stable at 5° C. at pH 4-6, with ≥about 95% of CNP38 remaining after 15 weeks. When the temperature was raised to 25° C., at pH 4 about 85% of CNP38 remained after 15 weeks, at pH 5 about 85% remained after 15 weeks, and at pH 6 about 80% remained after 15 weeks. When the temperature was raised to 40° C., at pH 4 about 55-60% of CNP38 remained after 15 weeks, at pH 5 about 65% remained after 15 weeks, and at pH 6 about 40% remained after 15 weeks. FIG. **83** illustrates the observed plot of pseudo-first order degradation rate constant (K_{obs}) vs. pH from pH 3 to 8 and at 5° C., 25° C. and 40° C. The stability data for CNP38 in the preformulation studies suggests CNP formulations having a pH in the range from about 4 to about 6. An acidic pH (e.g., pH≤about 6) can promote the stability of a CNP variant by, e.g., minimizing or avoiding deamidation of asparagine and/or glutamine residue(s), isomerization of aspartic acid residue(s), or degradation of the CNP variant by other pathways.

CNP variants can be formulated in pharmaceutical carriers for administration to subjects affected by, e.g., bone growth conditions. In some embodiments, liquid formulations of CNP variants are formulated according to any combinations of the ingredients and their amounts or concentrations in Table 16.

TABLE 16

Ingredient Class	Ingredient	Concentration Range
Active ingredient	CNP variant	10 mg/mL ± 9.9 mg/mL
Buffering agent	Acetic acid/acetate	10 mM ± 5 mM, or pH 5 ± 1
Buffering agent	Citric acid/citrate	10 mM ± 5 mM, or pH 5 ± 1
Isotonicity-adjusting agent	NaCl	140 mM ± 20 mM
Isotonicity-adjusting agent	Sucrose	10% ± 5%
Preservative	m-Cresol	0.4% ± 0.1% or 0.2%
Preservative/anti-adsorbent	Benzyl alcohol	1.5% ± 0.5%
Stabilizer	Glycerin (glycerol)	5%-100% (neat)[1]
Stabilizer	Methionine	0.01%-0.2%
Stabilizer	Ascorbic acid/ ascorbate salt	0.1%-1%
Stabilizer	Thioglycerol	0.1%-1%
Anti-adsorbent	Polysorbate 20	0.001%-0.5%
	Polysorbate 80	0.001%-0.5%
	Benzyl alcohol	0.5%-1.5%

[1]Glycerin is used to minimize or prevent water-driven hydrolysis, deamidation, isomerization or cleavage of CNP variants. For lyophilized formulations, 4-6% or 6-20% mannitol or sucrose can be substituted for NaCl.

In certain embodiments, lyophilized formulations of CNP variants are prepared from formulations formulated according to any combinations of the ingredients and their amounts or concentrations in Table 17.

TABLE 17

Ingredient Class	Ingredient	Concentration Range
Active ingredient	CNP variant	10 mg/mL ± 9.9 mg/mL
Buffering agent	Acetic acid/acetate	10 mM ± 5 mM, or pH 5 ± 1
Buffering agent	Citric acid/citrate	10 mM ± 5 mM, or pH 5 ± 1
Isotonicity-adjusting agent/bulking agent	Sorbitol	5% ± 3%
Isotonicity-adjusting agent/bulking agent	Mannitol	5% ± 3%
Isotonicity-adjusting agent/bulking agent	Sucrose	10% ± 5%
Preservative	m-Cresol	0.4% ± 0.2%
Preservative/anti-adsorbent	Benzyl alcohol	1.5% ± 0.5%
Stabilizer	Glycerin (glycerol)	5%-100% (neat)[1]
Stabilizer	Methionine	0.01%-0.2%
Stabilizer	Ascorbic acid/ ascorbate salt	0.1%-1%
Stabilizer	Thioglycerol	0.1%-1%
Anti-adsorbent	Polysorbate 20	0.001%-0.5%
	Polysorbate 80	0.001%-0.5%
	Benzyl alcohol	0.5%-1.5%

[1]Glycerin is used to minimize or prevent water-driven hydrolysis, deamidation, isomerization or cleavage of CNP variants.

In certain embodiments, a formulation comprising a CNP variant has a pH of about 3-7, or about 3-6, or about 3.5-6.5, or about 4-6, or about 4-5, or about 4.5-5.5. In some embodiments, for pH 4-5.5 a suitable buffering agent is acetic acid/acetate (e.g., sodium acetate), and for pH 5.5-6 a suitable buffering agent is citric acid/citrate. Citric acid/citrate (e.g., sodium citrate) is also a suitable buffering agent in the range of pH 3-6 or pH 4-6. In certain embodiments, the buffering agent has a concentration in the formulation of about 2-50 mM, or about 2-40 mM, or about 2-30 mM, or about 5-30 mM, or about 2-20 mM, or about 5-20 mM, or about 5-15 mM.

To minimize or avoid deamidation of a CNP variant, the variant can be formulated in pharmaceutically acceptable organic cosolvents, such as glycerin, ethanol, and propylene

glycol. Because deamidation occurs by hydrolysis, substitution of an organic cosolvent for water minimizes contact of the CNP variant with water. The concentration of one or more organic solvents in an organic-aqueous solvent system can be, e.g., from about 10% to about 99%, or about 100% if water is not used.

Also to minimize or avoid deamidation of a CNP variant, water can be removed from the formulation by lyophilization. In some embodiments, lyophilized formulations contain any combinations of the following components:

- buffer: sodium acetate and acetic acid, or sodium citrate and citric acid;
- isotonicity/bulking agent: mannitol (e.g., 3-10%, 2-8% or 4-6%);
  sucrose (e.g., 6-20%, 5-15% or 8-12%);
- antioxidants: methionine and/or ascorbic acid with molal ratio of each antioxidant to CNP variant from about 0.1:1 to about 1:1, or from about 0.5:1 to about 5:1, or from about 1:1 to about 15:1, or from about 1:1 to about 10:1, or from about 3:1 to about 10:1.

Deamidation can also be minimized or avoided by storing a CNP composition (e.g., a liquid formulation or a lyophilized formulation) at lower temperature, such as at about 5° C., 0° C., −10° C., −20° C., −30° C., −40° C., −50° C., −60° C., −70° C., −80° C., −90° C., or −100° C.

To minimize or avoid oxidation of oxidizable residues (e.g., methionine) in a CNP variant, the variant can be formulated with one or more antioxidants. Exemplary antioxidants include, but are not limited to, methionine, ascorbic acid, and thioglycerol. Oxidation of, e.g., methionine residues can also be minimized or prevented by purging oxygen from a liquid medium (if a liquid formulation) with nitrogen or argon, and/or by purging oxygen from a container or packaging with nitrogen or argon.

In some embodiments, to minimize or prevent adsorption (e.g., adsorption of a CNP variant to plastic or glass), Polysorbate 20, Polysorbate 80 or benzyl alcohol, or a combination thereof, is added to a CNP formulation. In certain embodiments, each of the anti-adsorbent(s) is in a concentration from about 0.001% to about 0.5%, or from about 0.01% to about 0.5%, or from about 0.1% to about 1%, or from about 0.5% to about 1%, or from about 0.5% to about 1.5%, or from about 0.5% to about 2%, or from about 1% to about 2%. Exemplary range(s) of anti-adsorbent(s) in the formulation include without limitation from about 0.001% to about 0.5% of Polysorbate 20, from about 0.001% to about 0.5% of Polysorbate 80, and/or from about 0.5% to about 1.5% of benzyl alcohol.

In certain embodiments, a liquid CNP formulation comprises, or a lyophilized CNP formulation is prepared from a formulation that comprises, (1) an acetic acid/acetate (e.g., sodium acetate) buffer having a concentration of about 30 mM±5 or 10 mM buffering agent and a pH of about 4±0.5 or 1, and (2) benzyl alcohol (e.g., as a preservative and/or anti-adsorbent) at a concentration of about 1%±0.5%, and optionally (3) sucrose at a concentration of about 10%±5%.

Example 19

Clinical Evaluation of CNP Variants

The following example provides guidance on the parameters to be used for the clinical evaluation of compositions comprising CNP22 or variants thereof in the therapeutic methods of the present disclosure. As discussed herein, CNP22 or variants thereof will be used in the treatment of disorders responsive to CNP, including disorders of the bone and vascular smooth muscle. Clinical trials will be conducted which will provide an assessment of doses of CNP22 or variants thereof for safety, pharmacokinetics, and initial response of both surrogate and defined clinical endpoints. The trial will be conducted for a minimum, but not necessarily limited to, 24 weeks to collect sufficient safety information on about 100 evaluable patients. The initial dose for the trials will vary from about 0.001 to about 1.0 mg/kg/week, or any of the doses described herein. In the event that the initial dose in this range does not produce a significant direct clinical benefit, the dose should be increased within this range or beyond this range as necessary, and maintained for an additional minimal period of, but not necessarily limited to, 24 weeks to establish safety and to evaluate efficacy further.

Measurements of safety will include adverse events, allergic reactions, complete clinical chemistry panel (including kidney and liver functions), urinalysis, and CBC with differential. In addition, other parameters relevant to clinical benefit will be monitored. The present example also includes the determination of pharmacokinetic parameters of CNP22 or variants thereof, including absorption, distribution, metabolism, excretion, and half-life and bioavailability in the blood. It is anticipated that such analyses will help relate dose to clinical response.

Methods

Patients will undergo a baseline medical history and physical exam, and a standard set of clinical laboratory tests (including CBC, Panel 20, CH50, and UA). The patients will be followed closely with weekly visits to the clinic. The patients will return to the clinic for a complete evaluation one week after completing the treatment period. Should dose escalation be required, the patients will follow the same schedule outlined above. Safety will be monitored throughout the trial.

Diagnosis and Inclusion Criteria

The patients may be male or female, with a documented diagnosis of a potentially CNP-responsive disorder. A specific example of a potentially CNP-responsive, bone-related disorder is achondroplasia, which may be confirmed by genetic testing and other evidence of an FGFR-3 mutation or dysfunction. The ideal age range of achondroplasia patients will be from infant (<1 year of age) to pre-adolescent (<13 years of age). A patient will be excluded from this study if the patient is pregnant or lactating; has received an investigational drug within 30 days prior to study enrollment; or has a medical condition, serious intercurrent illness, or other extenuating circumstance that may significantly decrease study compliance.

Safety

Therapy with CNP22 or variants thereof will be determined to be safe if no significant acute or chronic drug reactions occur during the course of the study. The longer-term administration of the drug will be determined to be safe if no significant abnormalities are observed in the clinical examinations, clinical labs, or other appropriate studies.

It has been shown that compared to wild-type CNP22, certain CNP variants of the disclosure are much more resistant to NEP degradation in vitro, have a much longer plasma half-life and bioavailability in rats, stimulate a much higher level of cGMP production in rats, and/or induce a significantly greater increase in long bone length and body length in achondroplastic mice. Furthermore, it has been shown that short duration dose regimen treatments with CNP22 are nearly as effective as continuous CNP22 treatment in reversing FGF2-induced arrest of chondrocyte growth in vitro. These results, among others described herein, demonstrate the utility of CNP variants of the disclosure in treating CNP-responsive conditions or disorders such as, e.g., bone-related disorders and vascular smooth muscle disorders.

It is understood that every embodiment of the disclosure described herein may optionally be combined with any one or more of the other embodiments described herein. Every patent literature and every non-patent literature cited herein are incorporated herein by reference in their entirety.

Numerous modifications and variations to the disclosure, as set forth in the embodiments and illustrative examples described herein, are expected to occur to those skilled in the art. Consequently only such limitations as appear in the appended claims should be placed on the disclosure.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 237

<210> SEQ ID NO 1
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<220> FEATURE:
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<223> OTHER INFORMATION: wtCNP-22
<220> FEATURE:
<221> NAME/KEY: DISULFID
<222> LOCATION: (6)..(22)
<223> OTHER INFORMATION: Cysteines in positions 6 and 22 form cyclic domain via disulfide bond

<400> SEQUENCE: 1

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 2
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: CNP-17

<400> SEQUENCE: 2

Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly
1 5 10 15

Cys

<210> SEQ ID NO 3
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: CNP-27

<400> SEQUENCE: 3

Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu
1 5 10 15

Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25

<210> SEQ ID NO 4
<211> LENGTH: 53
<212> TYPE: PRT
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<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:

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<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: CNP-53

<400> SEQUENCE: 4

Asp Leu Arg Val Asp Thr Lys Ser Arg Ala Ala Trp Ala Arg Leu Leu
1 5 10 15

Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly
20 25 30

Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met
35 40 45

Ser Gly Leu Gly Cys
50

<210> SEQ ID NO 5
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
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<223> OTHER INFORMATION: Synthetic Polypeptide
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<221> NAME/KEY: MOD_RES
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<223> OTHER INFORMATION: Gly is optionally modified with an amino acid sequence derived from a natriuretic polypeptide, a non-natriuretic polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (22)..(22)
<223> OTHER INFORMATION: Cys is optionally modified with an amino acid sequence derived from a natriuretic polypeptide, a non-natriuretic polypeptide

<400> SEQUENCE: 5

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 6
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Gly is modified with a synthetic or natural polymeric group or combination thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: Xaa is Lys or a conservative amino acid substitution thereof, any natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amine on a side chain, Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Glu or Ser
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<221> NAME/KEY: MOD_RES
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is Lys or a conservative amino acid substitution thereof, any natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amine on a side chain, Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Glu or Ser
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (22)..(22)
<223> OTHER INFORMATION: Cys is optionally modified with a synthetic or natural polymeric group or combination thereof

<400> SEQUENCE: 6

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Gly Leu Ser Xaa Gly Cys Phe Gly Leu Xaa Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 7
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 7

Gly Ala Asn Lys Lys
1 5

<210> SEQ ID NO 8
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 8

Gly Ala Asn Arg Arg
1 5

<210> SEQ ID NO 9
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 9

Gly Ala Asn Pro Arg
1 5

<210> SEQ ID NO 10
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 10

Gly Ala Asn Gln Gln
1 5

<210> SEQ ID NO 11
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 11

Gly Ala Asn Ser Ser
1 5

<210> SEQ ID NO 12
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

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<400> SEQUENCE: 12

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1 5

<210> SEQ ID NO 13
<211> LENGTH: 5
<212> TYPE: PRT
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<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 13

Gly Ala Asn Arg Met
1 5

<210> SEQ ID NO 14
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 14

Gly Ala Asn Arg Thr
1 5

<210> SEQ ID NO 15
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 15

Gly Ala Asn Arg Ser
1 5

<210> SEQ ID NO 16
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 16

Ala Ala Trp Ala Arg Leu Leu Gln Glu His Pro Asn Ala
1 5 10

<210> SEQ ID NO 17
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 17

Ala Ala Trp Ala Arg Leu Leu Gln Glu His Pro Asn Ala Arg
1 5 10

<210> SEQ ID NO 18
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 18

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Asp Leu Arg Val Asp Thr Lys Ser Arg Ala Ala Trp Ala Arg
1 5 10

<210> SEQ ID NO 19
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 19

Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys
1 5 10 15

<210> SEQ ID NO 20
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 20

Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Arg Arg
1 5 10 15

<210> SEQ ID NO 21
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 21

Asp Leu Arg Val Asp Thr Lys Ser Arg Ala Ala Trp Ala Arg Leu Leu
1 5 10 15

Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys
20 25 30

<210> SEQ ID NO 22
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 22

Asp Leu Arg Val Asp Thr Lys Ser Arg Ala Ala Trp Ala Arg Leu Leu
1 5 10 15

Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Arg Arg
20 25 30

<210> SEQ ID NO 23
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 23

Ser Leu Arg Arg Ser Ser
1 5

<210> SEQ ID NO 24
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 24

Asn Ser Phe Arg Tyr
1 5

<210> SEQ ID NO 25
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 25

Ser Pro Lys Met Val Gln Gly Ser Gly
1 5

<210> SEQ ID NO 26
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 26

Met Val Gln Gly Ser Gly
1 5

<210> SEQ ID NO 27
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 27

Lys Val Leu Arg Arg Tyr
1 5

<210> SEQ ID NO 28
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 28

Lys Val Leu Arg Arg His
1 5

<210> SEQ ID NO 29
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 29

Gly Gln His Lys Asp Asp Asn Pro Asn Leu Pro Arg
1 5 10

<210> SEQ ID NO 30
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

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<400> SEQUENCE: 30

Gly Val Pro Gln Val Ser Thr Ser Thr
1 5

<210> SEQ ID NO 31
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 31

Gly Glu Arg Ala Phe Lys Ala Trp Ala Val Ala Arg Leu Ser Gln
1 5 10 15

<210> SEQ ID NO 32
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 32

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
1 5 10

<210> SEQ ID NO 33
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Amino acid extention

<400> SEQUENCE: 33

Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys
1 5 10 15

<210> SEQ ID NO 34
<211> LENGTH: 37
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC FEATURE
<223> OTHER INFORMATION: CNP-22 with amino acid extention
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (19)..(19)
<223> OTHER INFORMATION: Xaa is Lys or a conservative amino acid
substitution thereof, any natural or unnatural amino acid, Arg,
Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Glu or Ser
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (25)..(25)
<223> OTHER INFORMATION: Xaa is Lys or a conservative amino acid
substitution thereof, any natural or unnatural amino acid, Arg,
Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Glu or Ser
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (37)..(37)
<223> OTHER INFORMATION: Cys is optionally modified with a synthetic,
natural or combination polymeric group

<400> SEQUENCE: 34

Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly
1 5 10 15

-continued

Leu Ser Xaa Gly Cys Phe Gly Leu Xaa Leu Asp Arg Ile Gly Ser Met
20 25 30

Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 35
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: CNP22 (K4R)

<400> SEQUENCE: 35

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 36
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: GANRR-CNP22(K4R) - CNP27(Arg4)

<400> SEQUENCE: 36

Gly Ala Asn Arg Arg Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu
1 5 10 15

Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25

<210> SEQ ID NO 37
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: GANPR-CNP22(K4R) - CNP27(Pro4) - Analog CI

<400> SEQUENCE: 37

Gly Ala Asn Pro Arg Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu
1 5 10 15

Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25

<210> SEQ ID NO 38
<211> LENGTH: 24
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: ER-CNP22

<400> SEQUENCE: 38

Glu Arg Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile
1 5 10 15

Gly Ser Met Ser Gly Leu Gly Cys

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20

<210> SEQ ID NO 39
<211> LENGTH: 24
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BA - ER-CNP22(K4R)

<400> SEQUENCE: 39

Glu Arg Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile
1 5 10 15

Gly Ser Met Ser Gly Leu Gly Cys
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<210> SEQ ID NO 40
<211> LENGTH: 23
<212> TYPE: PRT
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<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: R-CNP22

<400> SEQUENCE: 40

Arg Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly
1 5 10 15

Ser Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 41
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog AZ - R-CNP22(K4R)

<400> SEQUENCE: 41

Arg Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly
1 5 10 15

Ser Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 42
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: CNP22 (K4R)(K10R)

<400> SEQUENCE: 42

Gly Leu Ser Arg Gly Cys Phe Gly Leu Arg Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 43

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<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: CNP-22 (Cys6-Phe7 variable bond)
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -CH2-NH-
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -C(=O)-N(R)- where R is methyl, ethyl, n-propyl, isopropyl, cyclopropyl, n-butyl, isobutyl, sec-butyl or tert-butyl
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -C(=O)-NH-CH2-
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -CH2-S-
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -CH2-S(O)n where n is 1 or 2
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -CH2-CH2-
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -CH=CH-
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -C(=O)-CH2-
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -CH(CN)-NH-
<220> FEATURE:
<221> NAME/KEY: MOD RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -CH(OH)-CH2-
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with -O-C(=O)-NH-
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Cys6-Phe7 peptide bond (-C(=O)-NH-) replaced with NHC(=O)NH-

<400> SEQUENCE: 43

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 44

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<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Xaa is 3-amino-2-phenylpropionic acid or D-Phe

<400> SEQUENCE: 44

Gly Leu Ser Lys Gly Cys Xaa Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 45
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: CNP22 - modified Cys6
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(6)
<223> OTHER INFORMATION: Xaa is Cys, homocysteine, penicillamine, 2-mercaptopropionic acid, or 3-mercaptopropionic acid.

<400> SEQUENCE: 45

Gly Leu Ser Lys Gly Xaa Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 46
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Gly is optionally modified with a bone or cartilage targeting compound, a biphosphonate, polyAsp, polyGlu, osteopontin, osteocalcin, or sialoprotein peptides, polymers, PEG, carbohydrates, hydrophobic acids, or amino acids
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: Xaa is Lys or a conservative amino acid substitution thereof, any natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amine on a side chain, Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser, or Glu
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Xaa is Cys with peptide bond isosteres between positions 6 and 7
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Xaa is L-Phe, D-Phe, 3-amino-2-phenylpropionic acid, or N-alkylated derivatives of Phe
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Xaa is Gly, tert-butyl-Gly (tBu-Gly), Thr, Ser, Val or Asn
<220> FEATURE:

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<221> NAME/KEY: MOD_RES
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: Xaa is Leu, Ser, Thr or peptide bond isosteres thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is Lys or a conservative amino acid substitution thereof, any natural or unnatural amino acid, Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (11)..(11)
<223> OTHER INFORMATION: Xaa is Leu or peptide bond isosteres thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (14)..(14)
<223> OTHER INFORMATION: Xaa is Ile, tBu-Gly and peptide bond isosteres thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (17)..(17)
<223> OTHER INFORMATION: Xaa is Met, Val, Asn, beta-Cl-Ala, 2-aminobutyric acid (Abu) or 2-amino-isobutyric acid (Aib)
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Xaa is Leu, norleucine (Nle), homoleucine (Hleu), Val, tert-butyl-Ala (tBu-Ala), Ser, Thr, Arg or peptide bond isosteres thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (22)..(22)
<223> OTHER INFORMATION: Cys is optionally modified with a bone or cartilage targeting compound, a bisphosphonate, polyAsp, polyGlu, osteopontin, osteocalcin or sialoprotein peptides, or amino acid sequences derived from non-CNP polypeptides

<400> SEQUENCE: 46

Gly Leu Ser Xaa Gly Xaa Xaa Xaa Xaa Xaa Xaa Asp Arg Xaa Gly Ser
1 5 10 15

Xaa Ser Gly Xaa Gly Cys
20

<210> SEQ ID NO 47
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Gly is optionally modified with a peptide sequence containing one to five amino acids from a natriuretic or non-natriuretic polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Gly is optionally modified with a peptide sequence containing one to five amino acids from a natriuretic or non-natriuretic polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: Xaa is Lys or a conservative amino acid substitution thereof, any natural or unnatural amino acid or peptidomimetic, Arg, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser, or Glu
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(6)
<223> OTHER INFORMATION: Xaa is Cys or decarboxy Cys
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Peptide bond between positions 6 and 7 may be

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      replaced with -CH2-NH-
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Peptide bond between positions 6 and 7 may be
      replaced with -C(=O)-N(R)- where R is methyl, ethyl, n-propyl,
      isopropyl, cyclopropyl, n-butyl, isobutyl, sec-butyl or tert-butyl
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Peptide bond between positions 6 and 7 may be
      replaced with -C(=O)-NH-CH2-
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Peptide bond between positions 6 and 7 may be
      replaced with -CH2-S-
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Peptide bond between positions 6 and 7 may be
      replaced with -CH2-S(O)n where n is 1 or 2
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Peptide bond between positions 6 and 7 may be
      replaced with -CH2-CH2-
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Peptide bond between positions 6 and 7 may be
      replaced with -CH=CH-
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Peptide bond between positions 6 and 7 may be
      replaced with -C(=O)-CH2-
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Peptide bond between positions 6 and 7 may be
      replaced with -CH(CN)-NH-
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Peptide bond between positions 6 and 7 may be
      replaced with -CH(OH)-CH2-
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Peptide bond between positions 6 and 7 may be
      replaced with -O-C(=O)-NH-
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Peptide bond between positions 6 and 7 may be
      replaced with -NHC(=O)NH-
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Xaa is L-Phe, D-Phe, 3-amino-2-phenylpropionic
      acid, or N-alkylated derivatives of Phe
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Xaa is Gly, tert-butyl-Gly (tBu-Gly), Val, Ser,
      Thr or Asn
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: Xaa is Leu, Ser, Thr or peptide bond isosteres
      thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is Arg, Gly, 6-hydroxy-norleucine,
      citrulline (Cit), Gln, Ser or Glu
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (11)..(11)
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<223> OTHER INFORMATION: Xaa is Leu or peptide isosteres thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (14)..(14)
<223> OTHER INFORMATION: Xaa is Ile, tBu-Gly or peptide isosteres thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (17)..(17)
<223> OTHER INFORMATION: Xaa is Met, Val, Asn, beta-Cl-Ala, 2-aminobutyric acid (Abu) or 2-amino-isobutyric acid (Aib)
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Xaa is Leu, norleucine (Nle), homoleucine (Hleu), Val, tert-butyl-Ala (tBu-Ala), Ser, Thr, Arg, or peptide bond isosteres thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (22)..(22)
<223> OTHER INFORMATION: Cys is optionally modified with a synthetic bone targeting compound, a bisphosphonate, polyAsp, polyGlu, osteopontin, osteocalcin or sialoprotein peptides, or charged PEG molecules

<400> SEQUENCE: 47

Gly Leu Ser Xaa Gly Xaa Xaa Xaa Xaa Xaa Xaa Asp Arg Xaa Gly Ser
1 5 10 15

Xaa Ser Gly Xaa Gly Cys
20

<210> SEQ ID NO 48
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Xaa is a synthetic or natural polymeric group or combination thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is absent or may be one or more amino acids from Gly-Leu-Ser-Lys-Gly and/or substitutions at one or more of these positions
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Xaa is Lys, a conservative amino acid substitution, any natural or unnatural amino acid or peptidomimetic that does have a reactive primary amine on a side chain
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (19)..(19)
<223> OTHER INFORMATION: Cys is optionally modified with a synthetic or natural polymeric group or a combination thereof

<400> SEQUENCE: 48

Xaa Xaa Cys Phe Gly Leu Xaa Leu Asp Arg Ile Gly Ser Met Ser Gly
1 5 10 15

Leu Gly Cys

<210> SEQ ID NO 49
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES

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<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Xaa is absent or may be a peptide fragment or water soluble polymer
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is Cys or peptide bond isosteres thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (3)..(3)
<223> OTHER INFORMATION: Xaa is L-Phe, D-Phe, 3-amino-2-phenylpropionic acid or N-alkylated derivatives of Phe
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: Xaa is Gly, tert-butyl-Gly (tBu-Gly), Val, Ser, Thr or Asn
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (5)..(5)
<223> OTHER INFORMATION: Xaa is Leu, Ser, Thr or N-Me-Leu
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(6)
<223> OTHER INFORMATION: Xaa is Lys or a conservative amino acid substitution thereof, any natural or unnatural amino acid, Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Xaa is Leu and peptide bond isosteres thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is Ile, tBu-Gly or peptide bond isosteres thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (13)..(13)
<223> OTHER INFORMATION: Xaa is Met, Val, Asn, beta-Cl-Ala, 2-aminobutyric acid (Abu) or 2-amino-isobutyric acid (Aib)
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (16)..(16)
<223> OTHER INFORMATION: Xaa is Leu, norleucine (Nle), homoleucine (Hleu), Val, tert-butyl-Ala (tBu-Ala), Ser, Thr, Arg or peptide bond isosteres thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (18)..(18)
<223> OTHER INFORMATION: Cys is optionally modified with amino acid sequences useful in bone/cartilage targeting, polyAsp or polyGlu, osteopontin, osteocalcin, sialoprotein or sequences derived from NPPC or non-CNP polypeptides

<400> SEQUENCE: 49

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asp Arg Xaa Gly Ser Xaa Ser Gly Xaa
1 5 10 15

Gly Cys

<210> SEQ ID NO 50
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Gly is optionally modified with bone targeting compound, moieties to reduce renal clearance, charged PEG molecules or hydrophilic polymers
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: Xaa is Lys or a conservative amino acid substitution thereof, any natural or unnatural amino acid, Arg,

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      Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(6)
<223> OTHER INFORMATION: Xaa is Cys or peptide isosteres between
      positions 6 and 7
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Xaa is L-Phe, D-Phe, 3-amino-2-phenylpropionic
      acid or N-alkylated derivatives of Phe
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Xaa is Gly, tert-butyl-Gly, Thr, Ser, Val or
      Asn
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: Xaa is Leu, Ser, Thr or peptide bond isosteres
      thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is Lys or a conservative amino acid
      substitution thereof, any natural or unnatural amino acid, Arg,
      Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (11)..(11)
<223> OTHER INFORMATION: Xaa is Leu, Asn or peptide bond isosteres
      thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (14)..(14)
<223> OTHER INFORMATION: Xaa is Ile, tert-butyl-Gly (tBu-Gly), Asn or
      peptide bond isosteres thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: Xaa is Gly, Arg, Ser or Asn
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (17)..(17)
<223> OTHER INFORMATION: Xaa is Met, Val, Asn, beta-Cl-Ala,
      2-aminobutyric acid (Abu) or 2-amino-isobutyric acid (Aib)
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Xaa is Leu, norleucine (Nle), homoleucine
      (Hleu), Val, tert-butyl-Ala (tBu-Ala), Arg, Thr, Ser or peptide
      bond isoteres thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (22)..(22)
<223> OTHER INFORMATION: Cys is optionally modified with bone targeting
      compound, moieties to reduce renal clearance, charged PEG
      molecules or hydrophilic polymers

<400> SEQUENCE: 50

Gly Leu Ser Xaa Gly Xaa Xaa Xaa Xaa Xaa Xaa Asp Arg Xaa Xaa Ser
1               5                   10                  15

Xaa Ser Gly Xaa Gly Cys
            20


<210> SEQ ID NO 51
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Xaa is Arg or Glu

<400> SEQUENCE: 51
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Xaa Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 52
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (5)..(5)
<223> OTHER INFORMATION: Xaa is Arg, Gln or Ser

<400> SEQUENCE: 52

Gly Leu Ser Lys Xaa Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 53
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: Xaa is Ser, Asn, Arg, or Cit

<400> SEQUENCE: 53

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Xaa Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 54
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD RES
<222> LOCATION: (19)..(19)
<223> OTHER INFORMATION: Xaa is Ser, Arg, or Asn

<400> SEQUENCE: 54

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Xaa Leu Gly Cys
20

<210> SEQ ID NO 55
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (21)..(21)
<223> OTHER INFORMATION: Xaa is Ser, Thr, or Arg

<400> SEQUENCE: 55

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Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Xaa Cys
20

<210> SEQ ID NO 56
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog A
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (6)..(6)
<223> OTHER INFORMATION: CH2NH

<400> SEQUENCE: 56

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 57
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analogs B
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Xaa replaced with N-Me-Phe

<400> SEQUENCE: 57

Gly Leu Ser Lys Gly Cys Xaa Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 58
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog F
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: tBu

<400> SEQUENCE: 58

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 59
<211> LENGTH: 22
<212> TYPE: PRT

-continued

<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog H
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Xaa is replaced with NHCH2-CH(Phe)CO

<400> SEQUENCE: 59

Gly Leu Ser Lys Gly Cys Xaa Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 60
<211> LENGTH: 37
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BL - CNP37

<400> SEQUENCE: 60

Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly
1 5 10 15

Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met
20 25 30

Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 61
<211> LENGTH: 35
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CA

<400> SEQUENCE: 61

Ala Ala Trp Ala Arg Leu Leu Gln Glu His Pro Asn Ala Gly Leu Ser
1 5 10 15

Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly
20 25 30

Leu Gly Cys
35

<210> SEQ ID NO 62
<211> LENGTH: 36
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CB

<400> SEQUENCE: 62

Ala Ala Trp Ala Arg Leu Leu Gln Glu His Pro Asn Ala Arg Gly Leu
1 5 10 15

Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser

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            20                  25                  30

Gly Leu Gly Cys
        35


<210> SEQ ID NO 63
<211> LENGTH: 36
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CC

<400> SEQUENCE: 63

Asp Leu Arg Val Asp Thr Lys Ser Arg Ala Ala Trp Ala Arg Gly Leu
1               5                   10                  15

Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser
            20                  25                  30

Gly Leu Gly Cys
        35


<210> SEQ ID NO 64
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: GANQQ-CNP22

<400> SEQUENCE: 64

Gly Ala Asn Gln Gln Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu
1               5                   10                  15

Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
            20                  25


<210> SEQ ID NO 65
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: GANRR-CNP22

<400> SEQUENCE: 65

Gly Ala Asn Arg Arg Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu
1               5                   10                  15

Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
            20                  25


<210> SEQ ID NO 66
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: GANPR-CNP22

<400> SEQUENCE: 66

Gly Ala Asn Pro Arg Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu
1               5                   10                  15
```

Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25

<210> SEQ ID NO 67
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: GANSS-CNP22

<400> SEQUENCE: 67

Gly Ala Asn Ser Ser Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu
1 5 10 15

Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25

<210> SEQ ID NO 68
<211> LENGTH: 32
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequene
<220> FEATURE:
<223> OTHER INFORMATION: Sythetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CD
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: N-terminus BNP tail
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (32)..(32)
<223> OTHER INFORMATION: N-terminus BNP tail

<400> SEQUENCE: 68

Ser Pro Lys Met Val Gln Gly Ser Gly Cys Phe Gly Leu Lys Leu Asp
1 5 10 15

Arg Ile Gly Ser Met Ser Gly Leu Gly Cys Lys Val Leu Arg Arg His
20 25 30

<210> SEQ ID NO 69
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: GANQQ-CNP22(K4R) - Analog CH

<400> SEQUENCE: 69

Gly Ala Asn Gln Gln Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu
1 5 10 15

Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25

<210> SEQ ID NO 70
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: GANSS-CNP22(K4R) - Analog CG

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<400> SEQUENCE: 70

Gly Ala Asn Ser Ser Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu
1 5 10 15

Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25

<210> SEQ ID NO 71
<211> LENGTH: 38
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CS

<400> SEQUENCE: 71

Gly Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Pro Lys
1 5 10 15

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
20 25 30

Met Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 72
<211> LENGTH: 38
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CT

<400> SEQUENCE: 72

Gly Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Gln Lys
1 5 10 15

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
20 25 30

Met Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 73
<211> LENGTH: 38
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CU

<400> SEQUENCE: 73

Gly Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Gln Gln
1 5 10 15

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
20 25 30

Met Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 74
<211> LENGTH: 38
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CW

<400> SEQUENCE: 74

Gly Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Pro
1 5 10 15

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
20 25 30

Met Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 75
<211> LENGTH: 38
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 75

Gly Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys
1 5 10 15

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
20 25 30

Met Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 76
<211> LENGTH: 37
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CQ

<400> SEQUENCE: 76

Gly His His Ser His Glu Gln His Pro His Gly Ala Asn Gln Gln Gly
1 5 10 15

Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met
20 25 30

Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 77
<211> LENGTH: 38
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CR

<400> SEQUENCE: 77

Gly Ala His His Pro His Glu His Asp Thr His Gly Ala Asn Gln Gln
1 5 10 15

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
20 25 30

Met Ser Gly Leu Gly Cys
35

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<210> SEQ ID NO 78
<211> LENGTH: 37
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequencce
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CX

<400> SEQUENCE: 78

Gly His His Ser His Glu Gln His Pro His Gly Ala Asn Pro Arg Gly
1 5 10 15

Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met
20 25 30

Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 79
<211> LENGTH: 36
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CF

<400> SEQUENCE: 79

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gly Leu
1 5 10 15

Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser
20 25 30

Gly Leu Gly Cys
35

<210> SEQ ID NO 80
<211> LENGTH: 39
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CY

<400> SEQUENCE: 80

Gly Gln His Lys Asp Asp Asn Pro Asn Leu Pro Arg Gly Ala Asn Pro
1 5 10 15

Arg Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly
20 25 30

Ser Met Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 81
<211> LENGTH: 37
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CE

<400> SEQUENCE: 81

Gly Glu Arg Ala Phe Lys Ala Trp Ala Val Ala Arg Leu Ser Gln Gly
1 5 10 15

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Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met
20 25 30

Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 82
<211> LENGTH: 35
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CZ

<400> SEQUENCE: 82

Phe Gly Ile Pro Met Asp Arg Ile Gly Arg Asn Pro Arg Gly Leu Ser
1 5 10 15

Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly
20 25 30

Leu Gly Cys
35

<210> SEQ ID NO 83
<211> LENGTH: 40
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog DA

<400> SEQUENCE: 83

Gly Lys Arg Thr Gly Gln Tyr Lys Leu Gly Ser Lys Thr Gly Pro Gly
1 5 10 15

Pro Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile
20 25 30

Gly Ser Met Ser Gly Leu Gly Cys
35 40

<210> SEQ ID NO 84
<211> LENGTH: 37
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CK

<400> SEQUENCE: 84

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Gly Ala Asn Gln Gln Gly
1 5 10 15

Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met
20 25 30

Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 85
<211> LENGTH: 36
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

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<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CL

<400> SEQUENCE: 85

Gly Val Pro Gln Val Ser Thr Ser Thr Gly Ala Asn Gln Gln Gly Leu
1 5 10 15

Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser
20 25 30

Gly Leu Gly Cys
35

<210> SEQ ID NO 86
<211> LENGTH: 37
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CM

<400> SEQUENCE: 86

Gly Gln Pro Ser Ser Ser Ser Gln Ser Thr Gly Ala Asn Gln Gln Gly
1 5 10 15

Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met
20 25 30

Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 87
<211> LENGTH: 37
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CN

<400> SEQUENCE: 87

Gly Gln Thr His Ser Ser Gly Thr Gln Ser Gly Ala Asn Gln Gln Gly
1 5 10 15

Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met
20 25 30

Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 88
<211> LENGTH: 37
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CO

<400> SEQUENCE: 88

Gly Ser Thr Gly Gln Trp His Ser Glu Ser Gly Ala Asn Gln Gln Gly
1 5 10 15

Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met
20 25 30

Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 89
<211> LENGTH: 37
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CP

<400> SEQUENCE: 89

Gly Ser Ser Ser Ser Ser Ser Ser Ser Ser Gly Ala Asn Gln Gln Gly
1 5 10 15

Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met
20 25 30

Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 90
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Gly is optionally modified with bone targeting compound, a bisphosphonates, polyAsp, polyGlu, osteopontin, osteocalcin, or sialoprotein peptides, negatively charged PEG molecules or natural polymers
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(6)
<223> OTHER INFORMATION: Xaa is Cys or another natural or unnatural amino acid
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Xaa is Phe or another natural or unnatural amino acid
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Xaa is Gly, a larger natural or unnatural amino acid or peptidomimetic
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (22)..(22)
<223> OTHER INFORMATION: Cys is optionally modified with bone targeting compound, a bisphosphonates, polyAsp, polyGlu, osteopontin, osteocalcin, or sialoprotein peptides, negatively charged PEG molecules or natural polymers

<400> SEQUENCE: 90

Gly Leu Ser Lys Gly Xaa Xaa Xaa Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 91
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog J
<220> FEATURE:
<221> NAME/KEY: MOD_RES

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<222> LOCATION: (6)..(6)
<223> OTHER INFORMATION: Xaa is CH2-NH
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: N-Me
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: N-Me

<400> SEQUENCE: 91

Gly Leu Ser Lys Gly Xaa Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 92
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog K
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: N-Me
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: N-Me

<400> SEQUENCE: 92

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 93
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog L
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: N-Me
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (11)..(11)
<223> OTHER INFORMATION: N-Me
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: N-Me

<400> SEQUENCE: 93

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 94
<211> LENGTH: 22

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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog M
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: N-Me
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (11)..(11)
<223> OTHER INFORMATION: N-Me

<400> SEQUENCE: 94

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 95
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog Z

<400> SEQUENCE: 95

Gly Leu Ser Arg Gly Cys Tyr Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 96
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog AA

<400> SEQUENCE: 96

Gly Leu Ser Arg Gly Cys Phe Val Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 97
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog AB

<400> SEQUENCE: 97

Gly Leu Ser Arg Gly Cys Phe Ser Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

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<210> SEQ ID NO 98
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog AC

<400> SEQUENCE: 98

Gly Leu Ser Arg Gly Cys Phe Thr Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 99
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog AD

<400> SEQUENCE: 99

Gly Leu Ser Arg Gly Cys Phe Gly Thr Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 100
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog AE

<400> SEQUENCE: 100

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Arg Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 101
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog AF
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: Xaa is Cit

<400> SEQUENCE: 101

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Xaa Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 102
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog AG

<400> SEQUENCE: 102

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Val Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 103
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog AJ

<400> SEQUENCE: 103

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Val Gly Cys
20

<210> SEQ ID NO 104
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog AK
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Xaa is tBu-Ala

<400> SEQUENCE: 104

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Xaa Gly Cys
20

<210> SEQ ID NO 105
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog AT

<400> SEQUENCE: 105

Glu Leu Ser Glu Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 106
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog AV
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: N-terminus modified with pentanoic acid

<400> SEQUENCE: 106

Glu Leu Ser Glu Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 107
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog AW
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Heptanoic acid modifying N-terminus

<400> SEQUENCE: 107

Glu Leu Ser Glu Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 108
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC FEATURE
<223> OTHER INFORMATION: Analog BB
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: N-terminus modified with heptanoic acid

<400> SEQUENCE: 108

Glu Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 109
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BC

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<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: N-terminus modified with pentanoic acid

<400> SEQUENCE: 109

Glu Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 110
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BF
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is Cit

<400> SEQUENCE: 110

Gly Leu Ser Arg Gly Cys Phe Gly Leu Xaa Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 111
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BG

<400> SEQUENCE: 111

Gly Leu Ser Arg Gly Cys Phe Gly Leu Gln Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 112
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BH

<400> SEQUENCE: 112

Gly Leu Ser Arg Gly Cys Phe Gly Leu Arg Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 113
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BJ

<400> SEQUENCE: 113

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Asn Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 114
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BK

<400> SEQUENCE: 114

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Ser Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 115
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog N
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(22)
<223> OTHER INFORMATION: All D-amino acids

<400> SEQUENCE: 115

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 116
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BD
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: N-Me

<400> SEQUENCE: 116

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 117

-continued

<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BN
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (11)..(11)
<223> OTHER INFORMATION: N-Me

<400> SEQUENCE: 117

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 118
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BE
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: N-Me

<400> SEQUENCE: 118

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 119
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog V
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: 3,4-dichloro

<400> SEQUENCE: 119

Gly Leu Ser Gly Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 120
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog X
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (7)..(7)

<223> OTHER INFORMATION: 3-methyl

<400> SEQUENCE: 120

Gly Leu Ser Gly Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 121
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BP

<400> SEQUENCE: 121

Gly Leu Ser Arg Ser Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 122
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BO

<400> SEQUENCE: 122

Gly Leu Ser Arg Arg Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 123
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog P

<400> SEQUENCE: 123

Gly Leu Ser Gly Gly Cys Phe Gly Leu Arg Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 124
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BY

<400> SEQUENCE: 124

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Gly Leu Ser Arg Gly Cys Phe Gly Leu Ser Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 125
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BZ

<400> SEQUENCE: 125

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Gln
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 126
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BX

<400> SEQUENCE: 126

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Asn Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 127
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BQ

<400> SEQUENCE: 127

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Ser Leu Gly Cys
20

<210> SEQ ID NO 128
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BR

<400> SEQUENCE: 128

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Arg Leu Gly Cys

20

<210> SEQ ID NO 129
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BS

<400> SEQUENCE: 129

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Arg Gly Cys
20

<210> SEQ ID NO 130
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BT

<400> SEQUENCE: 130

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Ser Cys
20

<210> SEQ ID NO 131
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BU

<400> SEQUENCE: 131

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Thr Cys
20

<210> SEQ ID NO 132
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog BW

<400> SEQUENCE: 132

Gly Leu Ser Arg Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Arg Cys
20

<210> SEQ ID NO 133

<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog CJ

<400> SEQUENCE: 133

Arg Ser Ser Cys Phe Gly Gly Arg Ile Asp Arg Ile Gly Ala Cys
1 5 10 15

<210> SEQ ID NO 134
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: N-terminal linked to CNP22 by disuccinimidyl glutarate

<400> SEQUENCE: 134

Gly Leu Ser Arg Gly Cys Phe Gly Leu Arg Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 135
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: N-terminal linked to CNP22 by Bis-PE05

<400> SEQUENCE: 135

Gly Leu Ser Arg Gly Cys Phe Gly Leu Arg Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 136
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog E
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Xaa is D-Phe

<400> SEQUENCE: 136

Gly Leu Ser Lys Gly Cys Xaa Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 137

-continued

<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Sythetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Analog G
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Xaa is 3-Cl-Phe

<400> SEQUENCE: 137

Gly Leu Ser Lys Gly Cys Xaa Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 138
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Xaa comprises one or more amino acids selected from Gly-Leu-Ser-Lys-Gly or substitutions at one or more of these amino acids and further comprises a hydrophilic or water soluable polymer

<400> SEQUENCE: 138

Xaa Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu
1 5 10 15

Gly Cys

<210> SEQ ID NO 139
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Cys is modified with a natural and/or synthetic polymer
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (17)..(17)
<223> OTHER INFORMATION: Cys is modified with a natural and/or synthetic polymer

<400> SEQUENCE: 139

Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly
1 5 10 15

Cys

<210> SEQ ID NO 140
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Gly is modified with a natural and/or synthetic polymer

-continued

<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (22)..(22)
<223> OTHER INFORMATION: Cys is modified with a natural and/or synthetic polymer

<400> SEQUENCE: 140

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
1 5 10 15

Met Ser Gly Leu Gly Cys
20

<210> SEQ ID NO 141
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Cys is modified with a natural and/or synthetic polymeric group or combination thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (5)..(5)
<223> OTHER INFORMATION: Xaa is Lys or a conservative amino acid substitution thereof, any natural or unnatural amino acid, Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Glu or Ser
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (17)..(17)
<223> OTHER INFORMATION: Cys is optionally modified with a natural and/or synthetic polymeric group or combination thereof

<400> SEQUENCE: 141

Cys Phe Gly Leu Xaa Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly
1 5 10 15

Cys

<210> SEQ ID NO 142
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Xaa is optionally modified with bone- or cartilage-targeting moieties, moieties that reduce renal clearance, hydrophilic polymers, carbohydrates, hydrophobic acids and combinations thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is absent or may comprise 1 to 5 amino acids from Gly-Leu-Ser-Lys-Gly, optionally substituted with a natural or unnatural amino acid
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Xaa is Lys, any natural or unnatural amino acid or peptidomimetic that does not have a reactive primary amine on a side chain
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (19)..(19)
<223> OTHER INFORMATION: Xaa is optionally modified with bone- or cartilage-targeting moieties, moieties that reduce renal clearance, hydrophilic polymers, carbohydrates, hydrophobic acids and combinations thereof

<400> SEQUENCE: 142

Xaa Xaa Cys Phe Gly Leu Xaa Leu Asp Arg Ile Gly Ser Met Ser Gly
1 5 10 15

Leu Gly Cys

<210> SEQ ID NO 143
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Gly is optionally modified with cartilage or bone targeting compounds, a bisphosphonate, polyAsp, polyGlu, osteopontin peptides, osteocalcin peptides, sialoprotein peptides, charged PEG molecules or hydrophilic polymers
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: Xaa is Lys or a conservative amino acid substitution thereof, any natural or unnatural amino acid, Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(6)
<223> OTHER INFORMATION: Xaa is Cys or peptide isosteres between positions 6 and 7
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Xaa is L-Phe, D-Phe, N-alkylated Phe, or Phe analog
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Xaa is Gly, tert-butyl-Gly, Thr, Ser, Val or Asn
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: Xaa is Leu, Ser, Thr or peptide bond isosteres thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is Lys or a conservative amino acid substitution thereof, any natural or unnatural amino acid, Arg, Gly, 6-hydroxy-norleucine, citrulline (Cit), Gln, Ser or Glu
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (11)..(11)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (14)..(14)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: Xaa is Gly, Arg, Ser or Asn
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (17)..(17)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Xaa is Leu, norleucine (Nle), homoleucine (Hleu), Val, tert-butyl-Ala (tBu-Ala), Arg, Thr, Ser or peptide bond isoteres thereof
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (22)..(22)
<223> OTHER INFORMATION: Cys is optionally modified with cartilage or bone targeting compounds, a bisphosphonate, polyAsp, polyGlu, osteopontin peptides, osteocalcin peptides, sialoprotein peptides,

charged PEG molecules or hydrophilic polymers

<400> SEQUENCE: 143

Gly Leu Ser Xaa Gly Xaa Xaa Xaa Xaa Xaa Xaa Asp Arg Xaa Xaa Ser
1 5 10 15

Xaa Ser Gly Xaa Gly Cys
20

<210> SEQ ID NO 144
<211> LENGTH: 38
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 144

Gly His Lys Ser Glu Val Ala His Arg Phe Lys Gly Ala Asn Lys Lys
1 5 10 15

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
20 25 30

Met Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 145
<211> LENGTH: 39
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 145

Pro Gly Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys
1 5 10 15

Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly
20 25 30

Ser Met Ser Gly Leu Gly Cys
35

<210> SEQ ID NO 146
<211> LENGTH: 52
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 146

Leu Arg Val Asp Thr Lys Ser Arg Ala Ala Trp Ala Arg Leu Leu Gln
1 5 10 15

Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu
20 25 30

Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser
35 40 45

Gly Leu Gly Cys
50

<210> SEQ ID NO 147
<211> LENGTH: 51
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 147

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Arg Val Asp Thr Lys Ser Arg Ala Ala Trp Ala Arg Leu Leu Gln Glu
1               5                   10                  15

His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser
            20                  25                  30

Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly
        35                  40                  45

Leu Gly Cys
    50


<210> SEQ ID NO 148
<211> LENGTH: 50
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 148

Val Asp Thr Lys Ser Arg Ala Ala Trp Ala Arg Leu Leu Gln Glu His
1               5                   10                  15

Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys
            20                  25                  30

Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu
        35                  40                  45

Gly Cys
    50


<210> SEQ ID NO 149
<211> LENGTH: 49
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 149

Asp Thr Lys Ser Arg Ala Ala Trp Ala Arg Leu Leu Gln Glu His Pro
1               5                   10                  15

Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly
            20                  25                  30

Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly
        35                  40                  45

Cys


<210> SEQ ID NO 150
<211> LENGTH: 48
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 150

Thr Lys Ser Arg Ala Ala Trp Ala Arg Leu Leu Gln Glu His Pro Asn
1               5                   10                  15

Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys
            20                  25                  30

Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
        35                  40                  45


<210> SEQ ID NO 151
<211> LENGTH: 47
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
```

<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 151

Lys Ser Arg Ala Ala Trp Ala Arg Leu Leu Gln Glu His Pro Asn Ala
1 5 10 15

Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe
20 25 30

Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
35 40 45

<210> SEQ ID NO 152
<211> LENGTH: 46
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 152

Ser Arg Ala Ala Trp Ala Arg Leu Leu Gln Glu His Pro Asn Ala Arg
1 5 10 15

Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly
20 25 30

Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
35 40 45

<210> SEQ ID NO 153
<211> LENGTH: 45
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 153

Arg Ala Ala Trp Ala Arg Leu Leu Gln Glu His Pro Asn Ala Arg Lys
1 5 10 15

Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu
20 25 30

Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
35 40 45

<210> SEQ ID NO 154
<211> LENGTH: 44
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 154

Ala Ala Trp Ala Arg Leu Leu Gln Glu His Pro Asn Ala Arg Lys Tyr
1 5 10 15

Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys
20 25 30

Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
35 40

<210> SEQ ID NO 155
<211> LENGTH: 43
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 155

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Ala Trp Ala Arg Leu Leu Gln Glu His Pro Asn Ala Arg Lys Tyr Lys
1               5                   10                  15

Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu
            20                  25                  30

Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
        35                  40


<210> SEQ ID NO 156
<211> LENGTH: 42
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 156

Trp Ala Arg Leu Leu Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly
1               5                   10                  15

Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp
            20                  25                  30

Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
        35                  40


<210> SEQ ID NO 157
<211> LENGTH: 41
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 157

Ala Arg Leu Leu Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala
1               5                   10                  15

Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg
            20                  25                  30

Ile Gly Ser Met Ser Gly Leu Gly Cys
        35                  40


<210> SEQ ID NO 158
<211> LENGTH: 40
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 158

Arg Leu Leu Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn
1               5                   10                  15

Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile
            20                  25                  30

Gly Ser Met Ser Gly Leu Gly Cys
        35                  40


<210> SEQ ID NO 159
<211> LENGTH: 39
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 159

Leu Leu Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys
1               5                   10                  15

Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly
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            20                  25                  30

Ser Met Ser Gly Leu Gly Cys
        35


<210> SEQ ID NO 160
<211> LENGTH: 38
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 160

Leu Gln Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys
1               5                   10                  15

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser
            20                  25                  30

Met Ser Gly Leu Gly Cys
        35


<210> SEQ ID NO 161
<211> LENGTH: 36
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 161

Glu His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu
1               5                   10                  15

Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser
            20                  25                  30

Gly Leu Gly Cys
        35


<210> SEQ ID NO 162
<211> LENGTH: 35
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 162

His Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser
1               5                   10                  15

Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly
            20                  25                  30

Leu Gly Cys
        35


<210> SEQ ID NO 163
<211> LENGTH: 34
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 163

Pro Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys
1               5                   10                  15

Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu
            20                  25                  30

Gly Cys
```

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<210> SEQ ID NO 164
<211> LENGTH: 33
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 164

Asn Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly
1 5 10 15

Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly
20 25 30

Cys

<210> SEQ ID NO 165
<211> LENGTH: 32
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 165

Ala Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys
1 5 10 15

Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25 30

<210> SEQ ID NO 166
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 166

Arg Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe
1 5 10 15

Gly Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25 30

<210> SEQ ID NO 167
<211> LENGTH: 30
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 167

Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly
1 5 10 15

Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25 30

<210> SEQ ID NO 168
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 168

Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu
1 5 10 15

Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys

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20 25

<210> SEQ ID NO 169
<211> LENGTH: 28
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 169

Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys
1 5 10 15

Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25

<210> SEQ ID NO 170
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 170

Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu
1 5 10 15

Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25

<210> SEQ ID NO 171
<211> LENGTH: 26
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 171

Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp
1 5 10 15

Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25

<210> SEQ ID NO 172
<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 172

Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg
1 5 10 15

Ile Gly Ser Met Ser Gly Leu Gly Cys
20 25

<210> SEQ ID NO 173
<211> LENGTH: 24
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Polypeptide

<400> SEQUENCE: 173

Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile
1 5 10 15

Gly Ser Met Ser Gly Leu Gly Cys