UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 8,129,343 B2
APPLICATION NO. : 11/908834
DATED : March 6, 2012
INVENTOR(S) : Jesper Lau et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On the Title Page:

The first or sole Notice should read --

Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 1046 days.

Signed and Sealed this
Seventeenth Day of October, 2017

Joseph Matal

Joseph Matal
Performing the Functions and Duties of the
Under Secretary of Commerce for Intellectual Property and
Director of the United States Patent and Trademark Office

Docket No.: 7140.204-US
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Letters Patent of:
Jesper Lau et al.

Patent No.: 8,129,343

Issued: March 6, 2012

For: Acylated GLP-1 Compounds

REQUEST FOR CERTIFICATE OF CORRECTION
PURSUANT TO 37 C.F.R. § 1.322

Attention: Certificate of Correction Branch
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Commissioner:

Upon reviewing the above-identified patent, Patentee noted a typographical error which should be corrected as follows:

On the Title Page

The first or sole Notice should read:

"Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 1046 days."

On June 23, 2015, the USPTO issued a Patent Term Adjustment Petition decision granting the present case 1046 days of PTA. The Certificate of Correction issued on September 1, 2015 incorrectly states the number of PTA days as 1114.

Transmitted herewith is a proposed Certificate of Correction effecting such amendment. Patentee respectfully solicits the granting of the requested Certificate of Correction.

The error was not in the application as filed by applicant; accordingly no fee is required. However, if a fee is due, please charge our Deposit Account No. 14-1447, under Order No. 7140.204-US from which the undersigned is authorized to draw.

Dated: September 18, 2017

Respectfully submitted,

Electronic signature: / Rosemarie R. Wilk-Orescan, Reg. No. 45,220 /

Rosemarie R. Wilk-Orescan
Registration No.: 45,220
NOVO NORDISK INC
800 Scudders Mill Road
Plainsboro, New Jersey 08536
(609) 987-5800
Attorney For Applicant

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO.	:	8,129,343
APPLICATION NO.	:	11/908,834
ISSUE DATE	:	March 6, 2012
INVENTOR(S)	:	Jesper Lau et al.

It is certified that an error appears or errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On the Title Page:

The first or sole Notice should read:

Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 1046 days.

MAILING ADDRESS OF SENDER (Please do not use Customer Number below):
Rosemarie R. Wilk-Orescan
NOVO NORDISK INC
800 Scudders Mill Road
Plainsboro, New Jersey 08536

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.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/908,834	03/06/2012	8129343	7140.204-US	8500

23650 7590 02/15/2012
NOVO NORDISK, INC.
INTELLECTUAL PROPERTY DEPARTMENT
100 COLLEGE ROAD WEST
PRINCETON, NJ 08540

ISSUE NOTIFICATION

The projected patent number and issue date are specified above.

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(application filed on or after May 29, 2000)

The Patent Term Adjustment is 750 day(s). Any patent to issue from the above-identified application will include an indication of the adjustment on the front page.

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (http://pair.uspto.gov).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Application Assistance Unit (AAU) of the Office of Data Management (ODM) at (571)-272-4200.

APPLICANT(s) (Please see PAIR WEB site http://pair.uspto.gov for additional applicants):

Jesper Lau, Farum, DENMARK;
Paw Bloch, Taastrup, DENMARK;
Thomas Kruse Hansen, Herlev, DENMARK;

Notice of References Cited	Application/Control No. 11/908,834	Applicant(s)/Patent Under Reexamination LAU ET AL.	
	Examiner MARCELA M. CORDERO	Art Unit 1654	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
*	A	US-~~20070302058~~	08-2007	LAU et al. Change(s) applied	A61K38/26
	B	US- 20070203058		to document,	
	C	US-		/J.F./	
	D	US-		2/7/2012	
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT					
				Application No.	11/908,834
				Filing Date	September 17, 2007
				Applicant	Lau et al.
				Art Unit	1654
				Examiner Name:	Marcela M. Cordero Garcia
Sheet	1	of	1	Atty. Docket No.	7140.204-US

U.S. PATENT DOCUMENTS

EXAMINER INITIALS	Cite No.	DOCUMENT NUMBER Number –Kind Code (if known)	Issue/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.	DOCUMENT NUMBER Number –Kind Code (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
		RU 2006107600 (corresponds to WO2005/027978)	10-27-2007	Novo Nordisk A/S		
		WO 96/29342	09-26-1996	Novo Nordisk A/S		
		WO 98/08871	03-05-1998	Novo Nordisk A/S		
		WO 99/43708	09-02-1999	Novo Nordisk A/S		
		WO 00/34331	06-15-2000	Societe de Conseils de Recherches et d' Applications Scientifiques		
		WO 00/69911	11-23-2000	Conjuchem Inc		
		WO 02/46227	06-13-~~2000~~ 2002	Eli Lilly & Co		
		WO 2005/014049	02-17-2005	Novo Nordisk A/S		
		WO 2005/027978 (corresponds to RU2006107600)	03-31-2005	Novo Nordisk A/S		

Change(s) applied to document, /M.A.H./ 11/4/2011

EXAMINER SIGNATURE	/Marcela Cordero Garcia/	DATE CONSIDERED	11/19/2010

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /M.M.C.G./

UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450
www.uspto.gov

NOVO NORDISK, INC.
INTELLECTUAL PROPERTY DEPARTMENT
100 COLLEGE ROAD WEST
PRINCETON NJ 08540

MAILED
JAN 12 2012
OFFICE OF PETITIONS

In re Application of :
Lau, et al. :DECISION ON APPLICATION
Application No.: 11/908,834 :FOR ADJUSTMENT
Filed: September 17, 2008 :PATENT TERM ADJUTSMENT
Attorney Docket No. **7140.204-US** :

This is in response to the "APPLICATION FOR PATENT TERM ADJUSTMENT UNDER 37 CFR 1.705(d)" filed December 14, 2011. The petition is properly treated under 37 CFR 1.705(b). Applicants request that the initial Determination of Patent Term Adjustment under 35 U.S.C. 154(b) be corrected from two hundred and twenty-six (226) days to one hundred and ninety-one (191) days.

The request for review of determination of the patent term adjustment is **GRANTED TO THE EXTENT INDICTED HEREIN.**

The Office has updated the PALM and PAIR screens to reflect that the correct Patent Term Adjustment (PTA) determination at the time of the mailing of the Notice of Allowance is one hundred and fifty-eight (158) days. A copy of the updated PALM screen, showing the corrected determination, is enclosed.

On October 3, 2011, a Determination of Patent Term Adjustment under 35 U.S.C. 154(b) was mailed indicating that the patent term adjustment to date was 226 days. On December 14, 2011, applicants submitted the instant petition. Applicants assert the Determination of Patent Term Adjustment under 35 U.S.C. 154(b) mailed October 3, 2011, is incorrect. Specifically, applicants state that:

> [a] Notice of DO/EO Missing Requirements was due on or before July 11, 2008. The USPTO mailed the Notice on April 11, 2008, and the response was filed on September 17,2 008, thereby according an Applicant Delay of 68 days.
>
> In addition, an Office Action following a reply was due on or before January 9, 2011 (the date that is 4 months after the date on which the Response to Election-of-

Species/Restriction was filed). The USPTO mailed an Office Action on November 26, 2010. However, this Office Action was subsequently withdrawn due to Examiner error of inconsistency in the version of the claims used. The USPTO mailed a Supplemental Office Action correcting the claim error on February 11, 2011. In this regard, Applicants filed a response on September 9, 2010, but did not receive an Office Action until February 11, 2011, thereby according a USPTO delay of 33 days.

Excerpt from "APPLICATION FOR PATENT TERM ADJUSTMENT UNDER 37 CFR 1.705(d)" filed December 14, 2011, *pages 1-2.*

The application history has been reviewed and it has been determined that applicant should have been assessed a delay under 37 CFR 1.704(b)[1] for filing a reply in excess of the three month period from the April 11, 2008, mailing date of the "Notification of Missing Requirements under 35 USC 371 in the United States Designated/Elected Office (DO/EO/US)". On April 11, 2008, a "Notification of Missing Requirements under 35 USC 371 in the United States Designated/Elected Office (DO/EO/US)". was mailed. On September 17,2 008, applicants filed a response to the "Notification of Missing Requirements under 35 USC 371 in the United States Designated/Elected Office (DO/EO/US)". Thus, applicants failed to engage in reasonable efforts to conclude processing or examination of this application. Accordingly, the period of adjustment set forth in § 1.703 should have been reduced under 37 CFR 1.704(b) by 37 days, the number of days in

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[1] 37 CFR 1.704(b) states:

> With respect to the grounds for adjustment set forth in §§ 1.702(a) through (e), and in particular the ground of adjustment set forth in § 1.702(b), an applicant shall be deemed to have failed to engage in reasonable efforts to conclude processing or examination of an application for the cumulative total of any periods of time in excess of three months that are taken to reply to any notice or action by the Office making any rejection, objection, argument, or other request, measuring such three-month period from the date the notice or action was mailed or given to the applicant, in which case the period of adjustment set forth in § 1.703 shall be reduced by the number of days, if any, beginning on the day after the date that is three months after the date of mailing or transmission of the Office communication notifying the applicant of the rejection, objection, argument, or other request and ending on the date the reply was filed. The period, or shortened statutory period, for reply that is set in the Office action or notice has no effect on the three-month period set forth in this paragraph.

the period beginning on the day after the date that is three months after the date of mailing of the "Notification of Missing Requirements under 35 USC 371 in the United States Designated/Elected Office (DO/EO/US)", July 12, 2008, and ending on the date the reply was filed, September 17, 2008. **A period of reduction of 68 days will be entered.**

Petitioner's argument that an additional adjustment to the patent term under 37 CFR 1.702(a)(2) is warranted has been considered, but found unpersuasive.

37 CFR 1.702 provides that:

(a) Failure to take certain actions within specified time frames. Subject to the provisions of 35 U.S.C. 154(b) and this subpart, the term of an original patent shall be adjusted if the issuance of the patent was delayed due to the failure of the Office to:

(2) Respond to a reply under 35 U.S.C. 132 or to an appeal taken under 35 U.S.C. 134 not later than four months after the date on which the reply was filed or the appeal taken;

37 CFR 1.703 provides, in pertinent part, that:

(a) The period of adjustment under § 1.702(a) is the sum of the following periods:

(2) The number of days, if any, in the period beginning on the day after the date that is four months after the date a reply under § 1.111 was filed and ending on the date of mailing of either an action under 35 U.S.C. 132, or a notice of allowance under 35 U.S.C. 151, whichever occurs first[.]

It is undisputed that the Office mailed an action under 35 U.S.C. 132 in the form of a non-final Office action on November 26, 2010, within four months of the reply under 37 CFR 1.111 filed September 9, 2010. The subsequent mailing of another Office action under 35 U.S.C. 132 does not alter the date used in calculation of the period of adjustment. Pursuant to 35 U.S.C. 154(b)(1)(A)(ii), applicants are only entitled to day-to-day restoration of term lost as a result of delay created by the Office, after four months from the filing of the reply under 37 CFR 1.111 or appeal taken. The fact that the Office later withdrew the non-final Office action does not negate the fact that the Office took action within the meaning of 37 CFR 1.702(a)(2) on November 26, 2010. Further, relative to the

determination of patent term adjustment, the examiner does not have the authority to vacate, rescind, or withdraw an Office action. Unless expunged from the record, for purposes of calculating patent term adjustment, the action originally mailed by the examiner on November 26, 2010, was properly used to calculate the adjustment to the patent term, if any, pursuant to 37 CFR 1.702(a)(2) and 37 CFR 1.703(a)(2). See Changes to Implement Patent Term Adjustment under Twenty-Year Patent Term; Final Rule, 65 Fed. Reg. 54366 (September 18, 2000). Accordingly, no period of adjustment to the patent term pursuant to 37 CFR 1.702(a)(2) will be entered.

In view thereof, the determination of the patent term adjustment at the time of the mailing of the notice of allowance is one hundred and fifty-eight (158) days 287 days of Office delay - 129 days of applicant delay.

The Office acknowledges submission of the $200.00 fee set forth in 37 CFR 1.18(e) for consideration of the application for patent term adjustment under 37 CFR 1.705(b).

Applicants are reminded that any delays by the Office pursuant to 37 CFR 1.702(a)(4) and 1.702(b) and any applicant delays under 37 CFR 1.704(c)(10) will be calculated at the time of the issuance of the patent and applicants will be notified of the revised patent term adjustment to be indicated on the patent in the Issue Notification letter that is mailed to applicants approximately three weeks prior to issuance.

Telephone inquiries regarding this specific matter should be directed to the undersigned at (571) 272-3222.

Kenya A. McLaughlin

Kenya A. McLaughlin
Petitions Attorney
Office of Petitions

Enclosure: Copy of REVISED PALM screen

Patent Term Adjustments

PTA/PTE Information | Patent Term Adjustment | Patent Term Extension

Application Number*: 11908834 Search Explanation of PTA Calculation Explanation of PTE Calculation

PTA Calculations for Application: 11908834

Application Filing Date	09/17/2008	OverLapping Days Between (A and B) or (A and C)	0
Issue Date of Patent		Non-Overlapping USPTO Delays:	287
A Delays	287	PTO Manual Adjustment	-68
B Delays	0	Applicant Delay (APPL)	61
C Delays	0	Total PTA (days)	158

* - Sorted Column

File Contents History

Action Number	Action Recorded Date	Action Due Date	Action Code	Action Description	Duration PTO	Duration APPL	Parent Action Number
113	01/10/2012		P028	Adjustment of PTA Calculation by PTO		68	0
85	10/03/2011		MN/=.	Mail Notice of Allowance			0
84	09/28/2011		OAR	Office Action Review			0
83	09/28/2011		OAR	Office Action Review			0
82	09/28/2011		OAR	Office Action Review			0
81	09/28/2011		IREV	Issue Revision Completed			0
80	09/22/2011		OAR	Office Action Review			0
79	09/22/2011		OAR	Office Action Review			0
78	09/22/2011		OAR	Office Action Review			0
77	09/22/2011		ACRE	Allowed Case Returned to the Examiner for Clerical Processing			0
76	09/22/2011		DVER	Document Verification			0
75	09/22/2011		N/=.	Notice of Allowance Data Verification Completed			0
74	09/22/2011		DOCK	Case Docketed to Examiner in GAU			0
72	09/22/2011		EX.A	Examiner's Amendment Communication			0
71	09/22/2011		CNTA	Allowability Notice			0
73	09/21/2011		EXIE	Interview Summary - Examiner Initiated			0
67	07/25/2011		FWDX	Date Forwarded to Examiner			0
70	07/11/2011		IDSC	Information Disclosure Statement considered			0
69	07/11/2011		RCAP	Reference capture on IDS			0
68	07/11/2011	07/11/2011	M844	Information Disclosure Statement (IDS) Filed			66
66	07/11/2011	05/11/2011	A...	Response after Non-Final Action		61	60
65	07/11/2011		XT/G	Request for Extension of Time - Granted			0
64	07/11/2011		WIDS	Information Disclosure Statement (IDS) Filed			0
62	02/11/2011		ELC_RVW	Electronic Review			0
61	02/11/2011		EML_NTF	Email Notification			0
60	02/11/2011		MSRNF	Mail Supplemental Non-Final Action			0
59	02/07/2011		SRNF	Supplemental Non-Final Action			0
58	11/29/2010		ELC_RVW	Electronic Review			0
57	11/26/2010		EML_NTF	Email Notification			0
56	11/26/2010		MCTNF	Mail Non-Final Rejection			0
55	11/22/2010		CTNF	Non-Final Rejection			0
49	09/10/2010		FWDX	Date Forwarded to Examiner			0
48	09/09/2010		ELC.	Response to Election / Restriction Filed			0
47	08/31/2010		ELC_RVW	Electronic Review			0
46	08/31/2010		EML_NTF	Email Notification			0
45	08/31/2010	11/17/2009	MCTRS	Mail Restriction Requirement	287		19
44	08/27/2010		CTRS	Restriction/Election Requirement			0
43	08/09/2010		A.PE	Preliminary Amendment			0
52	05/28/2010		IDSC	Information Disclosure Statement considered			0
42	05/28/2010		M844	Information Disclosure Statement (IDS) Filed			0
41	05/28/2010		WIDS	Information Disclosure Statement (IDS) Filed			0
51	04/29/2010		IDSC	Information Disclosure Statement considered			0
40	04/29/2010		M844	Information Disclosure Statement (IDS) Filed			0
39	04/29/2010		WIDS	Information Disclosure Statement (IDS) Filed			0
37	03/11/2010		EML_NTR	Email Notification			0
36	03/11/2010		MEXIN	Mail Examiner Interview Summary (PTOL - 413)			0
35	03/03/2010		EXIN	Examiner Interview Summary Record (PTOL - 413)			0
33	12/09/2009		DOCK	Case Docketed to Examiner in GAU			0
32	06/19/2009		EML_NTR	Email Notification			0
31	06/18/2009		PG-ISSUE	PG-Pub Issue Notification			0
30	04/16/2009		EML_NTR	Email Notification			0
29	04/16/2009		FLRCPT.C	Filing Receipt - Corrected			0
28	04/01/2009		EML_NTR	Email Notification			0
27	04/01/2009		FLRCPT.C	Filing Receipt - Corrected			0
26	03/26/2009		TSSCOMP	IFW TSS Processing by Tech Center Complete			0

22	12/17/2008	OIPE	Application Dispatched from OIPE	0
21	12/03/2008	EML_NTR	Email Notification	0
20	12/03/2008	EML_NTR	Email Notification	0
17	12/03/2008	M903	Notice of DO/EO Acceptance Mailed	0
16	12/03/2008	FLRCPT.O	Filing Receipt	0
18	12/02/2008	PGPC	Sent to Classification Contractor	0
10	10/23/2008	CRFE	CRF Is Good Technically / Entered into Database	0
19	09/17/2008	371COMP	371 Completion Date	0
15	09/17/2008	ADDFLFEE	Additional Application Filing Fees	0
13	09/17/2008	SEQLIST	A set of symbols and procedures, provided to the PTO on a set of computer listings, that describe in	0
12	09/17/2008	CRFL	CRF Disk Has Been Received by Preexam / Group / PCT	0
11	09/17/2008	OATHDECL	A statement by one or more inventors satisfying the requirement under 35 USC 115, Oath of the Applic	0
0.5	03/20/2006	NEFILE	International Filing date	0

Export to: Excel

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/908,834	09/17/2008	Jesper Lau	7140.204-US	8500

23650 7590 12/12/2011
NOVO NORDISK, INC.
INTELLECTUAL PROPERTY DEPARTMENT
100 COLLEGE ROAD WEST
PRINCETON, NJ 08540

EXAMINER
CORDERO GARCIA, MARCELA M

ART UNIT	PAPER NUMBER
1654	

NOTIFICATION DATE	DELIVERY MODE
12/12/2011	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):

nnipatent@novonordisk.com
KSHL@novonordisk.com
KISW@novonordisk.com

UNITED STATES DEPARTMENT OF COMMERCE
U.S. Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450

APPLICATION NO./ CONTROL NO.	FILING DATE	FIRST NAMED INVENTOR / PATENT IN REEXAMINATION	ATTORNEY DOCKET NO.
11/908,834	17 September, 2008	LAU ET AL.	7140.204-US

	EXAMINER	
NOVO NORDISK, INC. INTELLECTUAL PROPERTY DEPARTMENT 100 COLLEGE ROAD WEST PRINCETON, NJ 08540	MARCELA M. CORDERO GARCIA	
	ART UNIT	PAPER
	1654	20111202

DATE MAILED:

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner for Patents

In view of the papers filed 12/2/2011, the inventorship in this nonprovisional application has been changed by the deletion of Florencio Zaragoza Dorwald, Henrik Stephensen and Kjeld Madsen.
The application will be forwarded to the Office of Initial Patent Examination (OIPE) for issuance of a corrected filing receipt, and correction of Office records to reflect the inventorship as corrected.
Any inquiry concerning this communication or earlier communications from the examiner should be directed to MARCELA M. CORDERO GARCIA whose telephone number is (571)272-2939. The examiner can normally be reached on M-F 8:30-5:00.
If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Cecilia J. Tsang can be reached on (571) 272-0562. 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.

/MARCELA M CORDERO GARCIA/
Primary Examiner, Art Unit 1654

PTO-90C (Rev.04-03)

Attorney Docket No.: 7140.204-US PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Jesper Lau et al. Confirmation No.: 8500

Application No.: 11/908,834 Group Art Unit: 1654

Filed: September 17, 2008 Examiner: Marcela M. Cordero Garcia

For: ACYLATED GLP-1 COMPOUNDS

PETITION TO CORRECT INVENTORSHIP UNDER 37 C.F.R. §1.48(b)

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Applicants hereby petition to change the inventorship in the above-captioned application to delete inventors Florencio Zaragoza Dorwald, Henrik Stephensen, and Kjeld Madsen for the above-captioned patent application.

The correct inventors were named in the application when filed. However, the prosecution of the application has resulted in the amendment and cancellation of claims so that less than all of the originally named inventors are the actual inventors of the invention being claimed in the application.

Applicants therefore have deleted Florencio Zaragoza Dorwald, Henrik Stephensen, and Kjeld Madsen as inventors.

Applicants respectfully request the grant of this petition.

Please charge the required fee, currently $130.00, and any additional fees to our Deposit Account No. 14-1447.

Respectfully submitted,

Date: December 1, 2011

/Richard W. Bork, Reg. No. 36,459/
Richard W. Bork, Reg. No. 36,459
Novo Nordisk Inc.
Customer Number 23650
(609) 987-5800

Use the following customer number for all correspondence
23650
PATENT TRADEMARK OFFICE

UNITED STATES PATENT AND TRADEMARK OFFICE

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/908,834	09/17/2008	Jesper Lau	7140.204-US	8500

23650 7590 11/17/2011
NOVO NORDISK, INC.
INTELLECTUAL PROPERTY DEPARTMENT
100 COLLEGE ROAD WEST
PRINCETON, NJ 08540

EXAMINER
CORDERO GARCIA, MARCELA M

ART UNIT	PAPER NUMBER
1654	

NOTIFICATION DATE	DELIVERY MODE
11/17/2011	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):

nnipatent@novonordisk.com
KSHL@novonordisk.com
KISW@novonordisk.com

Examiner-Initiated Interview Summary	Application No.	Applicant(s)	
	11/908,834	LAU ET AL.	
	Examiner	Art Unit	
	MARCELA M. CORDERO GARCIA	1654	

All participants (applicant, applicant's representative, PTO personnel):

(1) *MARCELA M. CORDERO GARCIA*. (3)_____.

(2) *RICHARD BORK*. (4)_____.

Date of Interview: *09 November 2011*.

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: *N/A*.

Identification of prior art discussed: *2007/0203058*.

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...)

Applicant's representative contacted Examiner regarding reference US 2007/0203058 (corresponding to US application 10/572,348). Applicant's representative pointed out that it appeared, based on the search history, that Examiner had considered this reference previously. Indeed 2007/0203058 had been previously reviewed during examination and it is herein made formally of record (see attached PTO-892).

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

/MARCELA M CORDERO GARCIA/ Primary Examiner, Art Unit 1654	

Notice of References Cited	Application/Control No. 11/908,834	Applicant(s)/Patent Under Reexamination LAU ET AL.	
	Examiner MARCELA M. CORDERO GARCIA	Art Unit 1654	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
*	A	US-20070302058	08-2007	LAU et al.	A61K38/26
	B	US-			
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

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

NOTICE OF ALLOWANCE AND FEE(S) DUE

23650 7590 10/03/2011
NOVO NORDISK, INC.
INTELLECTUAL PROPERTY DEPARTMENT
100 COLLEGE ROAD WEST
PRINCETON, NJ 08540

EXAMINER
CORDERO GARCIA, MARCELA M

ART UNIT	PAPER NUMBER
1654	

DATE MAILED: 10/03/2011

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/908,834	09/17/2008	Jesper Lau	7140.204-US	8500

TITLE OF INVENTION: ACYLATED GLP-1 COMPOUNDS

APPLN. TYPE	SMALL ENTITY	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	NO	$1740	$300	$0	$2040	01/03/2012

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the SMALL ENTITY status shown above.

If the SMALL ENTITY is shown as YES, verify your current SMALL ENTITY status:

A. If the status is the same, pay the TOTAL FEE(S) DUE shown above.

B. If the status above is to be removed, check box 5b on Part B - Fee(s) Transmittal and pay the PUBLICATION FEE (if required) and twice the amount of the ISSUE FEE shown above, or

If the SMALL ENTITY is shown as NO:

A. Pay TOTAL FEE(S) DUE shown above, or

B. If applicant claimed SMALL ENTITY status before, or is now claiming SMALL ENTITY status, check box 5a on Part B - Fee(s) Transmittal and pay the PUBLICATION FEE (if required) and 1/2 the ISSUE FEE shown above.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.

PTOL-85 (Rev. 02/11)

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: Mail **Mail Stop ISSUE FEE**
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
or Fax (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

23650 7590 10/03/2011

NOVO NORDISK, INC.
INTELLECTUAL PROPERTY DEPARTMENT
100 COLLEGE ROAD WEST
PRINCETON, NJ 08540

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's name)

(Signature)

(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/908,834	09/17/2008	Jesper Lau	7140.204-US	8500

TITLE OF INVENTION: ACYLATED GLP-1 COMPOUNDS

APPLN. TYPE	SMALL ENTITY	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	NO	$1740	$300	$0	$2040	01/03/2012

EXAMINER	ART UNIT	CLASS-SUBCLASS
CORDERO GARCIA, MARCELA M	1654	514-011700

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

❑ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.

❑ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list

(1) the names of up to 3 registered patent attorneys or agents OR, alternatively,

(2) the name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1_____

2_____

3_____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE (B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent) : ❑ Individual ❑ Corporation or other private group entity ❑ Government

4a. The following fee(s) are submitted:

❑ Issue Fee

❑ Publication Fee (No small entity discount permitted)

❑ Advance Order - # of Copies _____

4b. Payment of Fee(s): (**Please first reapply any previously paid issue fee shown above**)

❑ A check is enclosed.

❑ Payment by credit card. Form PTO-2038 is attached.

❑ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment, to Deposit Account Number _____ (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)

❑ a. Applicant claims SMALL ENTITY status. See 37 CFR 1.27.

❑ b. Applicant is no longer claiming SMALL ENTITY status. See 37 CFR 1.27(g)(2).

NOTE: The Issue Fee and Publication Fee (if required) will not be accepted from anyone other than the applicant; a registered attorney or agent; or the assignee or other party in interest as shown by the records of the United States Patent and Trademark Office.

Authorized Signature _____ Date _____

Typed or printed name _____ Registration No. _____

This collection of information is required by 37 CFR 1.311. 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 12 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, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450.

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.

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.
11/908,834	09/17/2008	Jesper Lau	7140.204-US	8500

23650 7590 10/03/2011
NOVO NORDISK, INC.
INTELLECTUAL PROPERTY DEPARTMENT
100 COLLEGE ROAD WEST
PRINCETON, NJ 08540

EXAMINER
CORDERO GARCIA, MARCELA M

ART UNIT	PAPER NUMBER
1654	

DATE MAILED: 10/03/2011

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(application filed on or after May 29, 2000)

The Patent Term Adjustment to date is 226 day(s). If the issue fee is paid on the date that is three months after the mailing date of this notice and the patent issues on the Tuesday before the date that is 28 weeks (six and a half months) after the mailing date of this notice, the Patent Term Adjustment will be 226 day(s).

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (http://pair.uspto.gov).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

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.

Examiner-Initiated Interview Summary	Application No.	Applicant(s)	
	11/908,834	LAU ET AL.	
	Examiner	Art Unit	
	MARCELA M. CORDERO GARCIA	1654	

All participants (applicant, applicant's representative, PTO personnel):

(1) *MARCELA M. CORDERO GARCIA*. (3)_____.

(2) *RICHARD BORK*. (4)_____.

Date of Interview: *21 September 2011*.

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: *43-45*.

Identification of prior art discussed: _____.

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 contacted Applicant's representative to discuss possible claim amendments to place the claims in condition for allowance. Claims 43-45 have been amended to replace SEQ ID NO: 6 for SEQ ID NO: 7, which is the correct sequence for the formulas claimed. Applicant's representative approved the amendment in a telephonic communication on 9/21/2011.

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

/MARCELA M CORDERO GARCIA/ Primary Examiner, Art Unit 1654	

Notice of Allowability	Application No.	Applicant(s)	
	11/908,834	LAU ET AL.	
	Examiner	Art Unit	
	MARCELA M. CORDERO GARCIA	1654	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to *7/11/2011*.

2. ☐ 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.

3. ☒ The allowed claim(s) is/are *43-48*.

4. ☐ 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 the:
      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)).

   * Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ A SUBSTITUTE OATH OR DECLARATION must be submitted. Note the attached EXAMINER'S AMENDMENT or NOTICE OF INFORMAL PATENT APPLICATION (PTO-152) which gives reason(s) why the oath or declaration is deficient.

6. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   (a) ☐ including changes required by the Notice of Draftsperson's Patent Drawing Review (PTO-948) attached
      1) ☐ hereto or 2) ☐ to Paper No./Mail Date _____.
   (b) ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).

7. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☒ Notice of References Cited (PTO-892)
2. ☐ Notice of Draftperson's Patent Drawing Review (PTO-948)
3. ☒ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date 7/11/2011
4. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material
5. ☐ Notice of Informal Patent Application
6. ☒ Interview Summary (PTO-413), Paper No./Mail Date *20110921* .
7. ☒ Examiner's Amendment/Comment
8. ☐ Examiner's Statement of Reasons for Allowance
9. ☐ Other _____.

/MARCELA M CORDERO GARCIA/
Primary Examiner, Art Unit 1654

MPI EXHIBIT 1004 PAGE 25

EXAMINER'S AMENDMENT

An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

Authorization for this examiner's amendment was given in a telephone interview with Richard Bork on 9/21/2011.

The application has been amended as follows:

IN THE CLAIMS:

In claim 43, at line 3, the phrase "SEQ ID NO: 6" has been deleted and replaced by the phrase "SEQ ID NO: 7".

In claim 44, at line 3, the phrase "SEQ ID NO: 6" has been deleted and replaced by the phrase "SEQ ID NO: 7".

In claim 45, at line 3, the phrase "SEQ ID NO: 6" has been deleted and replaced by the phrase "SEQ ID NO: 7".

Any inquiry concerning this communication or earlier communications from the examiner should be directed to MARCELA M. CORDERO GARCIA whose telephone number is (571)272-2939. The examiner can normally be reached on M-F 8:30-5:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Cecilia J. Tsang can be reached on (571) 272-0562. 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.

/MARCELA M CORDERO GARCIA/
Primary Examiner, Art Unit 1654

MMCG 09/2011

Notice of References Cited	Application/Control No. 11/908,834	Applicant(s)/Patent Under Reexamination LAU ET AL.	
	Examiner MARCELA M. CORDERO	Art Unit 1654	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
	A	US-			
	B	US-			
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Simonovsky et al. Poly(ether urethane)s incorporating long alkyl side-chains with terminal carboxy groups as fatty acid mimics: synthesis, structural characterization and protein adsorption. Journal of Biomaterials Science, Polymer Edition, 2005, Vol. 16, No. 12, pages 1463-1483.
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /M.M.C.G./

INFORMATION DISCLOSURE STATEMENT BY APPLICANT					
				Application No.	11/908,834
				Filing Date	September 17, 2007
				Applicant	Lau et al.
				Art Unit	1654
				Examiner Name:	CORDERO GARCIA, MARCELA M
Sheet	1	of	2	Atty. Docket No.	7140.204-US

U.S. PATENT DOCUMENTS

EXAMINER INITIALS	Cite No.	DOCUMENT NUMBER Number –Kind Code(if known)	Issue/Publication Date MM-DD-YYYY	NAME of Patentee or Applicant of Cited Document	Pages, Columns, Lines Where Relevant Passages or Relevant Figures Appear
		5545618	08-13-1996	Buckley et al.	

FOREIGN PATENT DOCUMENTS

EXAMINER INITIALS	Cite No.	DOCUMENT NUMBER Number –Kind Code(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
		EP 05102171.5	03-18-2005	Novo Nordisk A/S		
		EP 1704165	09-27-2006	Novo Nordisk A/S		
		WO 91/11457	08-08-1991	Buckley et al.		
		WO 02/098446	12-12-2002	Nobex Corp		
		WO 03/040309	05-15-2003	Bayer Corp		
		WO 04/065621	08-05-2004	Dyax Corp		
		WO 2006/097537	09-21-2006	Novo Nordisk A/S		

EXAMINER SIGNATURE		DATE CONSIDERED	

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /M.M.C.G./

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application No.	11/908,834
				Filing Date	September 17, 2007
				Applicant	Lau et al.
				Art Unit	1654
				Examiner Name:	CORDERO GARCIA, MARCELA M
Sheet	2	of	2	Atty. Docket No.	7140.204-US

NON PATENT LITERATURE DOCUMENTS

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
		GREENWALD RB, "PEG DRUGS: AN OVERVIEW," JOURNAL OF CONTROLLED RELEASE, 2001, VOL. 74, PAGES 159-171	
		JI ET AL., "STEARYL POLY(ETHYLENE OXIDE) GRAFTED SURFACES FOR PREFERENTIAL ADSORPTION OF ALBMNIN," BIOMATERIALS, 2001, VOL. 22, PAGES 3015-3023	
		KURTZHALS, P, ET AL., "ALBUMIN BINDING OF INSULINS ACYLATED WITH FATTY ACIDS: CHARACTERIZATION OF THE LIGAND-PROTEIN INTERACTION AND CORRELATION BETWEEN BINDING AFFINITY AND TIMING OF THE INSULIN EFFECT IN VIVO," BIOCHEM J, 1995, VOL. 312, PAGES 725-731	
		SIMONOVSKY ET AL., "POLY(ETHERURETHANE)S INCORPORATING LONG ALKYL SIDE-CHAINS WITH TERMINAL CARBOXYL GROUPS AS FATTY ACID MIMICS: SYNTHESIS, STRUCTURAL CHARACTERIZATION AND PROTEIN ADSORPTION," J BIOMAT SEI POLYMER EDN, 2005, VOL. 16, PAGES 1463-1483	
		SOLTERO ET AL., "THE ORAL DELIVERY OF PROTEIN AND PEPTIDE DRUGS," INNOVATIONS IN PHARMACEUTICAL TECHNOLOGY, 2001, VOL. 1, NO. 9, PAGES 106-110	
		STILL JG, "DEVELOPMENT OF ORAL INSULIN: PROGRESS AND CURENT STATUS," DIABETES/METAB RES REV, 2002, VOL. 18, SUPPL 1, PAGES S29-S37	
		VERONESE FM, "PEPTIDE AND PROTEIN PEGYLATION: A REVIEW OF PROBLEMS AND SOLUTIONS," BIOMATERIALS, 2001, VOL. 22, PAGES 405-417	
		WATANABE ET AL., "STRUCTURE-ACTIVITY RELATIONSHIPS OF GLUCAGON-LIKE PEPTIDE-1 (7-36) AMIDE: INSULINOTROPIC ACTIVITIES IN PERFUSED RAT PANCREASES, AND RECEPTOR BINDING AND CYCLIC AMP PRODUCTION IN RINm5F CELLS," JOURNAL OF ENDOCRINOLOGY, 1994, VOL. 140, PAGES 45-52	

EXAMINER SIGNATURE	/Marcela Cordero Garcia/	DATE CONSIDERED	09/20/2011

Please use the following customer number for all correspondence regarding this application.
23650
PATENT TRADEMARK OFFICE

Attorney Docket No.: 7140.204-US PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Lau et al. Confirmation No. 8500

Application No.: 11/908,834 Group Art Unit: 1654

Filed: September 17, 2007 Examiner: CORDERO GARCIA, MARCELA M

For: Acylated GLP-1 Compounds

RESPONSE TO OFFICE ACTION AND AMENDMENT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

In response to the Office Action dated February 11, 2011, please amend the above-captioned application and consider the remarks herewith as follows:

AMENDMENTS TO THE CLAIMS are reflected in the Listing of Claims which begins on page 2 of this paper.

REMARKS begin on page 4 of this paper.

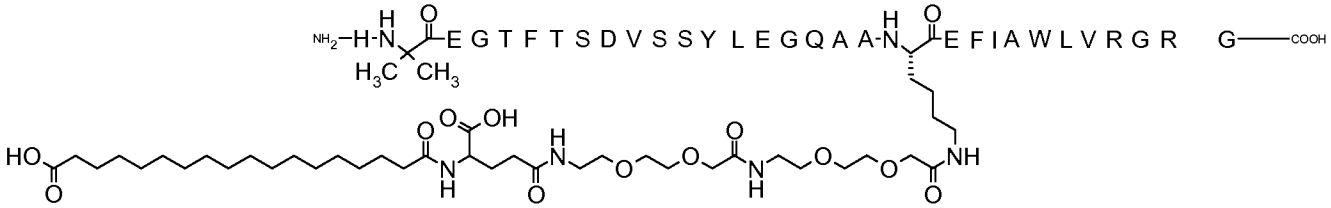
AMENDMENTS TO THE CLAIMS:

The following Listing of Claims replaces all prior versions, and listings, of claims.

LISTING OF CLAIMS

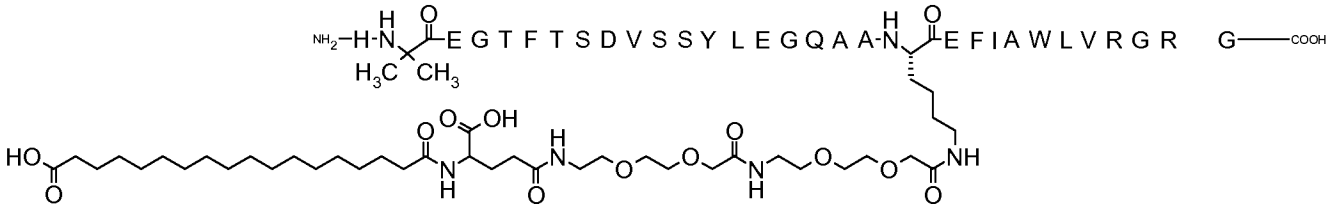
1-42. (Cancelled)

43. (New) A compound of the structure



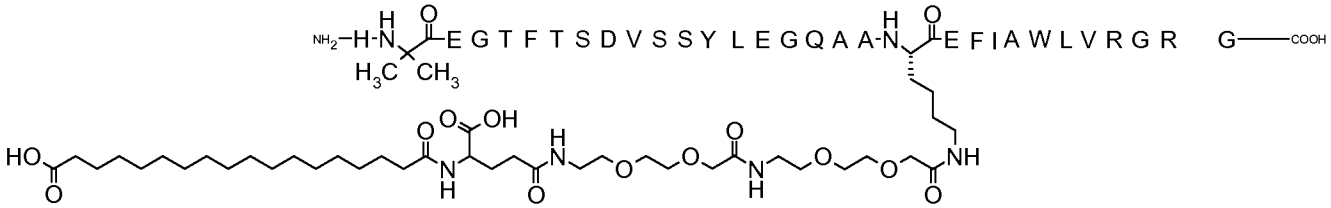
where the amino acid sequence is that of SEQ ID NO:6.

44. (New) A pharmaceutical composition comprising a compound of the structure



where the amino acid sequence is that of SEQ ID NO:6, and a pharmaceutically acceptable excipient.

45. (New) A method for treating type 2 diabetes in a subject, said method comprising administering to a subject in need of such treatment an effective amount of a pharmaceutical composition comprising a compound of the structure



where the amino acid sequence is that of SEQ ID NO:6, and a pharmaceutically acceptable excipient.

46. (New) A compound having the following name N-ε^{26}-[2-(2-[2-(2-[2-(2-[4-(17-Carboxyheptadecanoylamino)-4(S)-carboxybutyrylamino]ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][Aib8,Arg34]GLP-1-(7-37)peptide.

47. (New) A pharmaceutical composition comprising a compound having the following name N-ε^{26}-[2-(2-[2-(2-[2-(2-[4-(17-Carboxyheptadecanoylamino)-4(S)-carboxybutyrylamino]ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][Aib8,Arg34]GLP-1-(7-37)peptide and a pharmaceutically acceptable excipient.

48. (New) A method for treating type 2 diabetes in a subject, said method comprising administering to a subject in need of such treatment an effective amount of a pharmaceutical composition comprising a compound having the following name N-ε^{26}-[2-(2-[2-(2-[2-(2-[4-(17-Carboxyheptadecanoylamino)-4(S)-carboxybutyrylamino]ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][Aib8,Arg34]GLP-1-(7-37)peptide and a pharmaceutically acceptable excipient.

REMARKS:

Summary of Claim Amendments and Pending Claims

Upon entry of the present amendment, claims 1-42 are canceled without prejudice or disclaimer and claims 43-48 are added.

By entry of the present amendment, claims 43-48 are pending and at issue. Claims 43-48 find support inter alia in Example 4. No new matter is added by entry of the present Amendment.

Applicants reserve the right to file continuation and/or divisional applications directed to subject matter contained in canceled claims 1-42.

Supplemental Information Disclosure Statement

Applicants thank the Examiner for consideration of the Information Disclosure Statements filed November 28, 2008, April 29, 2010, and May 28, 2010, by returning an initialed copy of the Information Disclosure Statement submitted therein.

Applicants submit concurrently herewith a Supplemental Information Disclosure Statement citing references submitted by an opponent in an opposition to the European counterpart application that granted as EP patent 1 863 839. Applicants request that the Examiner review and initial the Supplemental Information Disclosure Statement included therein in the next communication from the Office.

Response to Objections

The Office Action objected to claims 9-12 and 15-16 as being dependent upon a rejected base claim. In reply, Applicants respectfully submit that this objection is rendered moot by the cancellation of claims 9-12 and 15-16.

The 35 U.S.C. §103(a) Rejections

The Office Action rejected claims 7-8, 20, 26-27, 31-32, and 37 under 35 U.S.C. §103(a) as being unpatentable over Knudsen et al. (US 6,268,343).

The Office Action rejected claims 21, 28, and 40-42 under 35 U.S.C. §103(a) as being unpatentable over Knudsen et al. (US 6,268,343) in view of Larsen et al., (US 6,528,486).

Applicants respectfully traverse these rejections.

Applicants note that the new claims are directed to the compound disclosed in Example 4 and that US Patent No. 6,268,343, either alone or in view of Larsen US Patent No. 6,528,486, neither teaches nor suggests this specific compound. Accordingly, withdrawal of these rejections is respectfully requested.

The Double Patenting Rejections

The Office Action provisionally rejected claims 7-8, 20, 26-27, 31-32, and 37 on the grounds of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-40 of copending US Patent No. 6,268,343.

The Office Action provisionally rejected claims 21, 28, and 40-42 on the grounds of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-40 of copending US Patent No. 6,268,343 in view of Larsen et al., (US 6,528,486).

Applicants respectfully traverse these rejections.

Applicants note that the new claims are directed to the compound shown in Example 4 and that the cited claims of US Patent No. 6,268,343, either alone or in view of Larsen US Patent No. 6,528,486, neither teach nor suggest this specific compound. Accordingly, withdrawal of these rejections is respectfully requested.

Conclusion

In view of the above, Applicant(s) submit(s) that the application is now in condition for allowance and issue and respectfully request(s) early action to that end. Applicant(s) believe(s) that no additional fees are due. However, should any fees be due, the Commissioner is hereby authorized to charge any fees in connection with this application and to credit any overpayments to Deposit Account No. 14-1447. The undersigned invites the Examiner to contact him/her by telephone if there are any questions concerning this amendment or application.

Respectfully submitted,

Date: July 11, 2011

/Richard W. Bork, Reg. No. 36,459/
Richard W. Bork, Reg. No. 36,459
Novo Nordisk Inc.
Customer Number 23650
(609) 987-5800

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23650

PATENT TRADEMARK OFFICE

INFORMATION DISCLOSURE STATEMENT BY APPLICANT					
				Application No.	11/908,834
				Filing Date	September 17, 2007
				Applicant	Lau et al.
				Art Unit	1654
				Examiner Name:	CORDERO GARCIA, MARCELA M
Sheet	1	of	2	Atty. Docket No.	7140.204-US

U.S. PATENT DOCUMENTS

EXAMINER INITIALS	Cite No.	DOCUMENT NUMBER Number –Kind Code(if known)	Issue/Publication Date MM-DD-YYYY	NAME of Patentee or Applicant of Cited Document	Pages, Columns, Lines Where Relevant Passages or Relevant Figures Appear
		5545618	08-13-1996	Buckley et al.	

FOREIGN PATENT DOCUMENTS

EXAMINER INITIALS	Cite No.	DOCUMENT NUMBER Number –Kind Code(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
		EP 05102171.5	03-18-2005	Novo Nordisk A/S		
		EP 1704165	09-27-2006	Novo Nordisk A/S		
		WO 91/11457	08-08-1991	Buckley et al.		
		WO 02/098446	12-12-2002	Nobex Corp		
		WO 03/040309	05-15-2003	Bayer Corp		
		WO 04/065621	08-05-2004	Dyax Corp		
		WO 2006/097537	09-21-2006	Novo Nordisk A/S		

EXAMINER SIGNATURE		DATE CONSIDERED	

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application No.	11/908,834
				Filing Date	September 17, 2007
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Sheet	2	of	2	Atty. Docket No.	7140.204-US

NON PATENT LITERATURE DOCUMENTS

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
		GREENWALD RB, "PEG DRUGS: AN OVERVIEW," JOURNAL OF CONTROLLED RELEASE, 2001, VOL. 74, PAGES 159-171	
		JI ET AL., "STEARYL POLY(ETHYLENE OXIDE) GRAFTED SURFACES FOR PREFERENTIAL ADSORPTION OF ALBMNIN," BIOMATERIALS, 2001, VOL. 22, PAGES 3015-3023	
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		STILL JG, "DEVELOPMENT OF ORAL INSULIN: PROGRESS AND CURENT STATUS," DIABETES/METAB RES REV, 2002, VOL. 18, SUPPL 1, PAGES S29-S37	
		VERONESE FM, "PEPTIDE AND PROTEIN PEGYLATION: A REVIEW OF PROBLEMS AND SOLUTIONS," BIOMATERIALS, 2001, VOL. 22, PAGES 405-417	
		WATANABE ET AL., "STRUCTURE-ACTIVITY RELATIONSHIPS OF GLUCAGON-LIKE PEPTIDE-1 (7-36) AMIDE: INSULINOTROPIC ACTIVITIES IN PERFUSED RAT PANCREASES, AND RECEPTOR BINDING AND CYCLIC AMP PRODUCTION IN RINm5F CELLS," JOURNAL OF ENDOCRINOLOGY, 1994, VOL. 140, PAGES 45-52	

EXAMINER SIGNATURE		DATE CONSIDERED	

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/908,834	09/17/2008	Jesper Lau	7140.204-US	8500

23650 7590 02/11/2011
NOVO NORDISK, INC.
INTELLECTUAL PROPERTY DEPARTMENT
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PRINCETON, NJ 08540

EXAMINER
CORDERO GARCIA, MARCELA M

ART UNIT	PAPER NUMBER
1654	

NOTIFICATION DATE	DELIVERY MODE
02/11/2011	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):

nnipatent@novonordisk.com
KSHL@novonordisk.com
KISW@novonordisk.com

Office Action Summary	Application No.	Applicant(s)	
	11/908,834	LAU ET AL.	
	Examiner	**Art Unit**	
	MARCELA M. CORDERO GARCIA	1654	

-- 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 3 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 *09 September 2010*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ 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

4)☒ Claim(s) *7-12,15,16,18,20,21,26-28,31,32,37 and 40-42* is/are pending in the application.

4a) Of the above claim(s) *18* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *7,8,20,21,26-28,31,32,37 and 40-42* is/are rejected.

7)☒ Claim(s) *9-12,15-16* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ 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).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

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) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)

3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.

4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

5) ☐ Notice of Informal Patent Application

6) ☐ Other: _____.

DETAILED ACTION

1. The Office Action mailed on 11/26/2010 is withdrawn and replaced by this Office Action. Examiner thanks Richard Bork for pointing out an inconsistency in the versions of the claims used. Please note that the IDSs of 11/28/2008, 4/29/2010 and 5/28/2010 were previously considered.

Election/Restrictions

2. Applicant's election of the species N-ε^{26}-[2-(2-[2-(2-[2-(2-[4-(17-Carboxyheptadecanoylamino)-4(S)-carboxybutyrylamino]ethoxy]acetylamino)-ethoxy]ethoxy)acetyl][Aib8,Arg34]GLP-1-(7-37) in the reply filed on 9/9/2010 is acknowledged. Applicant indicates that claims 7-12, 15-16, 20-21, 26-28, 31-32, 37 and 40-42 read on the elected species. Because applicant did not distinctly and specifically point out the supposed errors in the restriction requirement, the election has been treated as an election without traverse (MPEP § 818.03(a)).

Status of the claims

3. Claims 7-12, 15-16, 18, 20-21, 26-28, 31-32, 37 and 40-42 are currently pending. Claim 18 is withdrawn. Claims 7-12, 15-16, 20-21, 26-28, 31-32, 37 and 40-42 read on the elected species and are presented for examination on the merits. Upon searching, other species encompassed by the instant claims were found are herein examined for the sake of compact prosecution.

Claim Rejections - 35 USC § 103

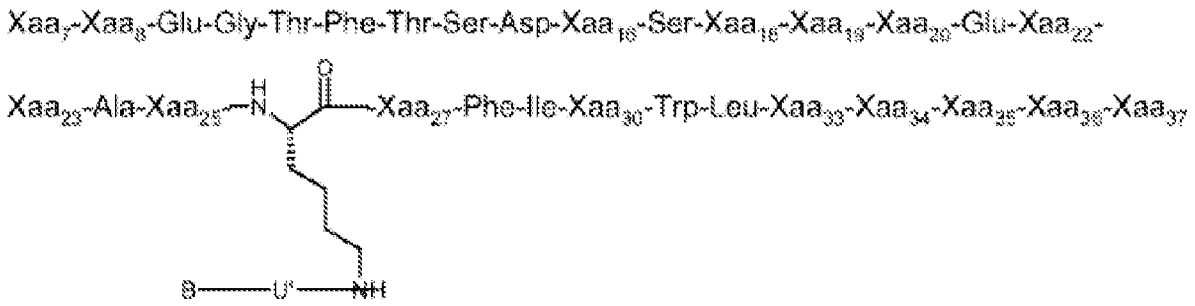
4. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

5. Claims 7-8, 20, 26-27, 31-32, 37 are rejected under 35 U.S.C. 103(a) as being unpatentable over Knudsen et al. (US 6,268,343, see PTO-892 dated 11/26/2010).

Knudsen et al. disclose a genus of GLP-1 analog which encompasses compounds of formula I



Formula I

Knudsen et al. teach GLP-1 analogs encompassing the instantly claimed generic sequence (e.g., cols. 9-10, 12-16). Please note that positions 37-45 in the formula set

forth at cols. 9-10 may be deleted and therefore read upon 'consisting of' as instantly claimed. Furthermore, in order to obtain a satisfactory protracted profile of action of the GLP-1 derivative, one or more lipophilic substituents are attached to a GLP-1 moiety. The lipophilic substituents (B) include, e.g., HOOC(CH2)mCO- wherein m is an integer from 4 to 24, more preferably m is 12, 14, 18, 20 (col. 19). U' may encompass, e.g., spacers such as (NH-CH(COOH)(CH2)2CO-) (e.g., (cols. 18-19). The lipophilic substituents are attached to the parent peptide by means of a spacer. For example, the lipophilic substituent may be attached to the GLP-1 moiety by means of a spacer in such a way that a carboxyl group of the spacer forms an amide bond with an amino group of the GLP-1 moiety. In a most preferred embodiment, the spacer is an alpha,w-amino acid. Examples of suitable spacers are succinic acid, Lys, Glu or Asp, or a dipeptide such as Gly-Lys. When the spacer is succininc acid, one carboxyl forms an amide bond with an amino group of the amino acid residue, and the other carboxyl group thereof may form an amide bond with an amino group of the lipophilic substituent. When the spacer is Lys, Glu or Asp, the carboxyl group thereof may form an amide bond with an amino group of the amino residue, and the amino group thereof may form an amide bond with a carboxyl group of the lipophylic substituent. When Lys is used as the spacer, a further spacer may in some instances be inserted between the epsilon-amino group of Lys and the lipophilic substituent. In one preferred embodiment, such further spacer is succinic acid which forms an amide bond with the epsilon amino group of Lys and with an amino group present in the lipophilic substituent. In another preferred embodiment such a further spacer is Glu or Asp which forms an amide bond with the

epsilon-amino group of Lys and another amide bond with a carboxyl group present in the lipophilic substituent, that is, the lipophilic spacer are N-epsilon(gamma-L-glutamyl, N-epsilon-(B-L-asparagyl), N-epsilon-glycyl, and N-epsilon(alpha-gamma-aminobutanoyl) (col. 9). The lipophilic substituent is, e.g., an acyl group of the formula HOOC(CH2)m(CO), wherein m is 4 to 38 and preferably 4 to 24, more preferably m is 14, 15, 18, 20. The analogs have interesting pharmacological properties, in particular they have a more protracted profile of action than the parent peptides. The GLP-1 derivatives of Knudsen et al. have insulinotropic activity, ability to decrease glucagon, ability to suppress gastric motility, ability to restore glucose competency to beta cells, and/or ability to suppress appetite/reduce weight (col. 8). The N-terminal L-histidine in position 7 of the GLP-1(7-37) sequence may comprise a modified amino acid (e.g., col. 9). A modified amino acid may include, e.g., 4-imidazopropionyl. A preferred embodiment comprises the parent peptide Arg34-GLP-1(7-37) (e.g., col. 20).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to select GLP-1 analogs, spacers and lipophilic substituents from amongst those taught by Knudsen et al. One of ordinary skill in the art at the time the invention was made would have been motivated to do so because Knudsen et al. teach such combinations produce analogs with a satisfactory protracted profile of action. One of ordinary skill in the art at the time the invention was made would have had a reasonable expectation of success given that Knudsen et al. teach derivatives at Lys 26, e.g., Lys26(Nε -tetradecanoyl)GLP-(7-37) (col. 27) and further Knudsen et al. teach lipophilic substituents (B) including, e.g., HOOC(CH2)mCO- wherein m is an integer

from 4 to 24, more preferably m is 12, 14, 18, 20 (col. 19) and U' may encompass, e.g., spacers such as (NH-CH(COOH)(CH2)2CO-), e.g., cols. 18-19 and claims. The composition may be used to treat diabetes and obesity.

Thus, the invention as a whole is prima facie obvious over the references, especially in the absence of evidence to the contrary.

6. Claim 21, 28, 40-42 are rejected under 35 U.S.C. 103(a) as being unpatentable over Knudsen et al. (US 6,268,343, see PTO-892 dated 11/26/2010) in view of Larsen et al. (US 6,528,486).

Knudsen et al. are relied upon as above.

Knudsen et al. do not expressly teach a GLP-1 analog comprising, e.g., Aib8.

Larsen et al. disclose peptide conjugates which have increased stability and are useful in the treatment of excess levels of blood glucose. Larsen et al. (col. 3) teach GLP-1(7-37) having at least one modification consisting of, e.g., alpha-amino-isobutyric (Aib) at position 8 and a lipophilic substituent (e.g., col. 4-5). The lipophilic substituent may include, e.g., COOH(CH2)eCO, with e from 8 to 24 (e.g., col. 6).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to change the amino acid at position 8 of the GLP-1 (7-37) analog for Aib as taught by Larsen et al. One of ordinary skill in the art at the time the invention was made would have been motivated to do so in order to increase stability of the conjugates. One of ordinary skill in the art at the time the invention was made would have had a reasonable expectation of success given that the conjugates of Larsen et al.

and Knudsen et al. were drawn to GLP-1 (7-37) analogs and had similar lipophilic substituents.

Thus, the invention as a whole is prima facie obvious over the references, especially in the absence of evidence to the contrary.

Double Patenting

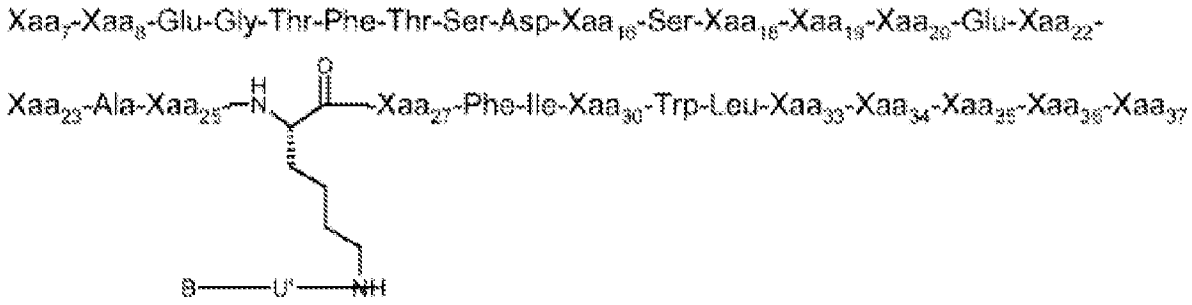
7. The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory obviousness-type double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent either is shown to

be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement.

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

8. Claims 7-8, 20, 26-27, 31-32, 37 are rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-40 of U.S. Patent No. 6,268,343, see PTO-892 dated 11/26/2010. Although the conflicting claims are not identical, they are not patentably distinct from each other because both the claimed invention and the invention of US '343 are drawn to GLP-1 compounds encompassed by formula I



Formula I

Knudsen et al. teach GLP-1 analogs encompassing the instantly claimed generic sequence (See, claims and guidance in the specification at cols. 9-10, 12-16 regarding amino acid substitutions). The lipophilic substituents (B) include, e.g., HOOC(CH2)mCO- wherein m is an integer from 4 to 24, more preferably m is 12, 14, 18, 20 (col. 19). U' may encompass, e.g., spacers such as (NH-CH(COOH)(CH2)2CO-) See, claims and guidance in disclosure at, e.g., cols. 18-19. The analogs have

interesting pharmacological properties, in particular they have a more protracted profile of action than the parent peptides. The GLP-1 derivatives of Knudsen et al. have insulinotropic activity, ability to decrease glucagon, ability to suppress gastric motility, ability to restore glucose competency to beta cells, and/or ability to suppress appetite/reduce weight (col. 8). The N-terminal L-histidine in position 7 of the GLP-1(7-37) sequence may comprise a modified amino acid (e.g., col. 9). A modified amino acid may include, e.g., 4-imidazopropionyl. A preferred embodiment comprises the parent peptide Arg34-GLP-1(7-37) (e.g., col. 20).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the GLP-1 analog at claim 1 in order to obtain other active GLP-1 analogs, by varying the amino acid residues, spacers and lipophilic substituents based on the guidance provided by Knudsen et al. One of ordinary skill in the art at the time the invention was made would have been motivated to do so because Knudsen et al. teach such combinations produce analogs with a satisfactory protracted profile of action. One of ordinary skill in the art at the time the invention was made would have had a reasonable expectation of success given that Knudsen et al. teach derivatives at Lys 26, e.g., Lys 26(Nε -tetradecanoyl), Arg34,GLP-(7-37) (e.g., claims) and further Knudsen et al. teach lipophilic substituents (B) including, e.g., HOOC(CH2)mCO- wherein m is an integer from 4 to 24, more preferably m is 12, 14, 18, 20 (col. 19) and U' may encompass, e.g., spacers such as (NH-CH(COOH)(CH2)2CO-), e.g., cols. 18-19 and claims. The composition may be used to treat diabetes and obesity (see claims).

Thus, the invention as a whole is prima facie obvious over the references, especially in the absence of evidence to the contrary.

9. Claims 21, 28, 40-42 are rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-40 of U.S. Patent No. 6,268,343, see PTO-892 dated 11/26/2010, in view of Larsen et al. (US 6,528,486).

US '343 is relied upon as above.

US '343 does not expressly teach a GLP-1 analog comprising, e.g., Aib8.

Larsen et al. disclose peptide conjugates which have increased stability and are useful in the treatment of excess levels of blood glucose. Larsen et al. (col. 3) teach GLP-1(7-37) having at least one modification consisting of, e.g., substituting alanine for, e.g., alpha-amino-isobutyric (Aib) at position 8 and a lipophilic substituent (e.g., col. 4-5). The lipophilic substituent may include, e.g., COOH(CH2)eCO, with e from 8 to 24 (e.g., col. 6).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to change the amino acid at position 8 of the GLP-1 (7-37) analog for Aib as taught by Larsen et al. One of ordinary skill in the art at the time the invention was made would have been motivated to do so in order to increase stability of the conjugates. One of ordinary skill in the art at the time the invention was made would have had a reasonable expectation of success given that the conjugates of Larsen et al. and US '343 were drawn to GLP-1 (7-37) analogs and had similar lipophilic substituents.

Thus, the invention as a whole is prima facie obvious over the references, especially in the absence of evidence to the contrary.

Claim Objections

10. Claims 9-12, 15-16 are objected to as being dependent upon a rejected base claim, but would be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims.

Conclusion

11. No claim is allowed.

The prior art made of record and not relied upon is considered pertinent to applicant's disclosure.

12. Any inquiry concerning this communication or earlier communications from the examiner should be directed to MARCELA M. CORDERO GARCIA whose telephone number is (571)272-2939. The examiner can normally be reached on M-F 8:30-5:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Cecilia J. Tsang can be reached on (571) 272-0562. 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.

/Marcela M Cordero Garcia/
Primary Examiner, Art Unit 1654

MMCG 02/2011

Notice of References Cited	Application/Control No. 11/908,834	Applicant(s)/Patent Under Reexamination LAU ET AL.	
	Examiner MARCELA M. CORDERO GARCIA	Art Unit 1654	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
*	A	US-6,528,486	03-2003	Larsen et al.	514/6.8
	B	US-			
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/908,834	09/17/2008	Jesper Lau	7140.204-US	8500

23650 7590 11/26/2010
NOVO NORDISK, INC.
INTELLECTUAL PROPERTY DEPARTMENT
100 COLLEGE ROAD WEST
PRINCETON, NJ 08540

EXAMINER
CORDERO GARCIA, MARCELA M

ART UNIT	PAPER NUMBER
1654	

NOTIFICATION DATE	DELIVERY MODE
11/26/2010	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):

nnipatent@novonordisk.com
KSHL@novonordisk.com
KISW@novonordisk.com

Office Action Summary	Application No.	Applicant(s)	
	11/908,834	LAU ET AL.	
	Examiner	**Art Unit**	
	MARCELA M. CORDERO GARCIA	1654	

-- 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 3 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 *09 September 2010*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ 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

4)☒ Claim(s) *7-12, 15-16, 18, 20-21, 26-28, 31-32, 40-42* is/are pending in the application.

4a) Of the above claim(s) *8-12, 18, 20-21, 26-28, 31-32, 40-42* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *7,15 and 16* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ 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).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

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) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)

3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *See Continuation Sheet*.

4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

5) ☐ Notice of Informal Patent Application

6) ☐ Other: _____.

Continuation of Attachment(s) 3). Information Disclosure Statement(s) (PTO/SB/08), Paper No(s)/Mail Date :11/28/2007, 04/29/2010, 05/28/2010.

DETAILED ACTION

Election/Restrictions

1. Applicant's election of the species N-ε^{26}-[2-(2-[2-(2-[2-(2-[4-(17-Carboxyheptadecanoylamino)-4(S)-carboxybutyrylamino]ethoxy]acetylamino)-ethoxy]ethoxy)acetyl][Aib8,Arg34]GLP-1-(7-37) in the reply filed on 9/9/2010 is acknowledged. Because applicant did not distinctly and specifically point out the supposed errors in the restriction requirement, the election has been treated as an election without traverse (MPEP § 818.03(a)).

Status of the claims

2. Claims 1-32, 37-39 are pending. Applicant states that claims 7-12, 15-16, 20-21, 26-28, 31-32, 37 and 41-42 read on the elected species. However, please note that claims 8-12, 20-21, 26-28, 31-32 and 37 are dependent directly or indirectly on claim 1, currently withdrawn, and therefore are also withdrawn. Also, claims 41-42 are not present. Claims 38-39 are present but are also dependent on a withdrawn claim (claim 1). Therefore, claims 1-6, 13-14, 17-39 are currently withdrawn. Claim 7 and 15-16 are presented for examination on the merits.

Claim Rejections - 35 USC § 103

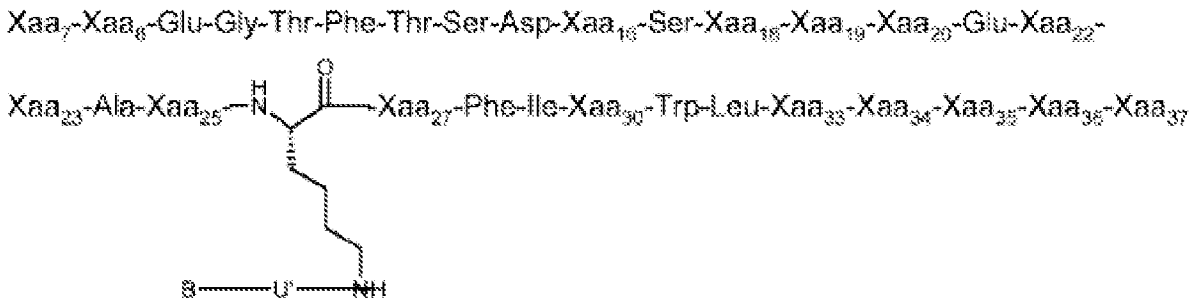
3. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

4. Claims 7 and 15-16 are rejected under 35 U.S.C. 103(a) as being unpatentable over Knudsen et al. (US 6,268,343).

Knudsen et al. disclose a genus of GLP-1 analog which encompasses compounds of formula I



Formula I

Knudsen et al. teach GLP-1 analogs encompassing the instantly claimed generic sequence (e.g., cols. 9-10, 12-16). Furthermore, in order to obtain a satisfactory protracted profile of action of the GLP-1 derivative, one or more lipophilic substituents are attached to a GLP-1 moiety. The lipophilic substituents (B) include, e.g., HOOC(CH2)mCO- wherein m is an integer from 4 to 24, more preferably m is 12, 14,

18, 20 (col. 19). U' may encompass, e.g., spacers such as (NH-CH(COOH)(CH2)2CO-) (e.g., (cols. 18-19). The analogs have interesting pharmacological properties, in particular they have a more protracted profile of action than the parent peptides. The GLP-1 derivatives of Knudsen et al. have insulinotropic activity, ability to decrease glucagon, ability to suppress gastric motility, ability to restore glucose competency to beta cells, and/or ability to suppress appetite/reduce weight (col. 8). The N-terminal L-histidine in position 7 of the GLP-1(7-37) sequence may comprise a modified amino acid (e.g., col. 9). A modified amino acid may include, e.g., 4-imidazopropionyl.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to select GLP-1 analogs, spacers and lipophilic substituents from amongst those taught by Knudsen et al. One of ordinary skill in the art at the time the invention was made would have been motivated to do so because Knudsen et al. teach such combinations produce analogs with a satisfactory protracted profile of action. One of ordinary skill in the art at the time the invention was made would have had a reasonable expectation of success given that Knudsen et al. teach derivatives at Lys 26, e.g., Lys26(Nε -tetradecanoyl)GLP-(7-37) (col. 27) and further Knudsen et al. teach lipophilic substituents (B) including, e.g., HOOC(CH2)mCO- wherein m is an integer from 4 to 24, more preferably m is 12, 14, 18, 20 (col. 19) and U' may encompass, e.g., spacers such as (NH-CH(COOH)(CH2)2CO-), e.g., cols. 18-19 and claims. The composition may be used to treat diabetes and obesity.

Thus, the invention as a whole is prima facie obvious over the references, especially in the absence of evidence to the contrary.

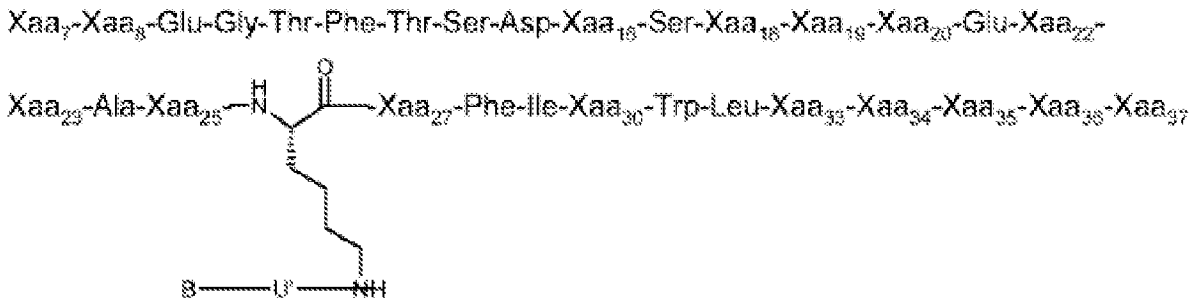
Double Patenting

5. The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory obviousness-type double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent either is shown to be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement.

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

6. Claims 7 and 15-16 are rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-40 of U.S. Patent No. 6,268,343. Although the conflicting claims are not identical, they are not patentably distinct from each other because both the claimed invention and the invention of US '343 are drawn to GLP-1 compounds encompassed by formula I



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37) sequence may comprise a modified amino acid (e.g., col. 9). A modified amino acid may include, e.g., 4-imidazopropionyl.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the GLP-1 analog at claim 1 in order to obtain other active GLP-1 analogs, by varying the amino acid residues, spacers and lipophilic substituents based on the guidance provided by Knudsen et al. One of ordinary skill in the art at the time the invention was made would have been motivated to do so because Knudsen et al. teach such combinations produce analogs with a satisfactory protracted profile of action. One of ordinary skill in the art at the time the invention was made would have had a reasonable expectation of success given that Knudsen et al. teach derivatives at Lys 26, e.g., Lys 26(Nε -tetradecanoyl), Arg34,GLP-(7-37) (e.g., claims) and further Knudsen et al. teach lipophilic substituents (B) including, e.g., HOOC(CH2)mCO- wherein m is an integer from 4 to 24, more preferably m is 12, 14, 18, 20 (col. 19) and U' may encompass, e.g., spacers such as (NH-CH(COOH)(CH2)2CO-), e.g., cols. 18-19 and claims. The composition may be used to treat diabetes and obesity (see claims).

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Conclusion

7. No claim is allowed.

The prior art made of record and not relied upon is considered pertinent to applicant's disclosure.

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/Marcela M Cordero Garcia/
Examiner, Art Unit 1654

MMCG 11/2010

Notice of References Cited	Application/Control No. 11/908,834	Applicant(s)/Patent Under Reexamination LAU ET AL.	
	Examiner MARCELA M. CORDERO GARCIA	Art Unit 1654	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
*	A	US-6,268,343	07-2001	Knudsen et al.	514/4.8
	B	US-			
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT					
				Application No.	11/908,834
				Filing Date	September 17, 2007
				Applicant	Lau et al.
				Art Unit	1654
				Examiner Name:	Marcela M. Cordero Garcia
Sheet	1	of	1	Atty. Docket No.	7140.204-US

U.S. PATENT DOCUMENTS

EXAMINER INITIALS	Cite No.	DOCUMENT NUMBER Number –Kind Code (if known)	Issue/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.	DOCUMENT NUMBER Number –Kind Code (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
		RU 2006107600 (corresponds to WO2005/027978)	10-27-2007	Novo Nordisk A/S		
		WO 96/29342	09-26-1996	Novo Nordisk A/S		
		WO 98/08871	03-05-1998	Novo Nordisk A/S		
		WO 99/43708	09-02-1999	Novo Nordisk A/S		
		WO 00/34331	06-15-2000	Societe de Conseils de Recherches et d' Applications Scientifiques		
		WO 00/69911	11-23-2000	Conjuchem Inc		
		WO 02/46227	06-13-2000	Eli Lilly & Co		
		WO 2005/014049	02-17-2005	Novo Nordisk A/S		
		WO 2005/027978 (corresponds to RU2006107600)	03-31-2005	Novo Nordisk A/S		

EXAMINER SIGNATURE	/Marcela Cordero Garcia/	DATE CONSIDERED	11/19/2010

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /M.M.C.G./

INFORMATION DISCLOSURE STATEMENT BY APPLICANT					
				Application No.	11/908,834
				Filing Date	September 17, 2007
				Applicant	Lau et al.
				Art Unit	1654
				Examiner Name:	Marcela M. Cordero Garcia
Sheet	1	of	1	Atty. Docket No.	7140.204-US

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EXAMINER INITIALS	Cite No.	DOCUMENT NUMBER Number –Kind Code (if known)	Issue/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.	DOCUMENT NUMBER Number –Kind Code (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
		WO 2004/99246	11-18-2004	Novo Nordisk A/S		

NON PATENT LITERATURE DOCUMENTS

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
		Green, Brian D., Biological Chemistry (2004), Vol. 385, No. 2, pp. 169-177.	

EXAMINER SIGNATURE	/Marcela Cordero Garcia/	DATE CONSIDERED	11/19/2010

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /M.M.C.G./

INFORMATION DISCLOSURE STATEMENT BY APPLICANT					
				Application No.	11/908,834
				Filing Date	September 17, 2007
				Applicant	Lau et al.
				Art Unit	TBD
				Examiner Name:	TBD
Sheet	1	of	2	Atty. Docket No.	7140.204-US

U.S. PATENT DOCUMENTS

EXAMINER INITIALS	Cite No.	DOCUMENT NUMBER Number –Kind Code(if known)	Issue/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.	DOCUMENT NUMBER Number –Kind Code(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
		WO 9629342	09-1996	Jonassen		
		WO9808871	03-1998	Bjorn		
		WO9943708	09-1999	Knudsen		
		WO0034331	06-2000	Dong		
		WO0069911	11-2000	Bridon		
		WO0246227	06-2002	Glaesner		
		WO2005014049	02-2005	Behrens		
		WO2005027978	03-2005	Bloch		

EXAMINER SIGNATURE		DATE CONSIDERED	

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /M.M.C.G./

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application No.	11/908,834
				Filing Date	September 17, 2007
				Applicant	Lau et al.
				Art Unit	TBD
				Examiner Name:	TBD
Sheet	2	of	2	Atty. Docket No.	7140.204-US

NON PATENT LITERATURE DOCUMENTS

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
		KNUDSEN, L.B. et al., Journal of Medicinal Chemistry, Vol. 43, pp. 1664-9 (2000)	
		KNUDSEN, L.B. et al., Journal of Medicinal Chemistry, Vol. 47, pp. 4128-34 (2004)	
		DEACON, C.F. et al., Diabetologia, Vol. 41, pp. 271-8 (1998)	

EXAMINER SIGNATURE	/Marcela Cordero Garcia/	DATE CONSIDERED	11/19/2010

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /M.M.C.G./

Attorney Docket No.: 7140.204-US PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Lau et al. Confirmation No.: 8500

Application No.: 11/908,834 Group Art Unit: 1654

Filed: September 17, 2007 Examiner: Marcela M. Cordero Garcia

For: Acylated GLP-1 Compounds

RESPONSE TO RESTRICTION REQUIREMENT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

This paper is being filed in response to the Office Action mailed August 31, 2010 that made an election of species requirement. Applicants were requested to elect a single and specific GLP-1 analog with all substituents fully assigned to which the claims shall be restricted if no genetic claim is finally held to be allowable.

In response to this requirement, Applicants hereby elect the compound recited in claim 28 as follows:

N-ε^{26}-[2-(2-[2-(2-[2-(2-[4-(17-Carboxyheptadecanoylamino)-4(S)-carboxybutyrylamino]ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][Aib8,Arg34]GLP-1-(7-37).

Claims 7-12, 15-16, 20-21, 26-28, 31-32, 37 and 40-42 read on the elected species. Applicants hereby reserve the right to file continuing applications directed to the nonelected subject matter.

The Examiner is hereby invited to contact the undersigned by telephone if there are any questions concerning this response or application.

Respectfully submitted,

Date: September 8, 2010

/Richard W. Bork, Reg. No. 36,459/
Richard W. Bork, Reg. No. 36,459
Novo Nordisk Inc.
Customer Number 23650
(609) 987-5800

Use the following customer number for all correspondence regarding this application.

23650
PATENT TRADEMARK OFFICE

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.
11/908,834	09/17/2008	Jesper Lau	7140.204-US	8500

23650 7590 08/31/2010
NOVO NORDISK, INC.
INTELLECTUAL PROPERTY DEPARTMENT
100 COLLEGE ROAD WEST
PRINCETON, NJ 08540

EXAMINER
CORDERO GARCIA, MARCELA M

ART UNIT	PAPER NUMBER
1654	

NOTIFICATION DATE	DELIVERY MODE
08/31/2010	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):

nnipatent@novonordisk.com
KSHL@novonordisk.com
KISW@novonordisk.com

Office Action Summary	Application No.	Applicant(s)	
	11/908,834	LAU ET AL.	
	Examiner	**Art Unit**	
	MARCELA M. CORDERO GARCIA	1654	

-- 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 09 August 2010.

2a)☐ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ 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

4)☒ Claim(s) 7-12,15,16,18,20,21,26-28,31,32,37 and 40-42 is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☐ Claim(s) _____ is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☒ Claim(s) 7-12, 15-16, 18, 20-21, 26-28, 31-32, 37, 40-42 are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ 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).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

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) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)

3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.

4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

5) ☐ Notice of Informal Patent Application

6) ☐ Other: _____.

DETAILED ACTION

Election/Restrictions

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack unity of invention because they are not so linked as to form a single general inventive concept under PCT Rule 13.1.

The species are as follows:

The many and multiple GLP-1 analogs consisting of formula I and methods of use thereof.

Applicant is required, in reply to this action, to elect a single species [i.e., elect a single and specific GLP-1 analog with all substituents fully assigned (e.g., one of compounds set forth in claim 28)] to which the claims shall be restricted if no generic claim is finally held to be allowable. The reply must also identify the claims readable on the elected species, including any claims subsequently added. An argument that a claim is allowable or that all claims are generic is considered non-responsive unless accompanied by an election.

Upon the allowance of a generic claim, applicant will be entitled to consideration of claims to additional species which are written in dependent form or otherwise require all the limitations of an allowed generic claim. Currently, the following claim(s) are generic: claims 7-12, 15-16, 18, 20-21, 26-28, 31-32, 37, 40-42.

REQUIREMENT FOR UNITY OF INVENTION

As provided in 37 CFR 1.475(a), a national stage application shall relate to one invention only or to a group of inventions so linked as to form a single general inventive

concept ("requirement of unity of invention"). Where a group of inventions is claimed in a national stage application, the requirement of unity of invention shall be fulfilled only when there is a technical relationship among those inventions involving one or more of the same or corresponding special technical features. The expression "special technical features" shall mean those technical features that define a contribution which each of the claimed inventions, considered as a whole, makes over the prior art. In the instant case, pursuant to PCT Rule 13.2 and PCT Administrative Instructions, Annex B, Part 1(f)(I)(B)(2), the species are not art recognized equivalents since they are drawn to different and distinct chemical structures

The determination whether a group of inventions is so linked as to form a single general inventive concept shall be made without regard to whether the inventions are claimed in separate claims or as alternatives within a single claim. See 37 CFR 1.475(e).

WHEN CLAIMS ARE DIRECTED TO MULTIPLE CATEGORIES OF INVENTIONS

As provided in 37 CFR 1.475(b), a national stage application containing claims to different categories of invention will be considered to have unity of invention if the claims are drawn only to one of the following combinations of categories:

(1) A product and a process specially adapted for the manufacture of said product; or

(2) A product and process of use of said product; or

(3) A product, a process specially adapted for the manufacture of the said product, and a use of the said product; or

(4) A process and an apparatus or means specifically designed for carrying out the said process; or

(5) A product, a process specially adapted for the manufacture of the said product, and an apparatus or means specifically designed for carrying out the said process.

Otherwise, unity of invention might not be present. See 37 CFR 1.475(c).

Applicant is advised that the reply to this requirement to be complete must include (i) an election of a species or 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 or species 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 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 or species.

Should applicant traverse on the ground that the inventions have unity of invention (37 CFR 1.475(a)), applicant must provide reasons in support thereof. Applicant may 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.

Where such evidence or admission is provided by applicant, 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.

Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 CFR 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a request under 37 CFR 1.48(b) and by the fee required under 37 CFR 1.17(i).

Any inquiry concerning this communication or earlier communications from the examiner should be directed to MARCELA M. CORDERO GARCIA whose telephone number is (571)272-2939. The examiner can normally be reached on M-F 8:30-5:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Cecilia J. Tsang can be reached on (571) 272-0562. 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.

/Marcela M Cordero Garcia/
Examiner, Art Unit 1654

MMCG 08/2010

Attorney Docket No.: 7140.204-US PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Lau et al.

Application No.: 11/908,834

Filed: September 17, 2007

For: Acylated GLP-1 Compounds

Confirmation No.: 8500

Group Art Unit: 1654

Examiner: Cordero Garcia, Marcela

PRELIMINARY AMENDMENT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Prior to examination of the above-identified application on the merits, kindly amend the above-captioned patent application as set forth below.

AMENDMENTS TO THE CLAIMS begin on page 2 of this paper.

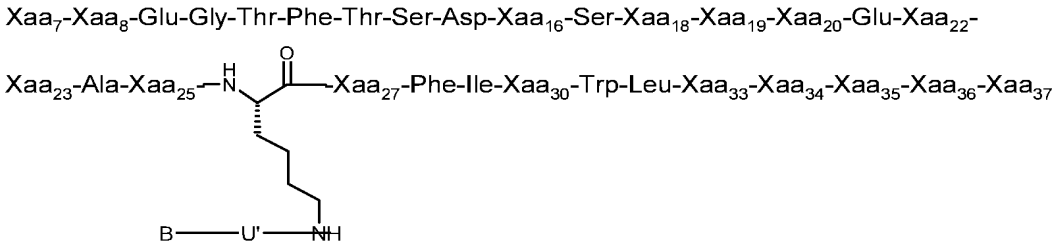
REMARKS begin on page 21 of this paper.

AMENDMENTS TO THE CLAIMS

CLAIM LISTING

1-6. (Cancelled)

7. (Currently Amended) A GLP-1 analog which is a compound consisting of formula I (SEQ ID No. 2):



Formula I

wherein

Xaa_7 is L-histidine, imidazopropionyl, α-hydroxy-histidine, D-histidine, desamino-histidine, 2-amino-histidine, β-hydroxy-histidine, homohistidine, N^{α}-acetyl-histidine, N^{α}-formyl-histidine, α-fluoromethyl-histidine, α-methyl-histidine, 3-pyridylalanine, 2-pyridylalanine or 4-pyridylalanine

Xaa_8 is ~~Ala,~~ Gly, Val, Leu, Ile, Thr, Ser, Lys, Aib, (1-aminocyclopropyl) carboxylic acid, (1-aminocyclobutyl) carboxylic acid, (1-aminocyclopentyl) carboxylic acid, (1-aminocyclohexyl) carboxylic acid, (1-aminocycloheptyl) carboxylic acid, or (1-aminocyclooctyl) carboxylic acid;

Xaa_{16} is Val or Leu;

Xaa_{18} is Ser, Lys or Arg;

Xaa_{19} is Tyr or Gln;

Xaa_{20} is Leu or Met;

Xaa_{22} is Gly, Glu or Aib;

Xaa_{23} is Gln, Glu, Lys or Arg;

Xaa_{25} is Ala or Val;

Xaa_{27} is Glu or Leu;

Xaa_{30} is Ala, Glu or Arg;

Xaa_{33} is Val or Lys;

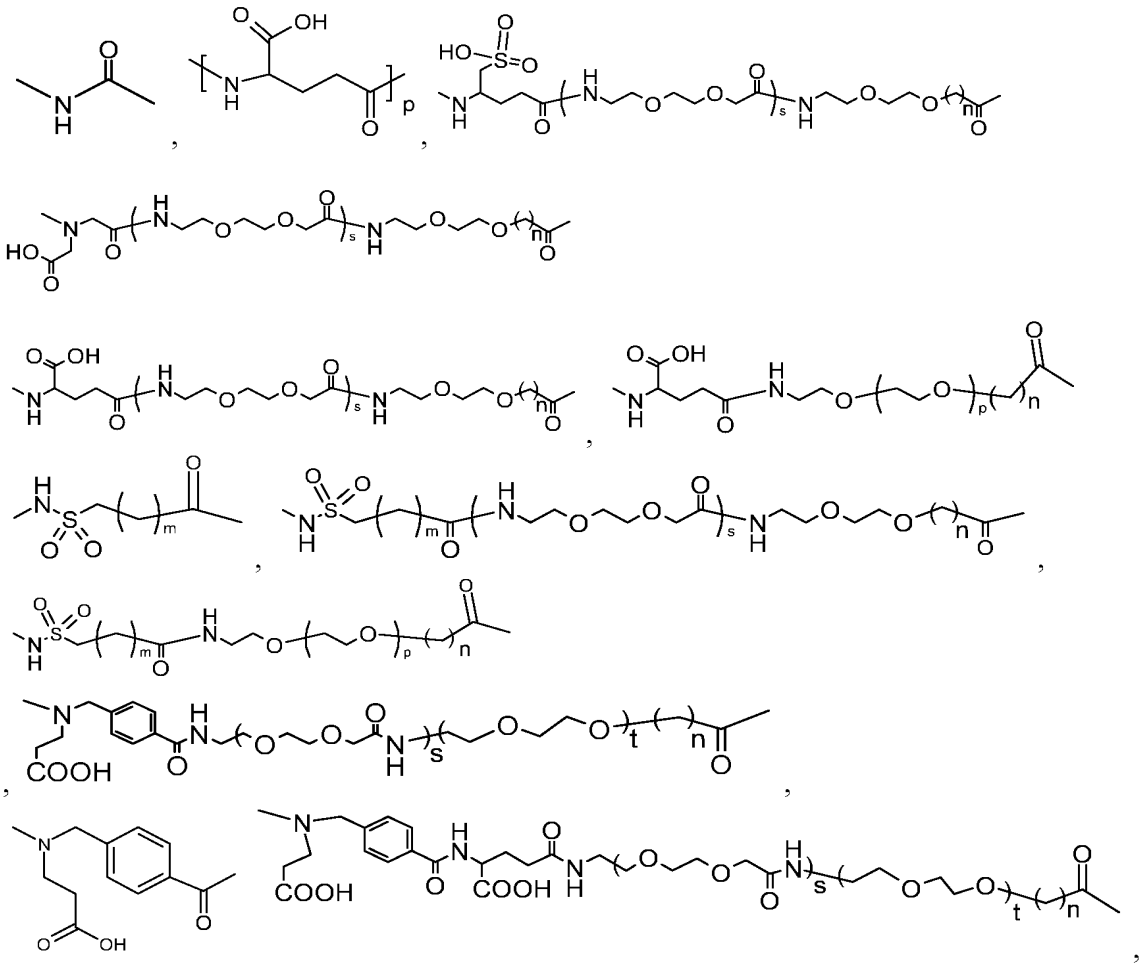
Xaa_{34} is Lys, Glu, Asn or Arg;

Xaa_{35} is Gly or Aib;

Xaa_{36} is Arg, Gly or Lys, or is absent;

Xaa_{37} is Gly, Ala, Glu, Pro, Lys, or is absent;

and B and U' together is the acylated moiety, where U' is selected from



m is 0, 1, 2, 3, 4, 5, or 6,

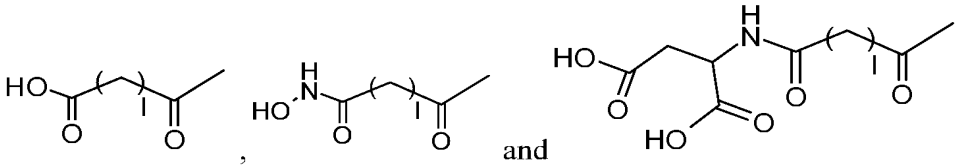
n is 1, 2 or 3

s is 0, 1, 2, or 3,

t is 0, 1, 2, 3, or 4

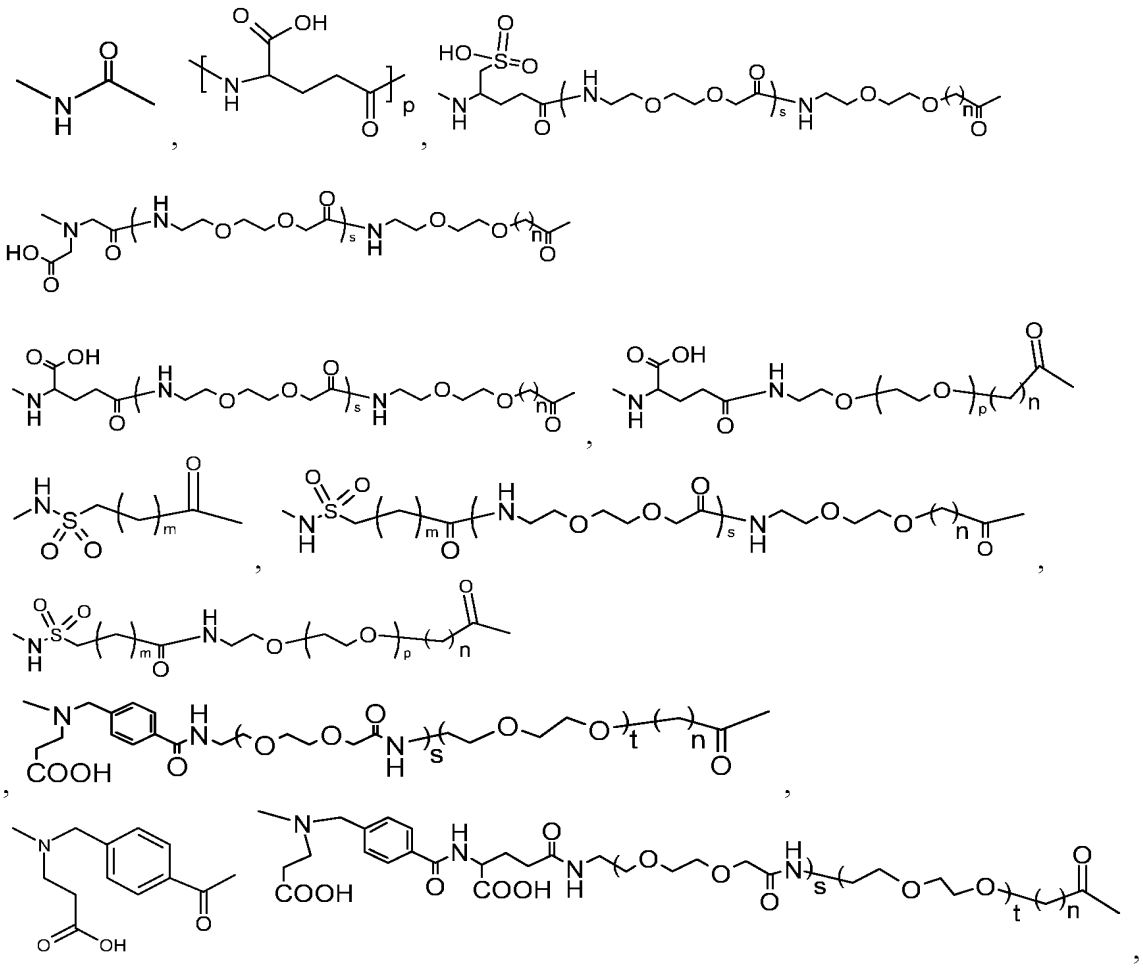
p is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17,18, 19, 20, 21, 22, or 23;

and where B is an acidic group selected from



where l is 12, 13, 14, 15, 16, 17, 18, 19 or 20;

8. (Currently Amended) A GLP-1 analog according to claim ~~6~~ 7, wherein U' is selected from



m is 2, 3, 4 or 5,

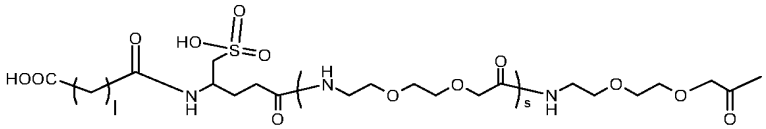
n is 1 or 2

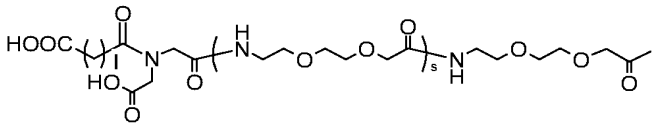
s is 0, 1, or 2,

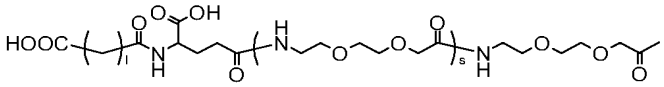
t is 0, 1, 2, or 3

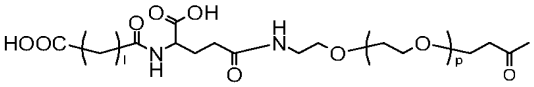
p is 1, 2, 3, 4, 7, 11 or 23

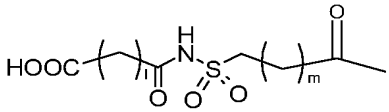
9. (Currently Amended) A GLP-1 analog according to claim ~~6~~ 8, wherein B-U'- is

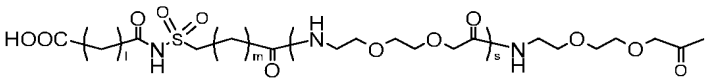


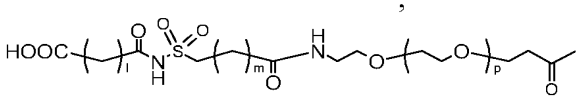


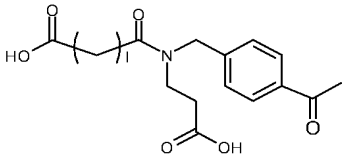


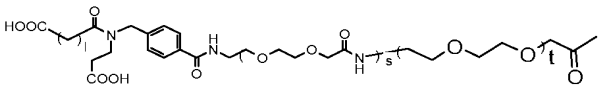


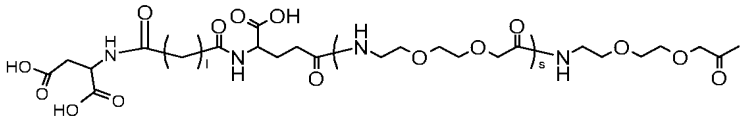


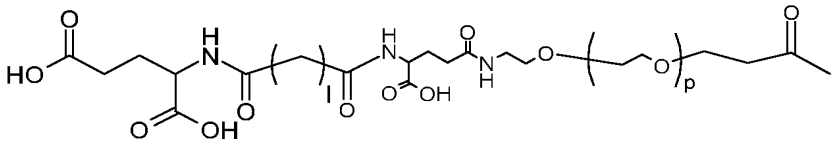












where l is 14, 15, 16, 17, 18, 19 or 20;

p is 1, 2, 3, 4, 7, 8 , 9, 10, 11 or 12.

s is 0, 1 or 2

t is 0 or 1;

m is 2, 3 or 4;

10. (Original) A GLP-1 analog according to claim 9, wherein

where l is 14, 15, 16, 17 or 18

p is 1, 2, 3, 4 or 11;

s is 0, 1 or 2;

t is 0 or 1;

11. (Currently Amended) A GLP-1 analog according to claim ~~6~~ 10, wherein s is 1.

12. (Currently Amended) A GLP-1 analog according to claim ~~6~~ 11, wherein l is 16

13-14 (Cancelled)

15. (Currently Amended) A GLP-1 analog according to claim ~~7~~ 12, wherein

Xaa_7 is His or desamino-histidine;

Xaa_8 is ~~Ala,~~ Gly, Val, Leu, Ile, Lys or Aib;

Xaa_{16} is Val;

Xaa_{18} is Ser;

Xaa_{19} is Tyr;

Xaa_{20} is Leu;

Xaa_{22} is Gly, Glu or Aib;

Xaa_{23} is Gln or Glu;

Xaa_{25} is Ala;

Xaa_{27} is Glu;

Xaa_{30} is Ala or Glu;

Xaa_{33} is Val;

Xaa_{34} is Lys or Arg;

Xaa_{35} is Gly or Aib;

Xaa_{36} is Arg or Lys

Xaa_{37} is Gly, amide or is absent;

16. (Original) A GLP-1 analog according to claim 15, wherein

Xaa_7 is His

Xaa_8 is Gly, or Aib;

Xaa_{16} is Val;

Xaa_{18} is Ser;

Xaa_{19} is Tyr;

Xaa_{20} is Leu;

Xaa_{22} is Glu or Aib;

Xaa_{23} is Gln;

Xaa_{25} is Ala;

Xaa_{27} is Glu;

Xaa_{30} is Ala;

Xaa_{33} is Val;

Xaa_{34} is Lys or Arg;

Xaa_{35} is Gly or Aib;

Xaa_{36} is Arg

Xaa_{37} is Gly

17. (Cancelled))

18. (Currently Amended) A GLP-1 analog according to claim ~~17~~ 7, wherein said GLP-1 analog comprises imidazopropionyl^7, α-hydroxy-histidine^7 or N-methyl-histidine^7, D-histidine^7, desamino-histidine^7, 2-amino-histidine^7, β-hydroxy-histidine^7, homohistidine^7, N^{α}-acetyl-histidine^7, α-fluoromethyl-histidine^7, α-methyl-histidine^7, 3-pyridylalanine^7, 2-pyridylalanine^7 or 4-pyridylalanine^7.

19. (Cancelled)

20. (Currently Amended) A GLP-1 analog according to claim ~~19~~ 7, wherein said GLP-1 analog comprises Aib^8, Gly^8, Val^8, Ile^8, Leu^8, Ser^8, Thr^8, (1-aminocyclopropyl) carboxylic acid, (1-aminocyclobutyl) carboxylic acid, (1-aminocyclopentyl) carboxylic acid, (1-aminocyclohexyl) carboxylic acid, (1-aminocycloheptyl) carboxylic acid, or (1-aminocyclooctyl) carboxylic acid.

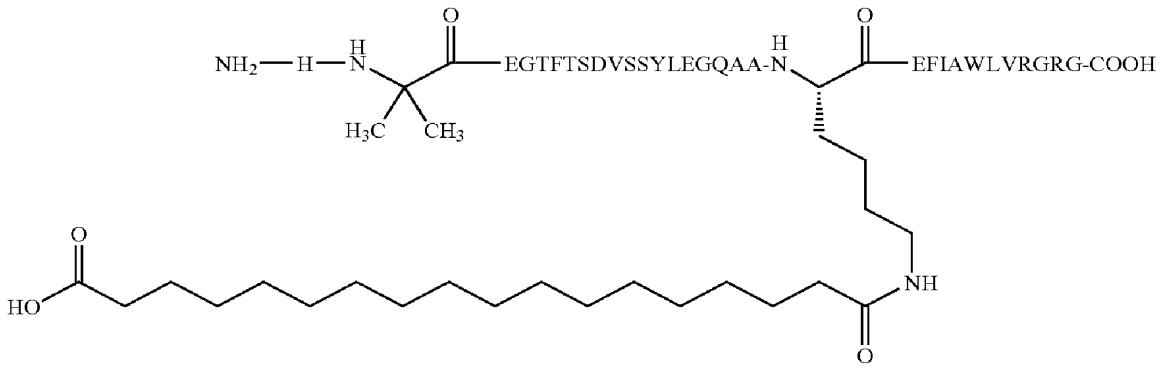
21. (Original) A GLP-1 analog according to claim 20, wherein said GLP-1 analog comprises Aib^8.

22-25. (Cancelled)

26. (Currently Amended) A GLP-1 analog according to claim ~~1~~ 7, wherein said GLP-1 analog comprises only one lysine residue.

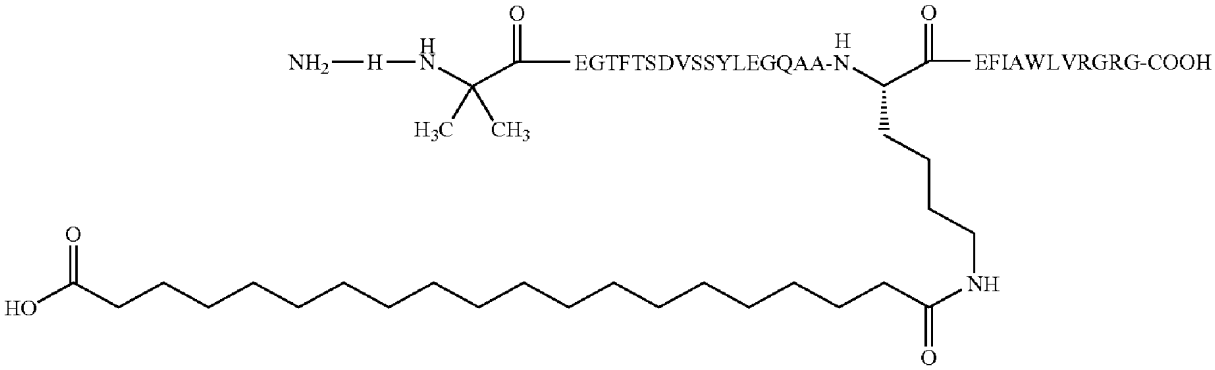
27. (Currently Amended) A GLP-1 analog according to claim ~~1~~ 7, which is
Aib^8,Arg^{34}-GLP-1(7-37)
$Aib^{8,22}$,Arg^{34}-GLP-1(7-37).
Arg^{34}-GLP-1(7-37).
[3-(4-Imidazolyl)Propionyl7,Arg34]GLP-1-(7-37)~~peptide~~
Gly^8,Arg^{34}-GLP-1(7-37) or
Aib^8,Arg^{34}, Pro^{37}-GLP-1(7-37)
~~$Aib^{8,22,27,30,35}$,Arg^{34},Pro^{37}-GLP-1(7-37)amide,~~
all of which are substituted by B-U' in position 26.

28. (Currently Amended) A compound according to claim ~~1~~ 7, which is selected from



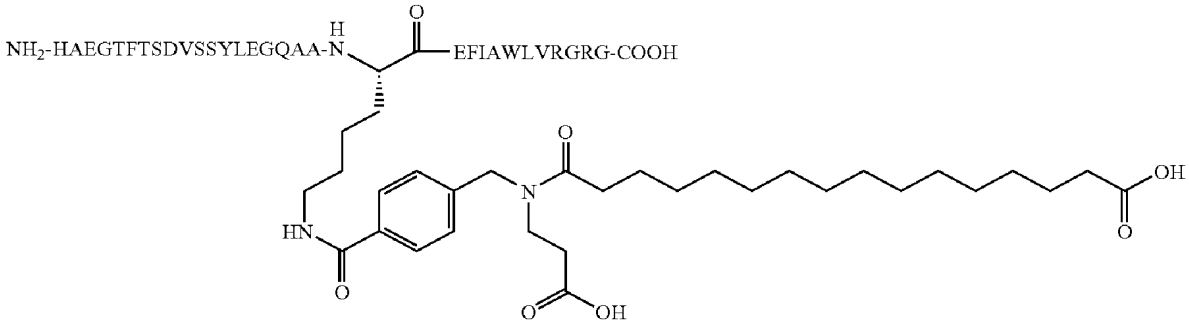
where the amino acid sequence is that of SEQ ID NO:4,

N-ε^{26}--(17-carboxyheptadecanoyl)-[Aib8,Arg34]GLP-1-(7-37)~~-peptide~~,



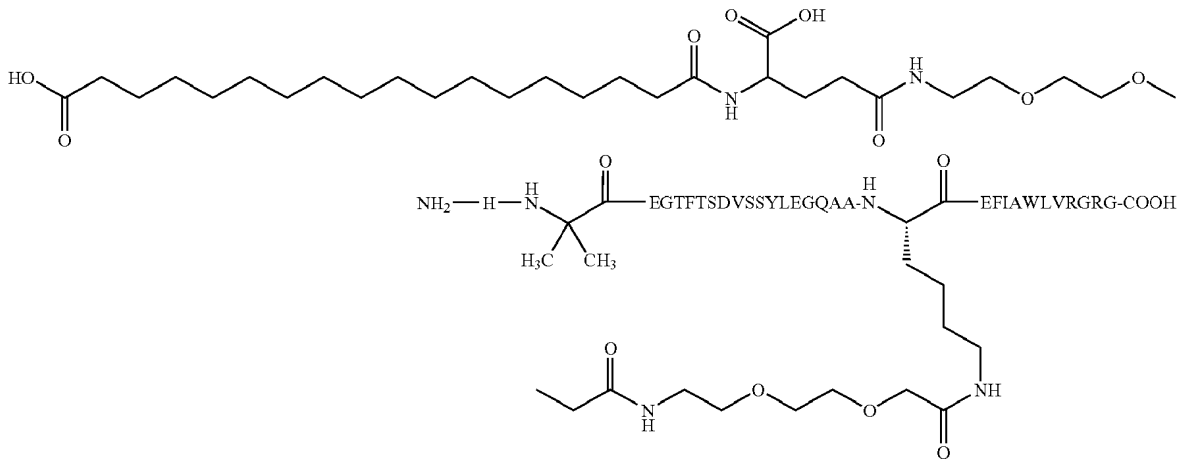
where the amino acid sequence is that of SEQ ID NO: 4,

N-ε^{26}-(19-carboxynonadecanoyl)-[Aib8,Arg34]GLP-1-(7-37)~~-peptide~~,



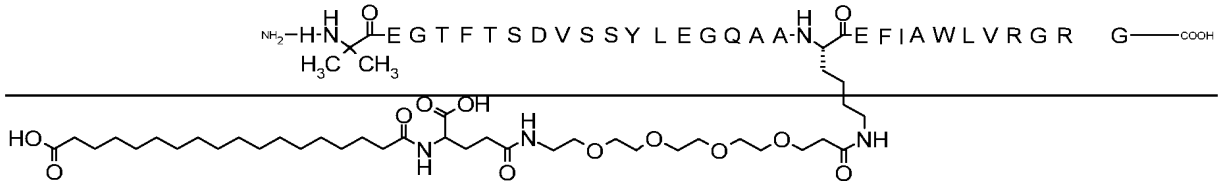
where the amino acid sequence is that of SEQ ID NO: 6,

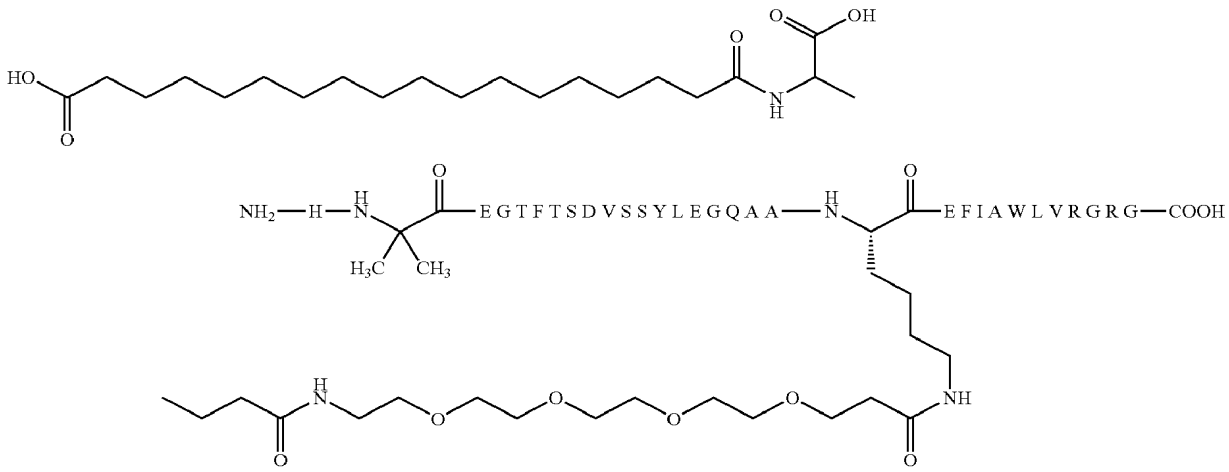
N-ε^{26}- (4-{[N-(2-carboxyethyl)-N-(15-carboxypentadecanoyl)amino]methyl}benzoyl)[Arg34]GLP-1-(7-37),



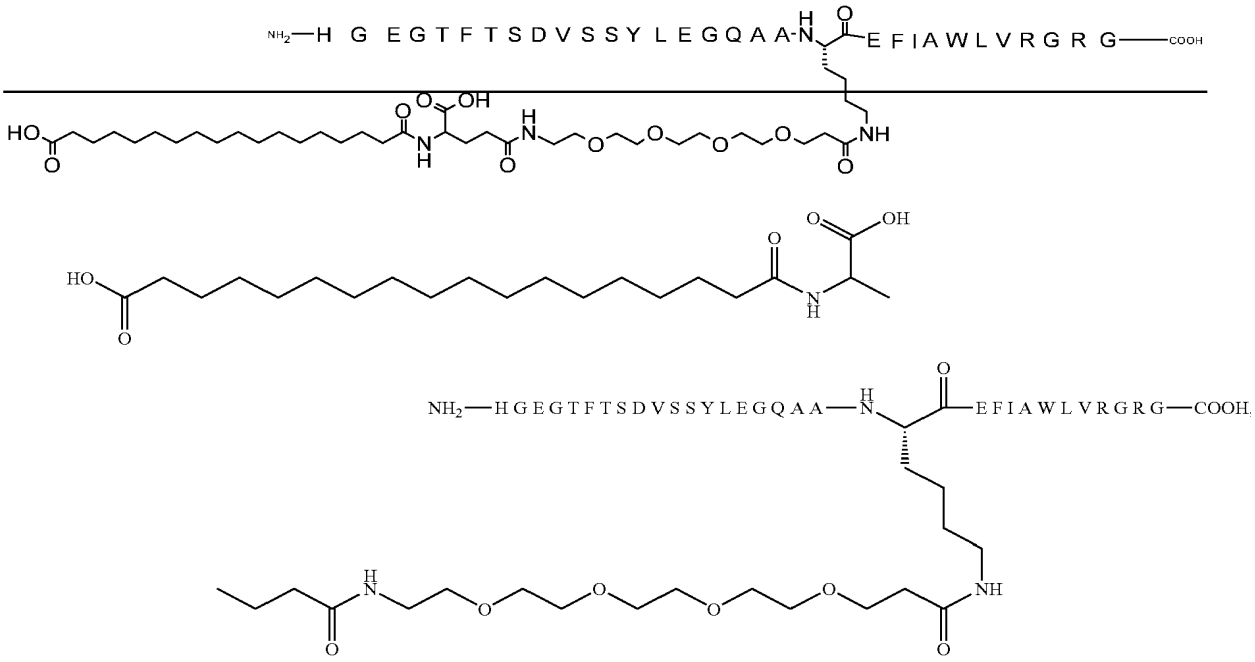
where the amino acid sequence is that of SEQ ID NO: 4,

N-ε^{26}-[2-(2-[2-(2-[2-(2-[4-(17-Carboxyheptadecanoylamino)-4(S)-carboxybutyrylamino]ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][Aib8,Arg34]GLP-1-(7-37)~~peptide~~,

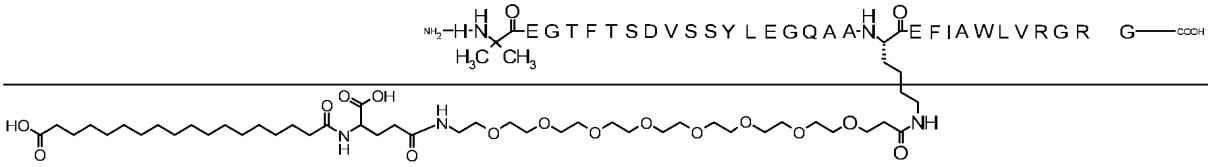


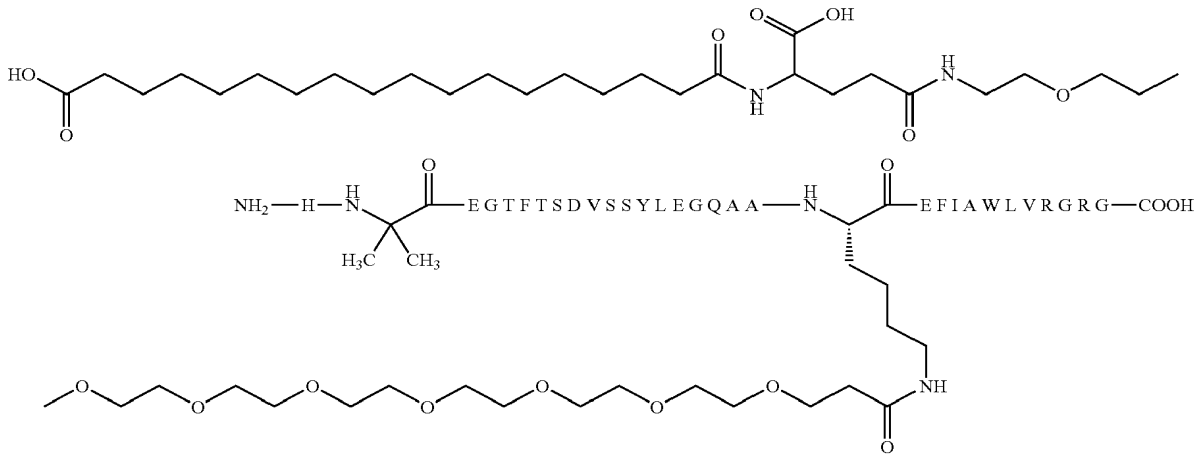


where the amino acid sequence is that of SEQ ID NO: 4,

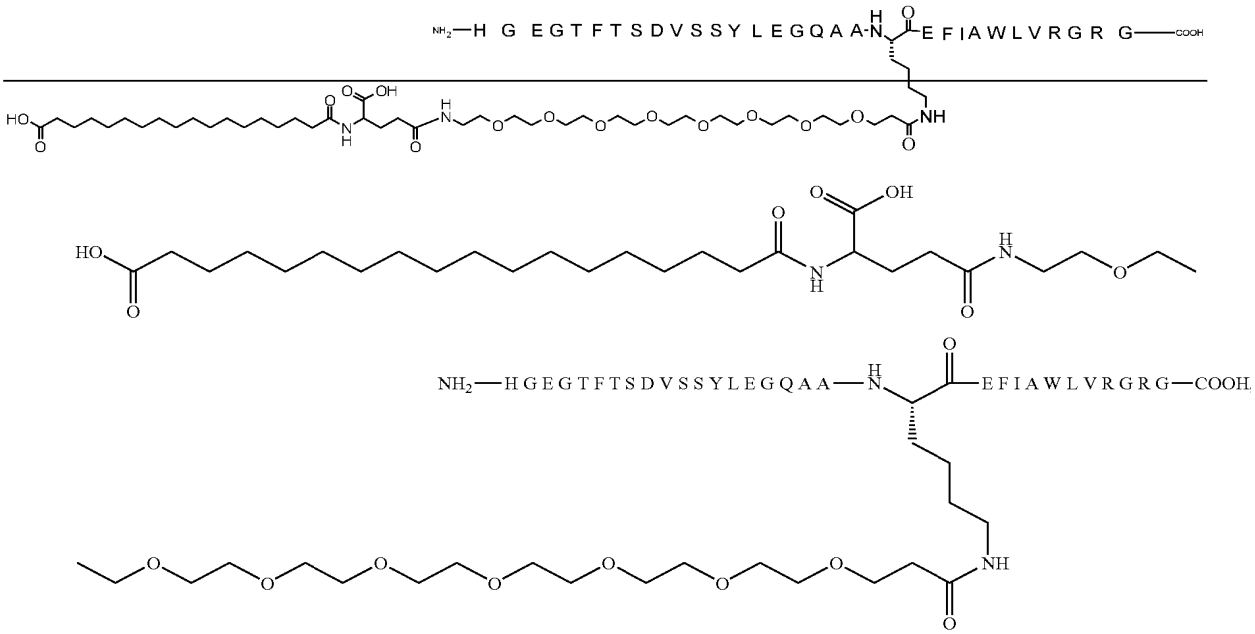


where the amino acid sequence is that of SEQ ID NO: 9,

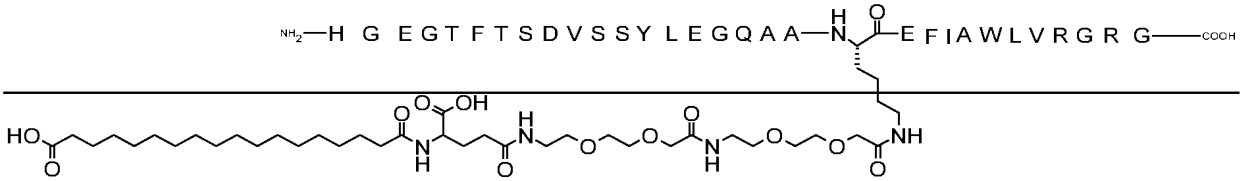


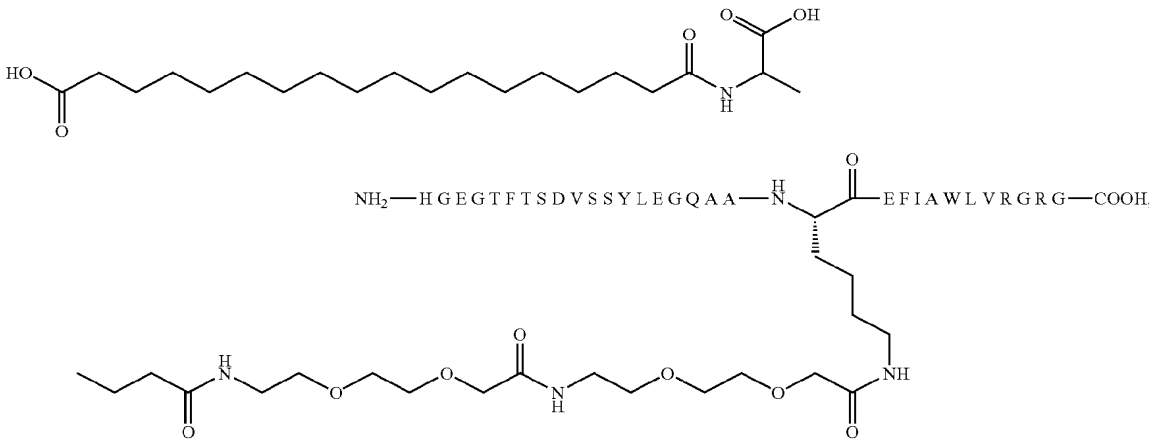


where the amino acid sequence is that of SEQ ID NO: 4,

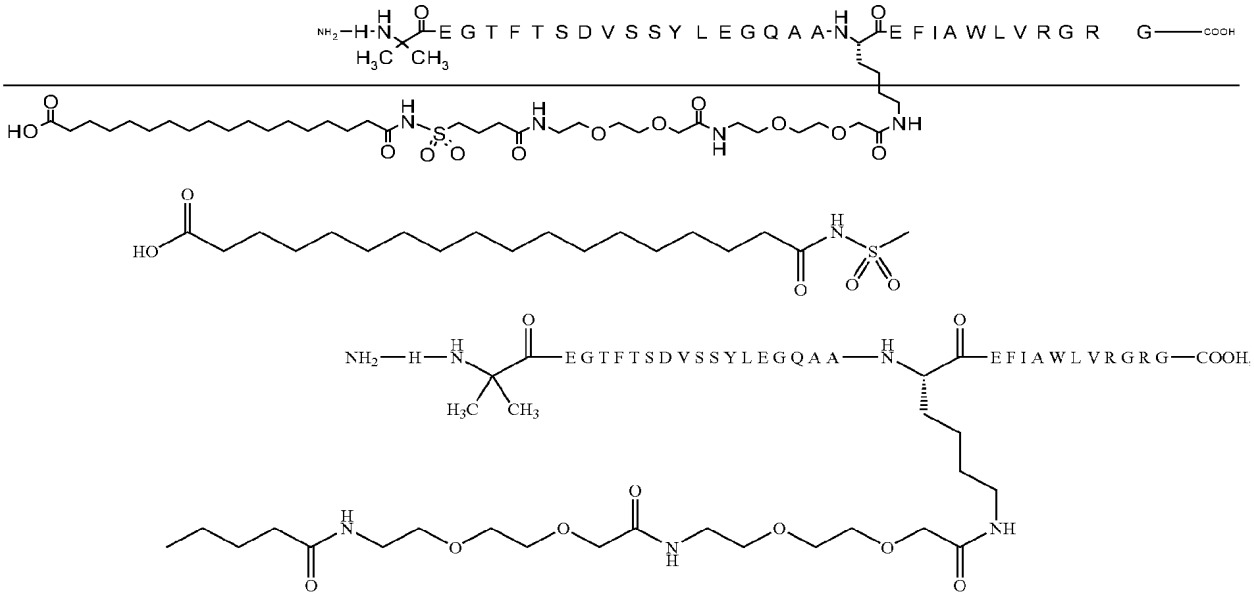


where the amino acid sequence is that of SEQ ID NO: 9,

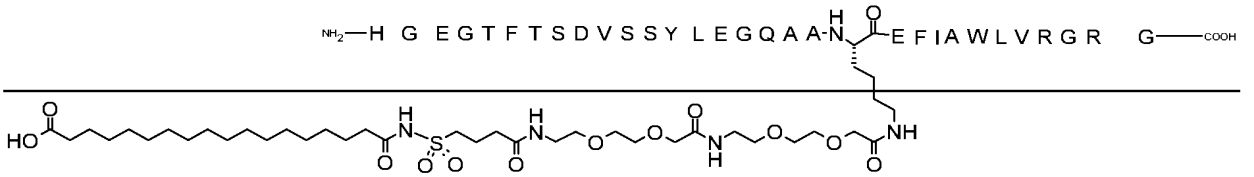


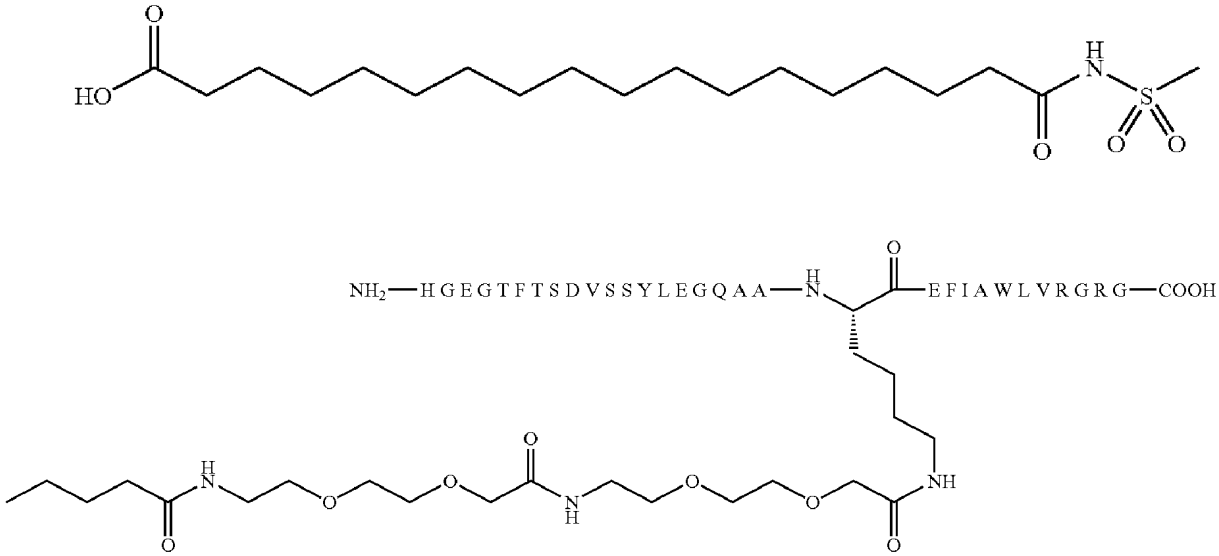


where the amino acid sequence is that of SEQ ID NO: 9,

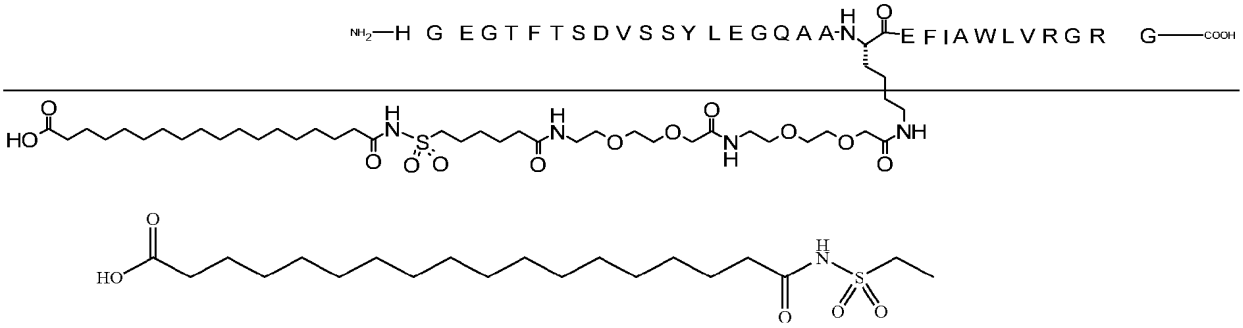


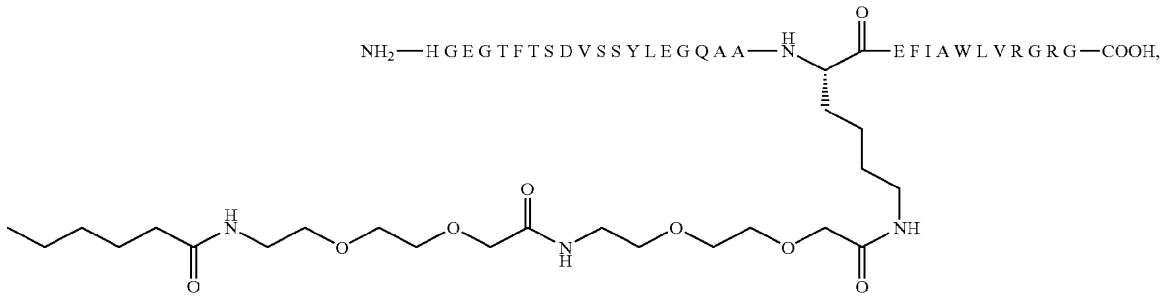
where the amino acid sequence is that of SEQ ID NO: 4,





where the amino acid sequence is that of SEQ ID NO: 9,





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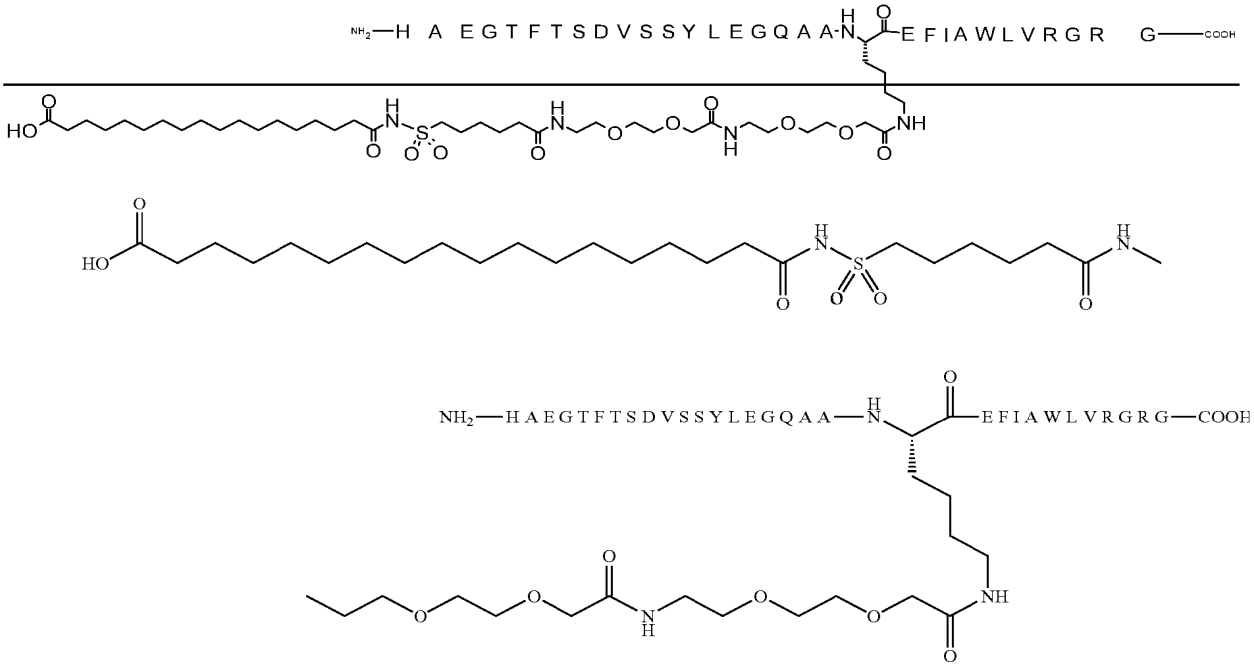
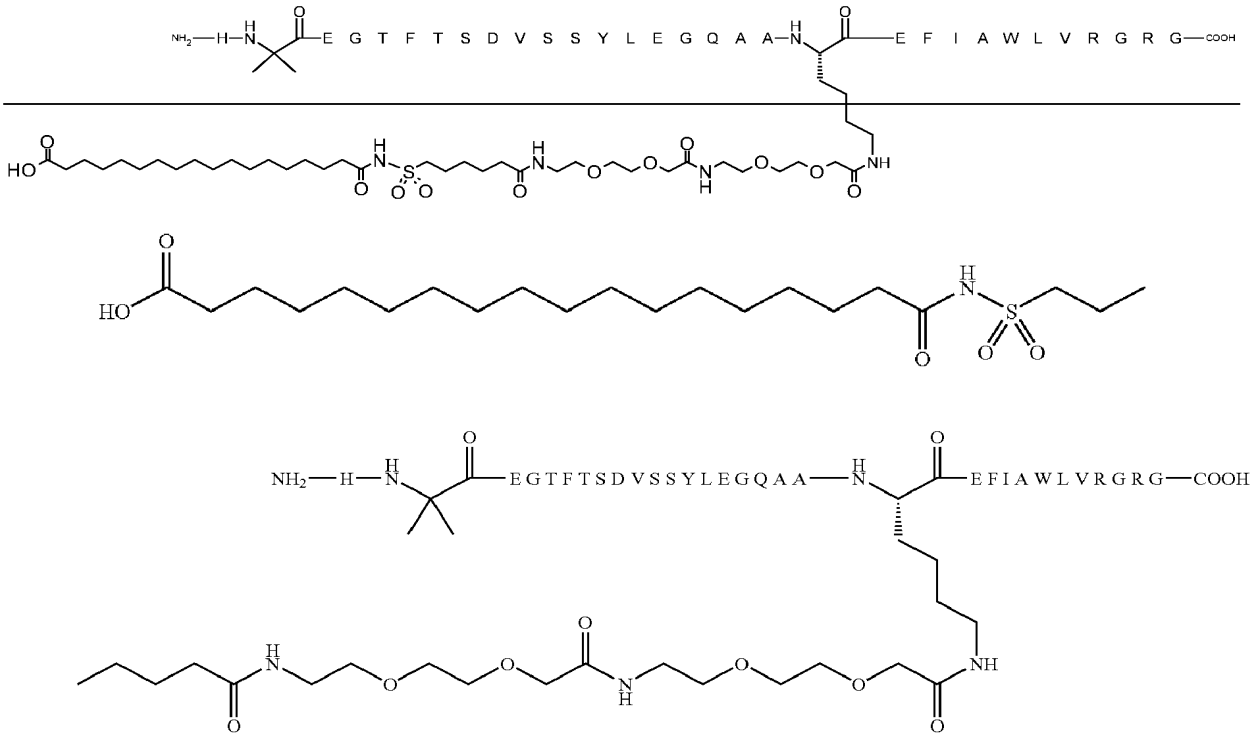
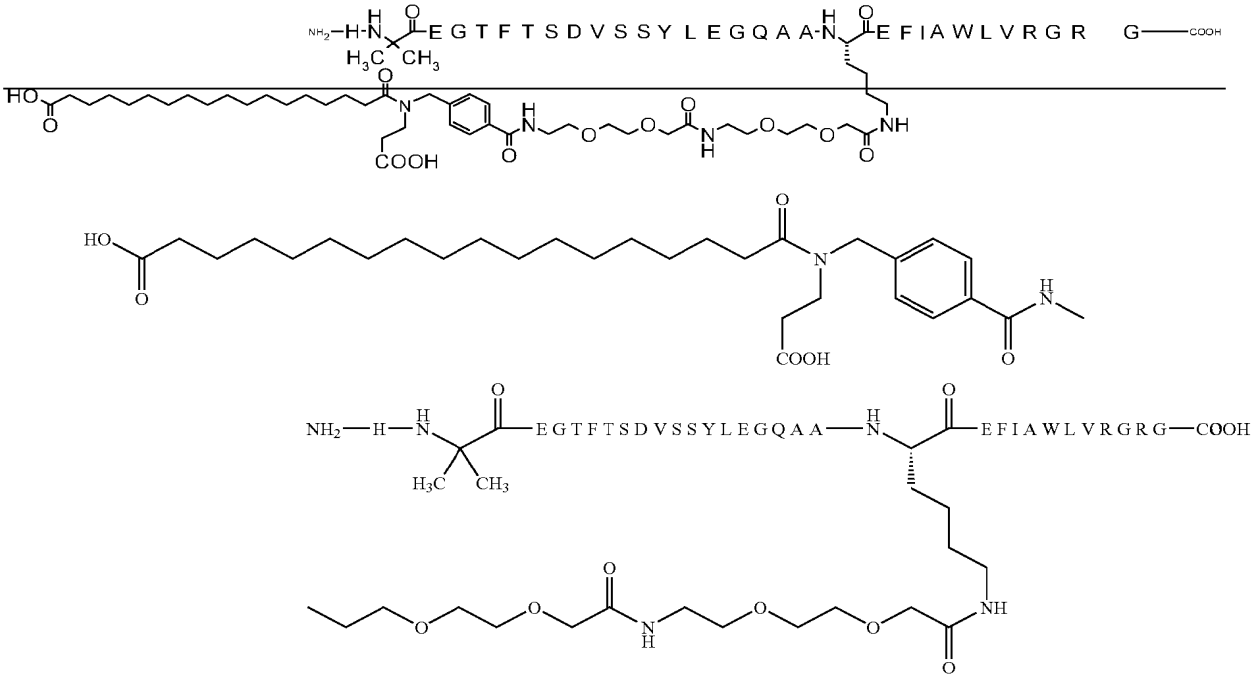


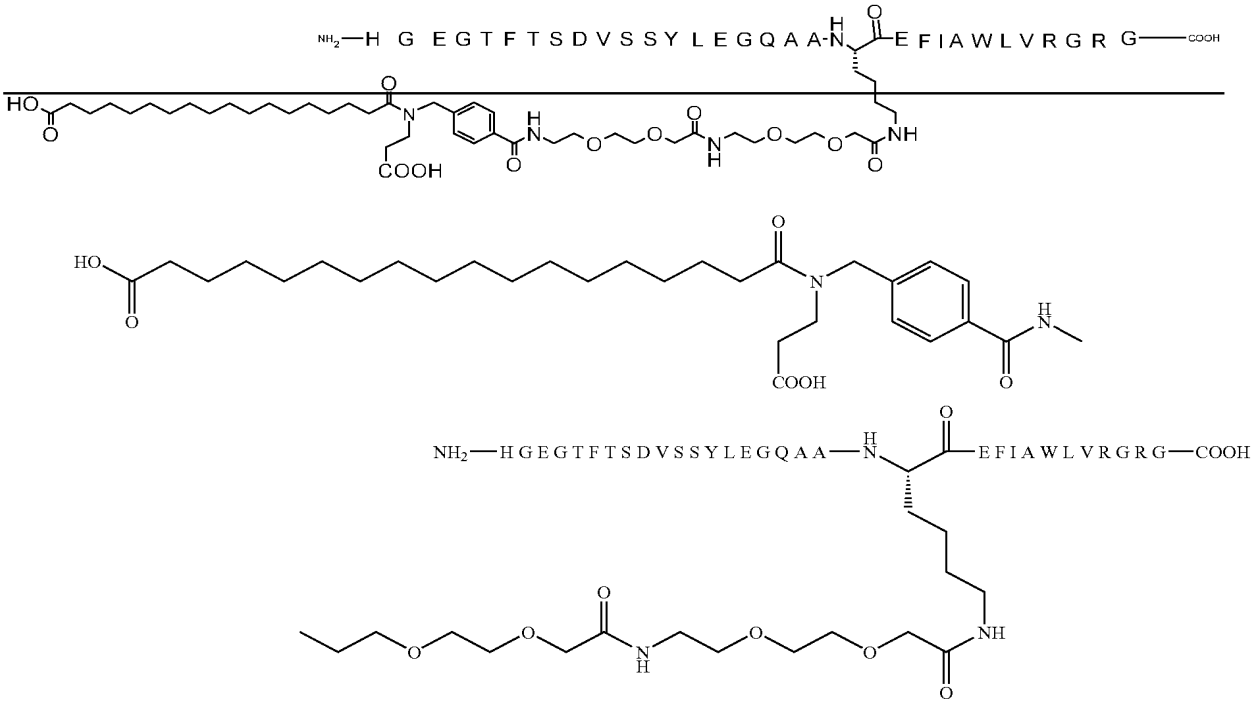
where the amino acid sequence is that of SEQ ID NO: 6,



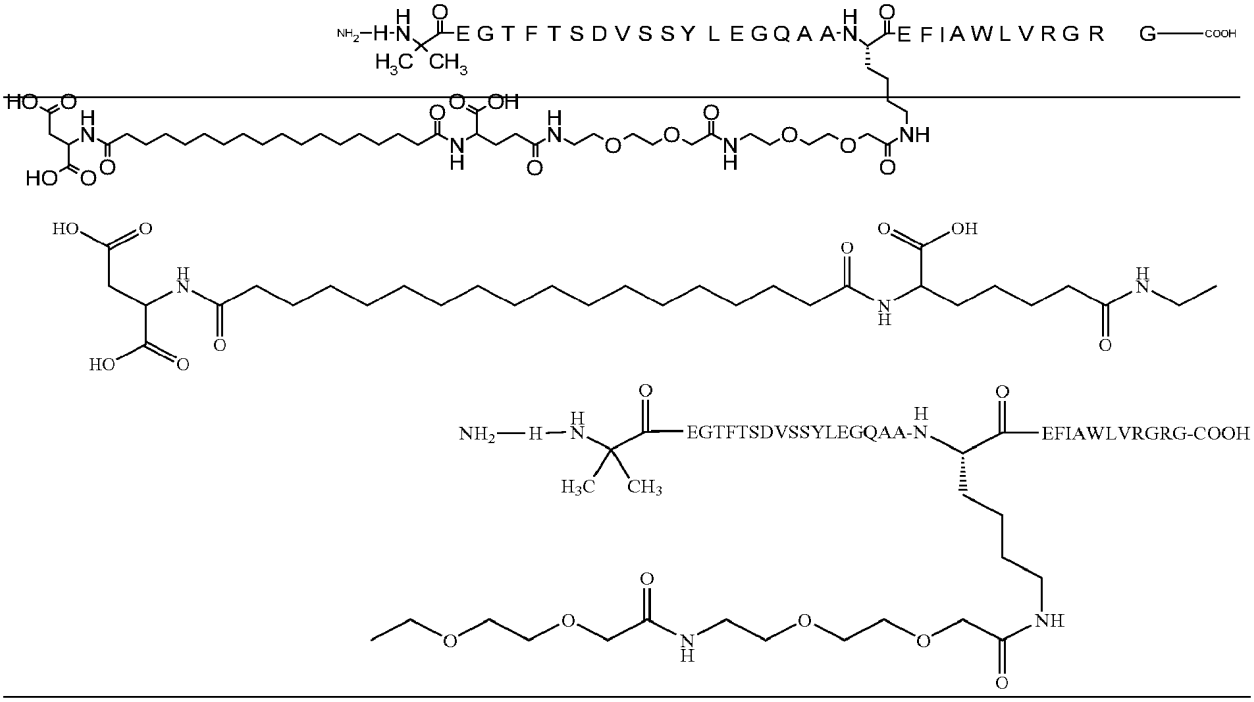
where the amino acid sequence is that of SEQ ID NO: 4,



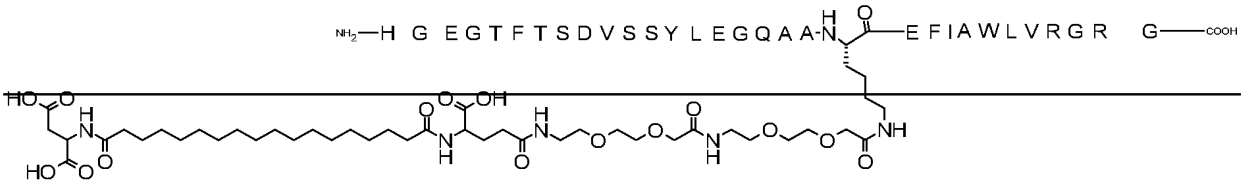
where the amino acid sequence is that of SEQ ID NO: 4,

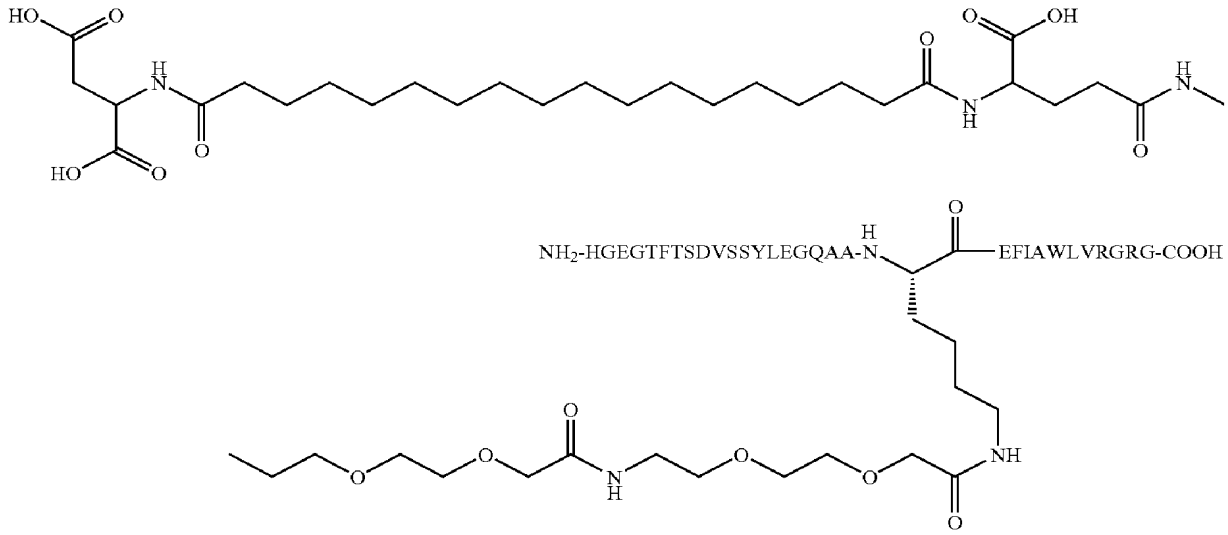


where the amino acid sequence is that of SEQ ID NO: 9,



where the amino acid sequence is that of SEQ ID NO: 4,





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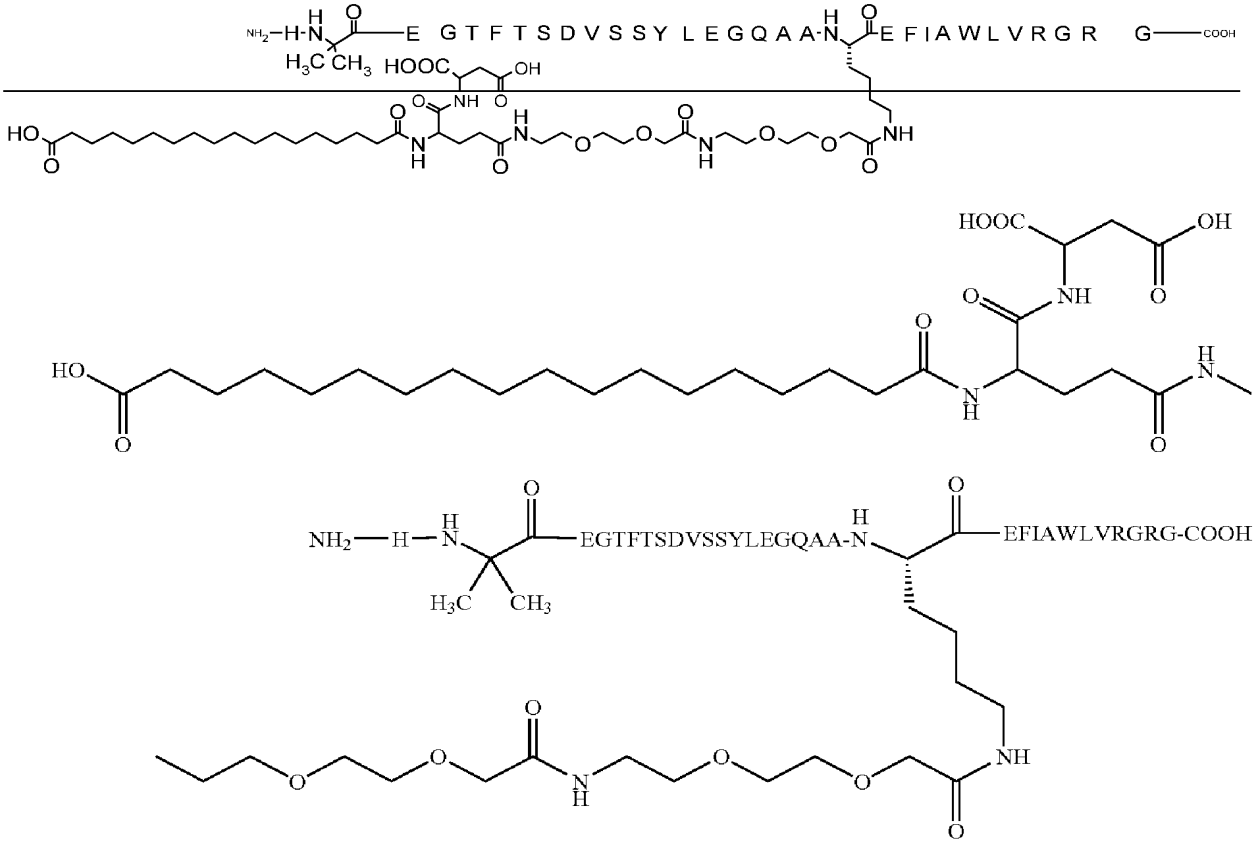
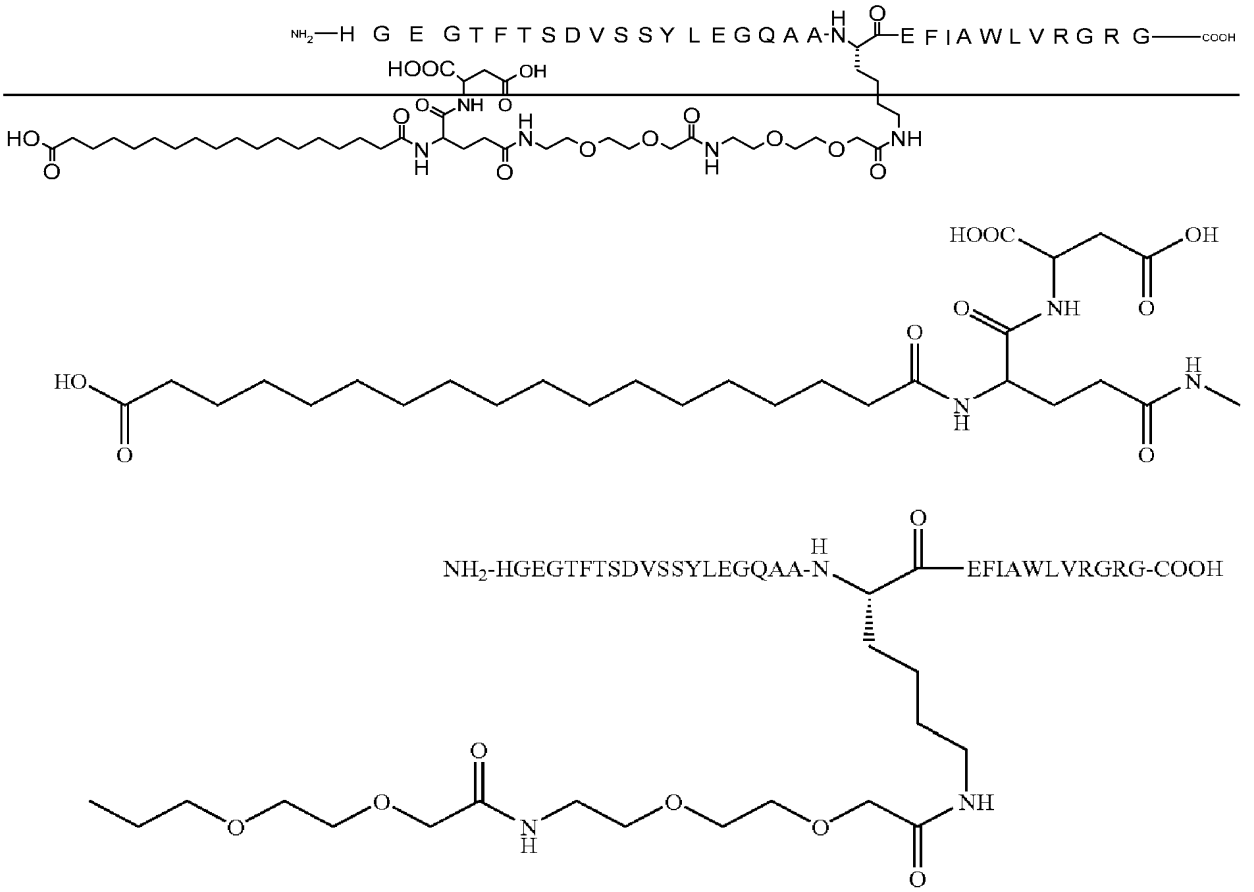


where the amino acid sequence is that of SEQ ID NO: 4, and



where the amino acid sequence is that of SEQ ID NO: 9.

29-30 (Cancelled)

31. (Currently Amended) A pharmaceutical composition comprising a compound according to claim ~~1~~ 7, and a pharmaceutically acceptable excipient.

32. (Original) The pharmaceutical composition according to claim 31, which is suited for parenteral administration.

33-36 (Cancelled)

37. (Currently Amended) A method for treating ~~hyperglycemia,~~ type 2 diabetes~~, impaired glucose tolerance, type 1 diabetes, obesity, hypertension, syndrome X, dyslipidemia, cognitive disorders, atheroschlerosis, myocardial infarction, coronary heart disease and other cardiovascular disorders, stroke, inflammatory bowel syndrome, dyspepsia and/or gastric ulcers~~ in a subject, said method comprising administering to a subject in need of such treatment an effective amount of a GLP-1 analog according to claim ~~1~~ 7.

38-39 (Cancelled)

40. (New) A pharmaceutical composition comprising a compound according to claim 28, and a pharmaceutically acceptable excipient.

41. (New) The pharmaceutical composition according to claim 40, which is suited for parenteral administration.

42. (New) A method for treating type 2 diabetes in a subject, said method comprising administering to a subject in need of such treatment an effective amount of a GLP-1 analog according to claim 28.

REMARKS

Claims 7-12, 15-16, 18, 20-21, 26-28, 31-32, 37 and 40-42 are pending following entry of this Amendment. The present amendment adds no new matter.

Applicants thank the Examiner for the courtesy extended during the telephone interview of March 3, 2010. The amendments presented herein, such as the amendments to the formulas presented in claim 28 to increase the font size of the formulas, are intended to address the comments made by the Examiner in the March 11, 2010 Interview Summary.

Applicants note that these amendments have not been made to overcome prior art and thus should be considered to have been made for a purpose unrelated to patentability, and no estoppel should be deemed to attach thereto. Moreover, any canceled subject matter is canceled without prejudice or disclaimer and Applicants reserve the right to pursue the canceled subject matter in future continuation applications. Thus, the cancelled subject matter has not been dedicated to the public.

It is believed that the claims are in condition for allowance, and a determination to that effect is earnestly solicited.

Respectfully submitted,
/Richard W. Bork, Reg. No. 36,459/

Date: August 9, 2010

Richard W. Bork, Reg. No. 36,459
Novo Nordisk Inc.
Customer Number 23650
(609) 987-5800

Use the following customer number for all correspondence regarding this application.

23650
PATENT TRADEMARK OFFICE

INFORMATION DISCLOSURE STATEMENT BY APPLICANT					
				Application No.	11/908,834
				Filing Date	September 17, 2007
				Applicant	Lau et al.
				Art Unit	1654
				Examiner Name:	Marcela M. Cordero Garcia
Sheet	1	of	1	Atty. Docket No.	7140.204-US

U.S. PATENT DOCUMENTS

EXAMINER INITIALS	Cite No.	DOCUMENT NUMBER Number –Kind Code (if known)	Issue/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.	DOCUMENT NUMBER Number –Kind Code (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
		WO 2004/99246	11-18-2004	Novo Nordisk A/S		

NON PATENT LITERATURE DOCUMENTS

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
		Green, Brian D., Biological Chemistry (2004), Vol. 385, No. 2, pp. 169-177.	

EXAMINER SIGNATURE		DATE CONSIDERED	

INFORMATION DISCLOSURE STATEMENT BY APPLICANT					
				Application No.	11/908,834
				Filing Date	September 17, 2007
				Applicant	Lau et al.
				Art Unit	1654
				Examiner Name:	Marcela M. Cordero Garcia
Sheet	1	of	1	Atty. Docket No.	7140.204-US

U.S. PATENT DOCUMENTS

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FOREIGN PATENT DOCUMENTS

EXAMINER INITIALS	Cite No.	DOCUMENT NUMBER Number –Kind Code(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
		RU 2006107600 (corresponds to WO2005/027978)	10-27-2007	Novo Nordisk A/S		
		WO 96/29342	09-26-1996	Novo Nordisk A/S		
		WO 98/08871	03-05-1998	Novo Nordisk A/S		
		WO 99/43708	09-02-1999	Novo Nordisk A/S		
		WO 00/34331	06-15-2000	Societe de Conseils de Recherches et d' Applications Scientifiques		
		WO 00/69911	11-23-2000	Conjuchem Inc		
		WO 02/46227	06-13-2000	Eli Lilly & Co		
		WO 2005/014049	02-17-2005	Novo Nordisk A/S		
		WO 2005/027978 (corresponds to RU2006107600)	03-31-2005	Novo Nordisk A/S		

EXAMINER SIGNATURE		DATE CONSIDERED	

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/908,834	09/17/2008	Jesper Lau	7140.204-US	8500

23650 7590 03/11/2010
NOVO NORDISK, INC.
INTELLECTUAL PROPERTY DEPARTMENT
100 COLLEGE ROAD WEST
PRINCETON, NJ 08540

EXAMINER
CORDERO GARCIA, MARCELA M

ART UNIT	PAPER NUMBER
1654	

NOTIFICATION DATE	DELIVERY MODE
03/11/2010	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):

nnipatent@novonordisk.com
KSHL@novonordisk.com
KISW@novonordisk.com

Interview Summary	Application No.	Applicant(s)	
	11/908,834	LAU ET AL.	
	Examiner	Art Unit	
	MARCELA M. CORDERO GARCIA	1654	

All participants (applicant, applicant's representative, PTO personnel):

(1) *MARCELA M. CORDERO GARCIA*. (3)_____.

(2) *RICHARD BORK*. (4)_____.

Date of Interview: *03 March 2010*.

Type: a)☒ Telephonic b)☐ Video Conference
c)☐ Personal [copy given to: 1)☐ applicant 2)☐ applicant's representative]

Exhibit shown or demonstration conducted: d)☐ Yes e)☒ No.
If Yes, brief description: _____.

Claim(s) discussed: *7,27 and 28*.

Identification of prior art discussed: *N/A*.

Agreement with respect to the claims f)☐ was reached. g)☐ was not reached. h)☒ N/A.

Substance of Interview including description of the general nature of what was agreed to if an agreement was reached, or any other comments: *See Continuation Sheet*.

(A fuller description, if necessary, and a copy of the amendments which the examiner agreed would render the claims allowable, if available, must be attached. Also, where no copy of the amendments that would render the claims allowable is available, a summary thereof must be attached.)

THE FORMAL WRITTEN REPLY TO THE LAST OFFICE ACTION MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW. (See MPEP Section 713.04). If a reply to the last Office action has already been filed, APPLICANT IS GIVEN A NON-EXTENDABLE PERIOD OF THE LONGER OF ONE MONTH OR THIRTY DAYS FROM THIS INTERVIEW DATE, OR THE MAILING DATE OF THIS INTERVIEW SUMMARY FORM, WHICHEVER IS LATER, TO FILE A STATEMENT OF THE SUBSTANCE OF THE INTERVIEW. See Summary of Record of Interview requirements on reverse side or on attached sheet.

/Marcela M Cordero Garcia/ Examiner, Art Unit 1654	

Summary of Record of Interview Requirements

Manual of Patent Examining Procedure (MPEP), Section 713.04, Substance of Interview Must be Made of Record
A complete written statement as to the substance of any face-to-face, video conference, or telephone interview with regard to an application must be made of record in the application whether or not an agreement with the examiner was reached at the interview.

Title 37 Code of Federal Regulations (CFR) § 1.133 Interviews
Paragraph (b)

In every instance where reconsideration is requested in view of an interview with an examiner, a complete written statement of the reasons presented at the interview as warranting favorable action must be filed by the applicant. An interview does not remove the necessity for reply to Office action as specified in §§ 1.111, 1.135. (35 U.S.C. 132)

37 CFR §1.2 Business to be transacted in writing.
All business with the Patent or Trademark Office should be transacted in writing. The personal attendance of applicants or their attorneys or agents at the Patent and Trademark Office is unnecessary. The action of the Patent and Trademark Office will be based exclusively on the written record in the Office. No attention will be paid to any alleged oral promise, stipulation, or understanding in relation to which there is disagreement or doubt.

The action of the Patent and Trademark Office cannot be based exclusively on the written record in the Office if that record is itself incomplete through the failure to record the substance of interviews.

It is the responsibility of the applicant or the attorney or agent to make the substance of an interview of record in the application file, unless the examiner indicates he or she will do so. It is the examiner's responsibility to see that such a record is made and to correct material inaccuracies which bear directly on the question of patentability.

Examiners must complete an Interview Summary Form for each interview held where a matter of substance has been discussed during the interview by checking the appropriate boxes and filling in the blanks. Discussions regarding only procedural matters, directed solely to restriction requirements for which interview recordation is otherwise provided for in Section 812.01 of the Manual of Patent Examining Procedure, or pointing out typographical errors or unreadable script in Office actions or the like, are excluded from the interview recordation procedures below. Where the substance of an interview is completely recorded in an Examiners Amendment, no separate Interview Summary Record is required.

The Interview Summary Form shall be given an appropriate Paper No., placed in the right hand portion of the file, and listed on the "Contents" section of the file wrapper. In a personal interview, a duplicate of the Form is given to the applicant (or attorney or agent) at the conclusion of the interview. In the case of a telephone or video-conference interview, the copy is mailed to the applicant's correspondence address either with or prior to the next official communication. If additional correspondence from the examiner is not likely before an allowance or if other circumstances dictate, the Form should be mailed promptly after the interview rather than with the next official communication.

The Form provides for recordation of the following information:
- Application Number (Series Code and Serial Number)
- Name of applicant
- Name of examiner
- Date of interview
- Type of interview (telephonic, video-conference, or personal)
- Name of participant(s) (applicant, attorney or agent, examiner, other PTO personnel, etc.)
- An indication whether or not an exhibit was shown or a demonstration conducted
- An identification of the specific prior art discussed
- An indication whether an agreement was reached and if so, a description of the general nature of the agreement (may be by attachment of a copy of amendments or claims agreed as being allowable). Note: Agreement as to allowability is tentative and does not restrict further action by the examiner to the contrary.
- The signature of the examiner who conducted the interview (if Form is not an attachment to a signed Office action)

It is desirable that the examiner orally remind the applicant of his or her obligation to record the substance of the interview of each case. It should be noted, however, that the Interview Summary Form will not normally be considered a complete and proper recordation of the interview unless it includes, or is supplemented by the applicant or the examiner to include, all of the applicable items required below concerning the substance of the interview.

A complete and proper recordation of the substance of any interview should include at least the following applicable items:
1) A brief description of the nature of any exhibit shown or any demonstration conducted,
2) an identification of the claims discussed,
3) an identification of the specific prior art discussed,
4) an identification of the principal proposed amendments of a substantive nature discussed, unless these are already described on the Interview Summary Form completed by the Examiner,
5) a brief identification of the general thrust of the principal arguments presented to the examiner,
   (The identification of arguments need not be lengthy or elaborate. A verbatim or highly detailed description of the arguments is not required. The identification of the arguments is sufficient if the general nature or thrust of the principal arguments made to the examiner can be understood in the context of the application file. Of course, the applicant may desire to emphasize and fully describe those arguments which he or she feels were or might be persuasive to the examiner.)
6) a general indication of any other pertinent matters discussed, and
7) if appropriate, the general results or outcome of the interview unless already described in the Interview Summary Form completed by the examiner.

Examiners are expected to carefully review the applicant's record of the substance of an interview. If the record is not complete and accurate, the examiner will give the applicant an extendable one month time period to correct the record.

Examiner to Check for Accuracy

If the claims are allowable for other reasons of record, the examiner should send a letter setting forth the examiner's version of the statement attributed to him or her. If the record is complete and accurate, the examiner should place the indication, "Interview Record OK" on the paper recording the substance of the interview along with the date and the examiner's initials.

Continuation of Substance of Interview including description of the general nature of what was agreed to if an agreement was reached, or any other comments: Examiner discussed a preliminary draft claim set sent by Richard Bork on 3/2/2010. It was noted that proposed claim 7 would be searched broadly as "comprising of" since the claim was not expressly claiming the compounds as "consisting of". Further, proposed claim 27 seemed to lack antecedent basis due to the missing limitation "all of which are substituted by B U' in position 26". With respect to proposed claim 28 Examiner noted that the formulas were too small and may present printer problems later on, the formulas are missing the SEQ ID NO: identifier and the combination of H with Aib formula as presently shown (e.g., first and second compounds) is a bit confusing and it would be preferably to either use a parenthesis to distinguish the residue Aib from the residue H or to identify Aib with its 3 letter identifier rather than its chemical formula, adding a wherein clause at the end of the claim to set forth the formula of Aib. Prior art was not discussed as the invention has not been searched by examiner.

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NOVO NORDISK, INC

FACSIMILE TRANSMITTAL SHEET

TO: MARCELA CORDERO GARCIA USPTO	FROM: LORI KLEWIN
	DATE: MARCH 2, 2010
FAX NUMBER: (571) 273-2939	TOTAL NO. OF PAGES INCLUDING COVER: 14
PHONE NUMBER:	SENDER'S PHONE NUMBER: (609) 987-5274
RE: U.S. Appln. No. 11/908,834 Our Ref: 7140.204-US	SENDER'S FAX NUMBER: 609-987-5440

FOR REVIEW ☐ PLEASE COMMENT ☐ PLEASE REPLY ☐ PLEASE RECYCLE

Attached is a DRAFT Preliminary Amendment for a telephone interview with Richard Bork on Wednesday March 3rd at 3 PM.

PLEASE NOTE: The information contained in this facsimile message is privileged and confidential, and is intended only for the use of the individual named above and others who have been specifically authorized to receive it. If you are not the intended recipient, you are hereby notified that any dissemination, distribution or copying of this communication is strictly prohibited. If you have received this communication in error, or if any problems occur with the transmission, please contact Karen Schlauch 609-987-5494.

DRAFT

Attorney Docket No.: 7140.204-US

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Lau et al.

Application No.: 11/908,834

Group Art Unit: 1654

Filed: September 17, 2007

Examiner: Cordero Garcia, Marcela

For: Acylated GLP-1 Compounds

PRELIMINARY AMENDMENT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Prior to examination of the above-identified application on the merits, kindly amend the above-captioned patent application as set forth below.

AMENDMENTS TO THE CLAIMS begin on page 2 of this paper.

REMARKS begin on page 13 of this paper.

DRAFT

371 National Stage of PCT/EP2006/060855
International Filing Date: March 20, 2006

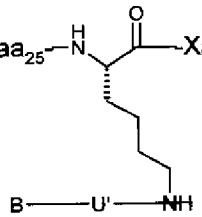
AMENDMENTS TO THE CLAIMS

CLAIM LISTING

1-6. (Cancelled)

7. (Original) A GLP-1 analog which is a compound of formula I (SEQ ID No. 2):

Xaa_7-Xaa_8-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Xaa_{16}-Ser-Xaa_{18}-Xaa_{19}-Xaa_{20}-Glu-Xaa_{22}-

Xaa_{23}-Ala-Xaa_{25}-  -Xaa_{27}-Phe-Ile-Xaa_{30}-Trp-Leu-Xaa_{33}-Xaa_{34}-Xaa_{35}-Xaa_{36}-Xaa_{37}

Formula I

wherein

Xaa_7 is L-histidine, imidazopropionyl, α-hydroxy-histidine, D-histidine, desamino-histidine, 2-amino-histidine, β-hydroxy-histidine, homohistidine, N^{α}-acetyl-histidine, N^{α}-formyl-histidine, α-fluoromethyl-histidine, α-methyl-histidine, 3-pyridylalanine, 2-pyridylalanine or 4-pyridylalanine

Xaa_8 is Ala, Gly, Val, Leu, Ile, Thr, Ser, Lys, Aib, (1-aminocyclopropyl) carboxylic acid, (1-aminocyclobutyl) carboxylic acid, (1-aminocyclopentyl) carboxylic acid, (1-aminocyclohexyl) carboxylic acid, (1-aminocycloheptyl) carboxylic acid, or (1-aminocyclooctyl) carboxylic acid;

Xaa_{16} is Val or Leu;

Xaa_{18} is Ser, Lys or Arg;

Xaa_{19} is Tyr or Gln;

Xaa_{20} is Leu or Met;

Xaa_{22} is Gly, Glu or Aib;

Xaa_{23} is Gln, Glu, Lys or Arg;

Xaa_{25} is Ala or Val;

DRAFT

Xaa_{27} is Glu or Leu;

Xaa_{30} is Ala, Glu or Arg;

Xaa_{33} is Val or Lys;

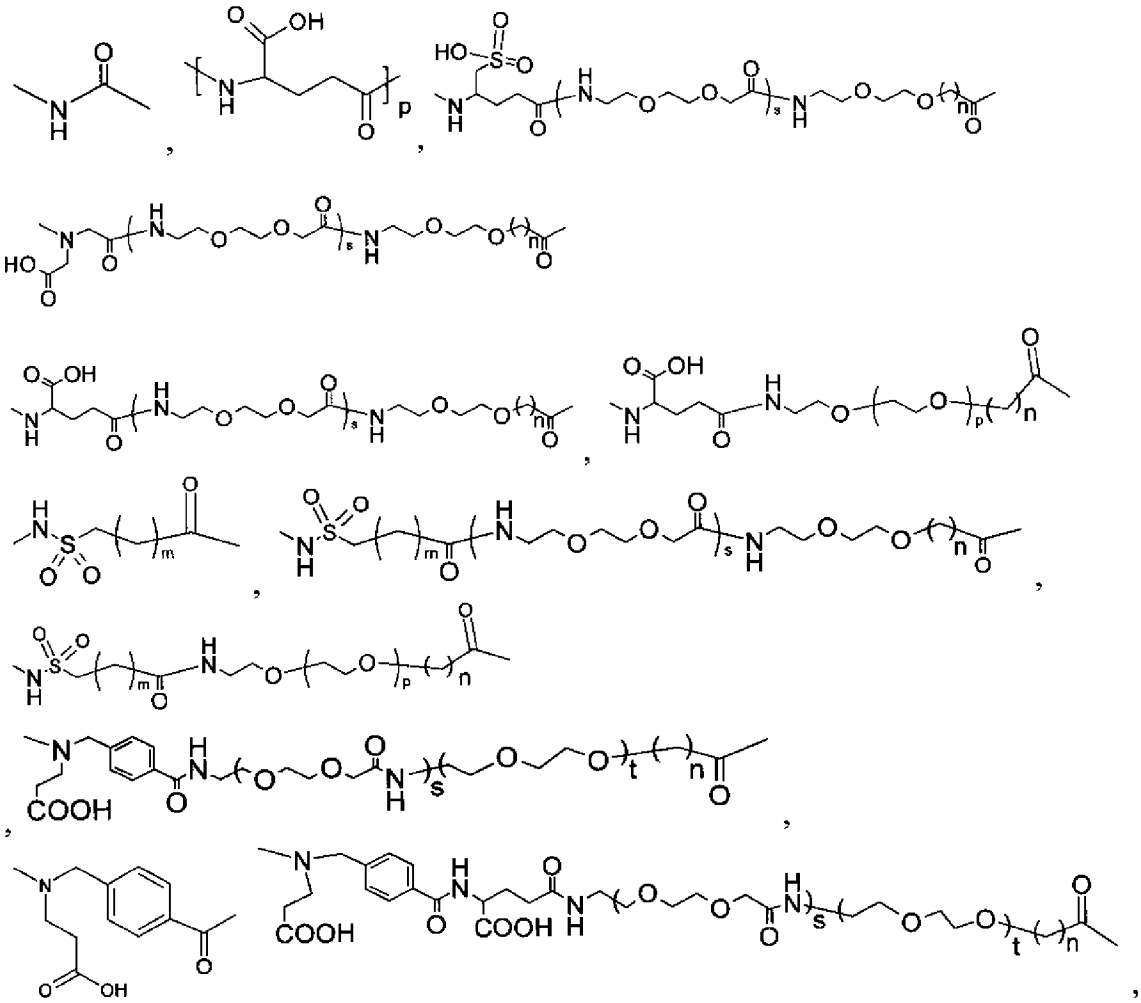
Xaa_{34} is Lys, Glu, Asn or Arg;

Xaa_{35} is Gly or Aib;

Xaa_{36} is Arg, Gly or Lys, or is absent;

Xaa_{37} is Gly, Ala, Glu, Pro, Lys, or is absent;

and B and U' together is the acylated moiety, where U' is selected from



m is 0, 1, 2, 3, 4, 5, or 6,

DRAFT

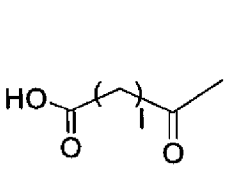
n is 1, 2 or 3

s is 0, 1, 2, or 3,

t is 0, 1, 2, 3, or 4

p is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17,18, 19, 20, 21, 22, or 23;

and where B is an acidic group selected from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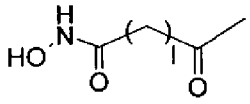
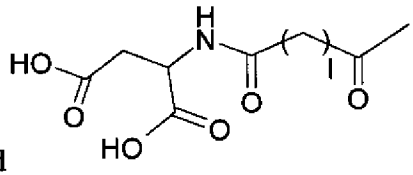
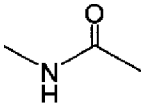

and


where l is 12, 13, 14, 15, 16, 17, 18, 19 or 20;

8. (Currently Amended) A GLP-1 analog according to claim ~~6~~ 7, wherein U' is selected from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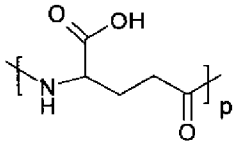
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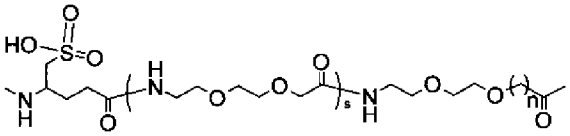

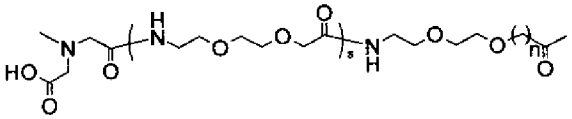

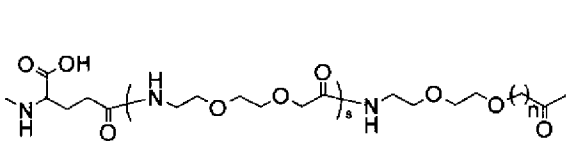
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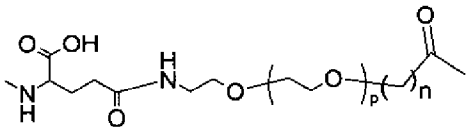

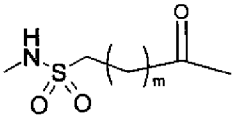
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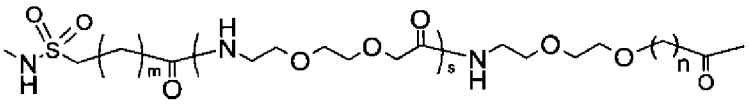
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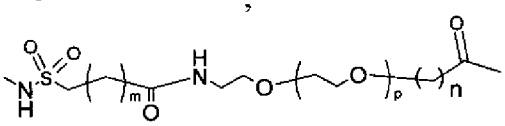

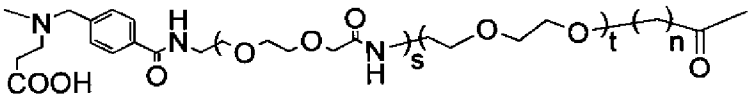
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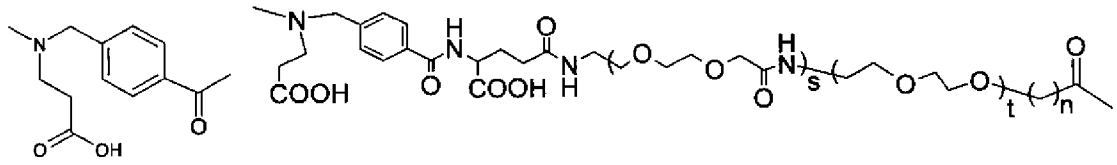


m is 2, 3, 4 or 5,

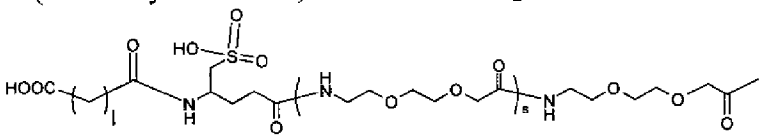
n is 1 or 2

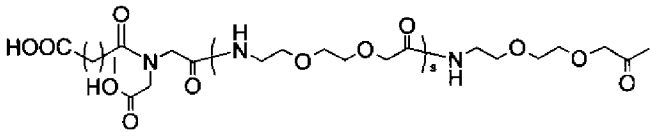
s is 0, 1, or 2,

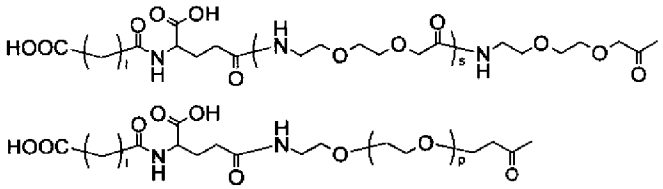
t is 0, 1, 2, or 3

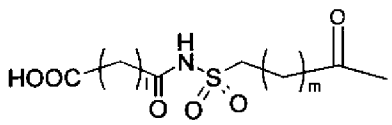
p is 1, 2, 3, 4, 7, 11 or 23

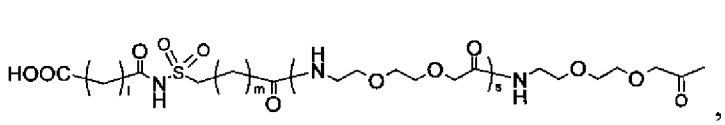
9. (Currently Amended) A GLP-1 analog according to claim ~~6~~ 8, wherein B-U'- is

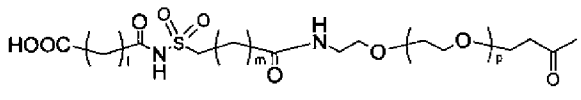


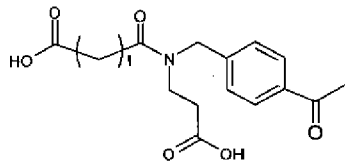


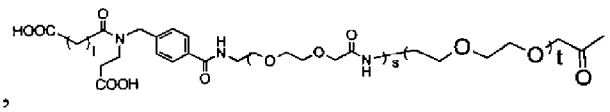


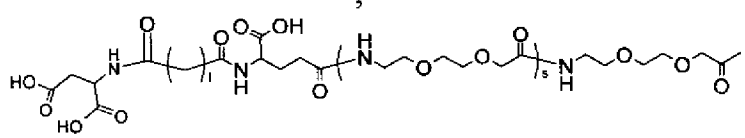












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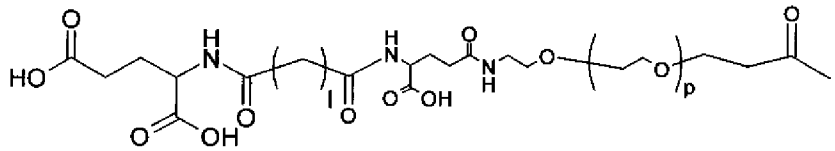

where l is 14, 15, 16, 17, 18, 19 or 20;

p is 1, 2, 3, 4, 7, 8 , 9, 10, 11 or 12.

s is 0, 1 or 2

t is 0 or 1;

m is 2, 3 or 4;

10. (Original) A GLP-1 analog according to claim 9, wherein

where l is 14, 15, 16, 17 or 18

p is 1, 2, 3, 4 or 11;

s is 0, 1 or 2;

t is 0 or 1;

11. (Currently Amended) A GLP-1 analog according to claim ~~6~~ 10, wherein s is 1.

12. (Currently Amended) A GLP-1 analog according to claim ~~6~~ 11, wherein l is 16

13-14 (Cancelled)

15. (Currently Amended) A GLP-1 analog according to claim ~~7~~ 12, wherein

Xaa_7 is His or desamino-histidine;

Xaa_8 is Ala, Gly, Val, Leu, Ile, Lys or Aib;

Xaa_{16} is Val;

Xaa_{18} is Ser;

Xaa_{19} is Tyr;

Xaa_{20} is Leu;

Xaa_{22} is Gly, Glu or Aib;

Xaa_{23} is Gln or Glu;

Xaa_{25} is Ala;

Xaa_{27} is Glu;

Xaa_{30} is Ala or Glu;

Xaa_{33} is Val;

Xaa_{34} is Lys or Arg;

Xaa_{35} is Gly or Aib;

Xaa_{36} is Arg or Lys

Xaa_{37} is Gly, amide or is absent;

16. (Original) A GLP-1 analog according to claim 15, wherein

Xaa_{7} is His

Xaa_{8} is Gly, or Aib;

Xaa_{16} is Val;

Xaa_{18} is Ser;

Xaa_{19} is Tyr;

Xaa_{20} is Leu;

Xaa_{22} is Glu or Aib;

Xaa_{23} is Gln;

Xaa_{25} is Ala;

Xaa_{27} is Glu;

Xaa_{30} is Ala;

Xaa_{33} is Val;

Xaa_{34} is Lys or Arg;

Xaa_{35} is Gly or Aib;

Xaa_{36} is Arg

Xaa_{37} is Gly

17. (Cancelled))

DRAFT

18. (Currently Amended) A GLP-1 analog according to claim ~~17~~ 7, wherein said GLP-1 analog comprises imidazopropionyl^{7}, α-hydroxy-histidine^{7} or N-methyl-histidine^{7}, D-histidine^{7}, desamino-histidine^{7}, 2-amino-histidine^{7}, β-hydroxy-histidine^{7}, homohistidine^{7}, N^{α}-acetyl-histidine^{7}, α-fluoromethyl-histidine^{7}, α-methyl-histidine^{7}, 3-pyridylalanine^{7}, 2-pyridylalanine^{7} or 4-pyridylalanine^{7}.

19. (Cancelled)

20. (Currently Amended) A GLP-1 analog according to claim ~~19~~ 7, wherein said GLP-1 analog comprises Aib^{8}, Gly^{8}, Val^{8}, Ile^{8}, Leu^{8}, Ser^{8}, Thr^{8}, (1-aminocyclopropyl) carboxylic acid, (1-aminocyclobutyl) carboxylic acid, (1-aminocyclopentyl) carboxylic acid, (1-aminocyclohexyl) carboxylic acid, (1-aminocycloheptyl) carboxylic acid, or (1-aminocyclooctyl) carboxylic acid.

21. (Original) A GLP-1 analog according to claim 20, wherein said GLP-1 analog comprises Aib^{8};

22-25. (Cancelled)

26. (Currently Amended) A GLP-1 analog according to claim ~~1~~ 7, wherein said GLP-1 analog comprises only one lysine residue.

27. (Currently Amended) A GLP-1 analog according to claim ~~1~~ 7, which is

Aib^{8},Arg^{34}-GLP-1(7-37)

Aib^{8,22},Arg^{34}-GLP-1(7-37).

Arg^{34}-GLP-1(7-37).

[3-(4-Imidazolyl)Propionyl7,Arg34]GLP-1-(7-37)~~peptide~~

Gly^{8},Arg^{34}-GLP-1(7-37) or

Aib^{8},Arg^{34}, Pro^{37}-GLP-1(7-37)

~~Aib^{8,22,27,30,35},Arg^{34},Pro^{37}-GLP-1(7-37)amide,~~

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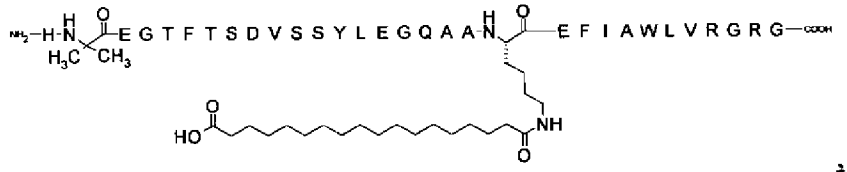
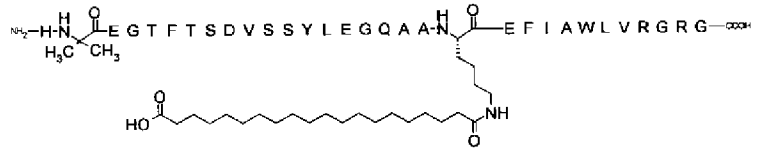
~~all of which are substituted by B-U' in position 26~~.

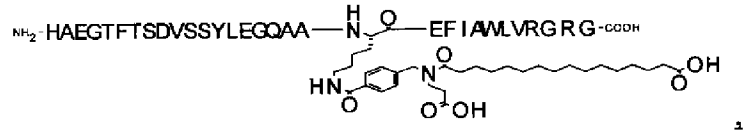
28. (Currently Amended) A compound according to claim ~~1~~ 7, which is selected from



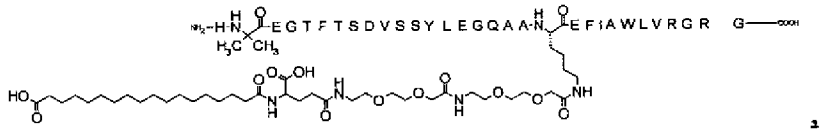
N-ε^{26}--(17-carboxyheptadecanoyl)-[Aib8,Arg34]GLP-1-(7-37)-peptide,



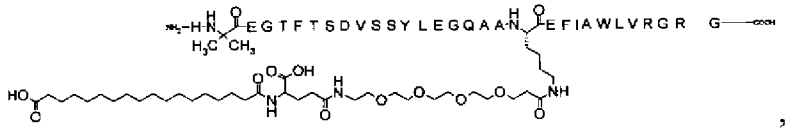
N-ε^{26}-(19-carboxynonadecanoyl)-[Aib8,Arg34]GLP-1-(7-37)-peptide,

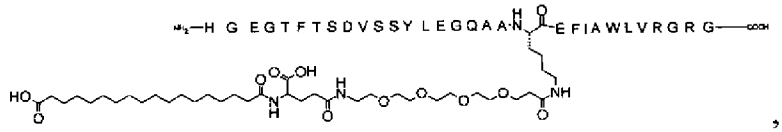


N-ε^{26}- (4-{[N-(2-carboxyethyl)-N-(15-carboxypentadecanoyl)amino]methyl}benzoyl)[Arg34]GLP-1-(7-37),

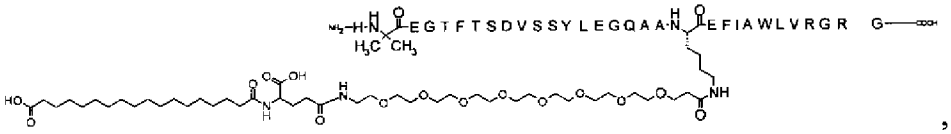


N-ε^{26}-[2-(2-[2-(2-[2-(2-[4-(17-Carboxyheptadecanoylamino)-4(S)-carboxybutyrylamino]ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][Aib8,Arg34]GLP-1-(7-37)peptide,





DRAFT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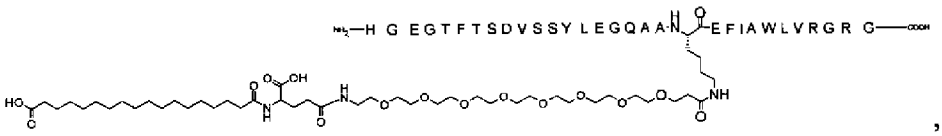
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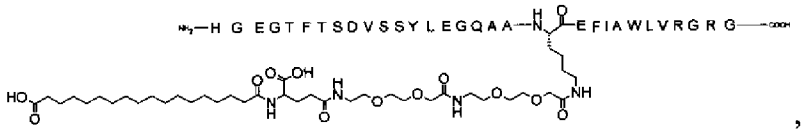
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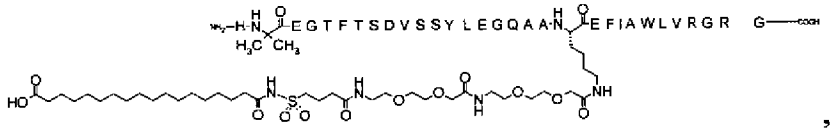
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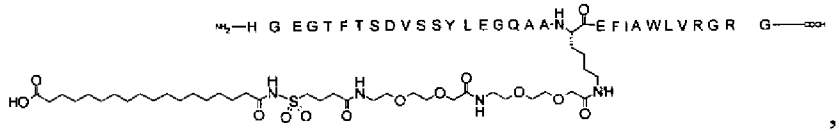
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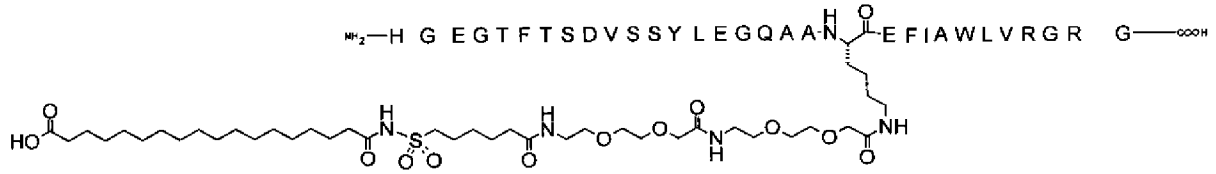
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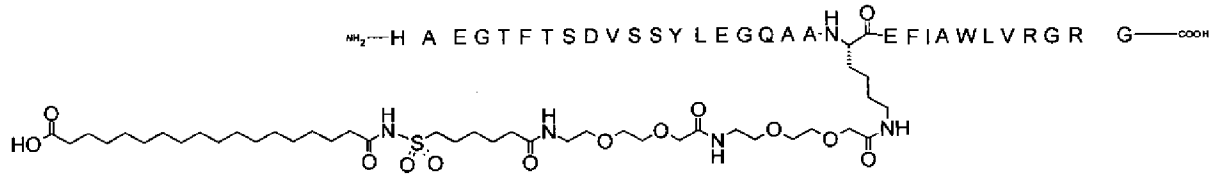
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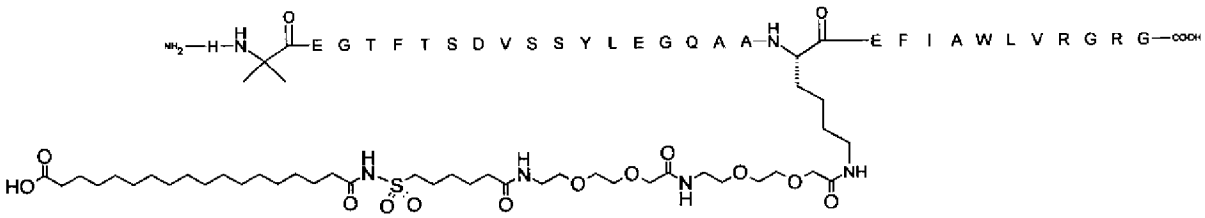
,

DRAFT



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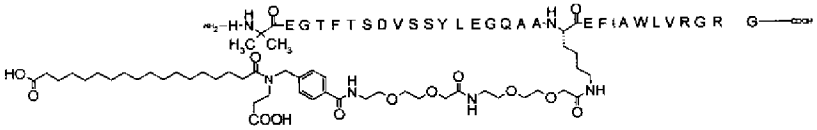

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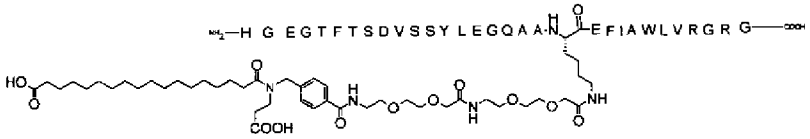

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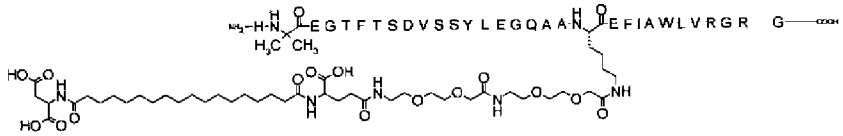

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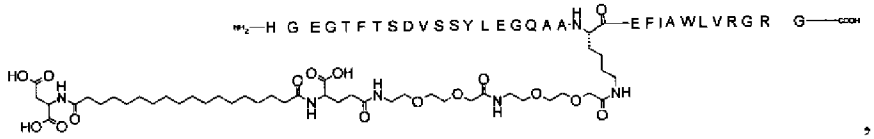

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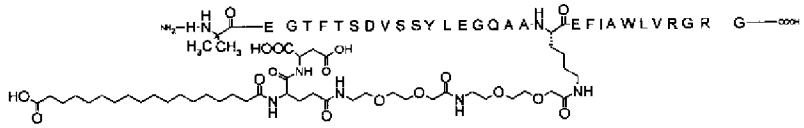

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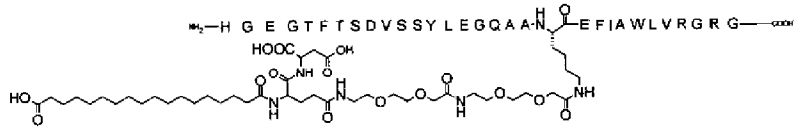

29-30 (Cancelled)

31. (Currently Amended) A pharmaceutical composition comprising a compound according to claim ~~1~~ 7, and a pharmaceutically acceptable excipient.

DRAFT

32. (Original) The pharmaceutical composition according to claim 31, which is suited for parenteral administration.

33-36 (Cancelled)

37. (Currently Amended) A method for treating ~~hyperglycemia,~~ type 2 diabetes~~, impaired glucose tolerance, type 1 diabetes, obesity, hypertension, syndrome X, dyslipidemia, cognitive disorders, atheroschlerosis, myocardial infarction, coronary heart disease and other cardiovascular disorders, stroke, inflammatory bowel syndrome, dyspepsia and/or gastric ulcers~~ in a subject, said method comprising administering to a subject in need of such treatment an effective amount of a GLP-1 analog according to claim ~~1~~ 7.

38-39 (Cancelled)

40. (New) A pharmaceutical composition comprising a compound according to claim 28, and a pharmaceutically acceptable excipient.

41. (New) The pharmaceutical composition according to claim 40, which is suited for parenteral administration.

42. (New) A method for treating type 2 diabetes in a subject, said method comprising administering to a subject in need of such treatment an effective amount of a GLP-1 analog according to claim 28.

DRAFT

REMARKS

Claims 7-12, 15-16, 18, 20-21, 26-28, 31-32, 37 and 40-42 are pending following entry of this Amendment. The present amendment adds no new matter.

Applicants note that these amendments have not been made to overcome prior art and thus should be considered to have been made for a purpose unrelated to patentability, and no estoppel should be deemed to attach thereto. Moreover, any canceled subject matter is canceled without prejudice or disclaimer and Applicants reserve the right to pursue the canceled subject matter in future continuation applications. Thus, the cancelled subject matter has not been dedicated to the public.

It is believed that the claims are in condition for allowance, and a determination to that effect is earnestly solicited.

Respectfully submitted,

Date: March ___2010

Richard W. Bork, Reg. No. 36,459
Novo Nordisk Inc.
Customer Number 23650
(609) 987-5800

Use the following customer number for all correspondence regarding this application.

23650

PATENT TRADEMARK OFFICE

STATEMENT UNDER 37 CFR 3.73(b)

ATTORNEY'S DOCKET NUMBER: 7140.204-US

APPLICANT/PATENT OWNER: Lau et al.

U.S. APPLICATION NO. 11/908,834 FILED/ISSUE DATE: September 17, 2007

TITLED: Acylated GLP-1 Compounds

NAME OF ASSIGNEE: NOVO NORDISK A/S, a CORPORATION
States that it is:

1. ☒ the assignee of the entire right, title and interest in:

2. ☐ an assignee of less than the entire right, title, and interest in
(The extent (by percentage) of its ownership interest is ___%); or

3. ☐ the assignee of an undivided interest in the entirety of (a complete assignment from one of the joint inventors was made) the patent application/patent identified above, by virtue of either:

A. ☒ 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 020070, Frame 0420, or for which a copy therefore is attached

OR

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 therefore is attached.

2. From: ___ To: ___
The document was recorded in the United States Patent and Trademark Office at Reel___, Frame ___, or for which a copy therefore is attached.

3. From: ___. To: ___
The document was recorded in the United States Patent and Trademark Office at Reel___, Frame ___, or for which a copy therefore is attached.

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

☐ As required by 37CFR 3.73(b)(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.

Signature	Date
Richard W. Bork	7/1/09
Printed or Typed Name	**Title**
Richard W. Bork, Reg. No. 36,459	Corporate Counsel IP

STATEMENT UNDER 37 CFR 3.73(b)

ATTORNEY'S DOCKET NUMBER: 7140.204-US

APPLICANT/PATENT OWNER: Lau et al.

U.S. **APPLICATION** NO. 111908,834 **FILED/ISSUE** DATE: September 17,2007

TITLED: Acylated GLP-1 Compounds

NAME OF ASSIGNEE: **NOVO NORDISK A/S,** a CORPORATION
States that it is:

1. ☐ the assignee of the entire right, title and interest in:

2. ☐ an assignee of less than the entire right, title, and interest in
(The extent (by percentage) of its ownership interest is %) ; or

3. ☐ the assignee of an undivided interest in the entirety of (a complete assignment from one of the joint inventors was made) the patent applicationlpatent identified above, by virtue of either:

A. ☐ An assignment from the **inventor(s)** of the patent applicationlpatent identified above. The assignment was recorded in the United States Patent and Trademark **Office** at Reel 020070 , Frame ___, or for which a copy therefore is attached

OR

B. ☐ A chain of title from the **inventor(s),** of the patent applicationlpatent 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 therefore is attached.

2. From: ___ To: ___
The document was recorded in the United States Patent and Trademark Office at **Reel___,** Frame ___, or for which a copy therefore is attached.

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

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

☐ As required by **37CFR 3.73(b)(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 302.081

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

Signature	Date
Richard W. Bork	7/1/09

Printed or Typed Name	Title
Richard W. Bork, Reg. No. 36,459	Corporate Counsel IP

Declaration for Utility or Design Patent Application (37 CFR 1.63)

[ ] **Declaration Submitted with Initial Filing or** [X] **Declaration Submitted after Initial Filing** (surcharge (37 CFR 1.16(e)) required)

DECLARATION FOR PATENT APPLICATION

Attorney Docket Number: **7140.204-US**

As a below named inventor, I hereby declare that:

My residence and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Acylated GLP-1 Compounds

The specification of which (check only one item below):
[ ] is attached hereto
[X] was filed as United States application

Application No. **11/908,834**

on **September 17, 2007**
and was amended
on **September 17, 2007**

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

PRIORITY CLAIMS
Pursuant to 37 CFR 1.63(c), Applicants hereby list any priority claims on an Application Data Sheet submitted herewith in accordance with 37 CFR 1.76.

Send Correspondence to: Customer Number 23650

Direct Telephone Calls To: Intellectual Property Department: (609) 987-5800

1 **Full Name of Inventor** (Last Name First):
LAU, Jesper
Country of Citizenship:
Denmark

2 **Full Name of Inventor** (Last Name First):
DÖRWALD, Florencio Zaragoza
Country of Citizenship:
Germany

3 **Full Name of Inventor** (Last Name First):
STEPHENSEN, Henrik
Country of Citizenship:
Denmark

Declaration	Attorney Docket No.: 7140.204-US

4	**Full Name of Inventor** (Last Name First): **BLOCH, Paw**
Country of Citizenship: **Denmark**	

5	**Full Name of Inventor** (Last Name First): **HANSEN, Thomas Kruse**
Country of Citizenship: **Denmark**	

6	**Full Name of Inventor** (Last Name First): **MADSEN, Kjeld**
Country of Citizenship: **Denmark**	

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signature of Inventor 1	Signature of Inventor 2	Signature of Inventor 3
Jesper [illegible]		Henrik Stephansen
Date 4/9 - 2008	Date	Date 4/9 - 2008
Signature of Inventor 4	**Signature of Inventor 5**	**Signature of Inventor 6**
Paw Bloch		
Date 4/9 - 2008	Date	Date

Declaration	Attorney Docket No.: 7140.204-US

4	**Full Name of Inventor** (Last Name First): **BLOCH, Paw**
Country of Citizenship: **Denmark**	

5	**Full Name of Inventor** (Last Name First): **HANSEN, Thomas Kruse**
Country of Citizenship: **Denmark**	

6	**Full Name of Inventor** (Last Name First): **MADSEN, Kjeld**
Country of Citizenship: **Denmark**	

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Signature of Inventor 1	Signature of Inventor 2	Signature of Inventor 3
	F. Zornoza	
Date	Date 5 Sep 2008	Date
Signature of Inventor 4	Signature of Inventor 5	Signature of Inventor 6
Date	Date	Date

Declaration	Attorney Docket No.: 7140.204-US

4	**Full Name of Inventor** (Last Name First): **BLOCH, Paw**
Country of Citizenship: **Denmark**	

5	**Full Name of Inventor** (Last Name First): **HANSEN, Thomas Kruse**
Country of Citizenship: **Denmark**	

6	**Full Name of Inventor** (Last Name First): **MADSEN, Kjeld**
Country of Citizenship: **Denmark**	

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Signature of Inventor 1	Signature of Inventor 2	Signature of Inventor 3
Date	Date	Date
Signature of Inventor 4	Signature of Inventor 5	Signature of Inventor 6
Date	Date 09/09/2008	Date

Declaration	Attorney Docket No.: 7140.204-US

4	**Full Name of Inventor** (Last Name First): **BLOCH, Paw**
Country of Citizenship: **Denmark**	

5	**Full Name of Inventor** (Last Name First): **HANSEN, Thomas Kruse**
Country of Citizenship: **Denmark**	

6	**Full Name of Inventor** (Last Name First): **MADSEN, Kjeld**
Country of Citizenship: **Denmark**	

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Signature of Inventor 1	Signature of Inventor 2	Signature of Inventor 3
Date	Date	Date
Signature of Inventor 4	Signature of Inventor 5	Signature of Inventor 6
Date	Date	Date 08-SEP-2008

INFORMATION DISCLOSURE STATEMENT BY APPLICANT					
				Application No.	11/908,834
				Filing Date	September 17, 2007
				Applicant	Lau et al.
				Art Unit	TBD
				Examiner Name:	TBD
Sheet	1	of	2	Atty. Docket No.	7140.204-US

U.S. PATENT DOCUMENTS

EXAMINER INITIALS	Cite No.	DOCUMENT NUMBER Number –Kind Code(if known)	Issue/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.	DOCUMENT NUMBER Number –Kind Code(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
		WO 9629342				
		WO9808871				
		WO9943708				
		WO0034331				
		WO0069911				
		WO0246227				
		WO2005014049				
		WO2005027978				

EXAMINER SIGNATURE		DATE CONSIDERED	

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application No.	11/908,834
				Filing Date	September 17, 2007
				Applicant	Lau et al.
				Art Unit	TBD
				Examiner Name:	TBD
Sheet	2	of	2	Atty. Docket No.	7140.204-US

NON PATENT LITERATURE DOCUMENTS

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
		KNUDSEN, L.B. et al., Journal of Medicinal Chemistry, Vol. 43, pp. 1664-9 (2000)	
		KNUDSEN, L.B. et al., Journal of Medicinal Chemistry, Vol. 47, pp. 4128-34 (2004)	
		DEACON, C.F. et al., Diabetologia, Vol. 41, pp. 271-8 (1998)	

EXAMINER SIGNATURE		DATE CONSIDERED	

7140.204-US

Declaration for Utility or Design Patent Application (37 CFR 1.63)

[ ] **Declaration Submitted with Initial Filing or** [X] **Declaration Submitted after Initial Filing** (surcharge (37 CFR 1.16(e)) required)

DECLARATION FOR PATENT APPLICATION

Attorney Docket Number: **7140.204-US**

As a below named inventor, I hereby declare that:

My residence and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Acylated GLP-1 Compounds

The specification of which (check only one item below):
[ ] is attached hereto
[X] was filed as United States application

Application No. **11/908,834**

on **September 17, 2007**
and was amended
on **September 17, 2007**

[ ] was filed as PCT international application
Number
on
and was amended under PCT Article 19
on

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability of this application in accordance with Title 37, Code of Federal Regulations, §1.56.

Send Correspondence to: Customer Number 23650

Direct Telephone Calls To: Intellectual Property Department: (609) 987-5800

1 **Full Name of Inventor** (Last Name First):
Lau, Jesper

Country of Citizenship:
Denmark

2 **Full Name of Inventor** (Last Name First):
Dorwald, Florencio Zaragoza

Country of Citizenship:
Germany

3 **Full Name of Inventor** (Last Name First):
Stephensen, Henrik

Country of Citizenship:
Denmark

MPI EXHIBIT 1004 PAGE 127

Declaration	Attorney Docket No.: 7140.204-US

4	**Full Name of Inventor** (Last Name First): **Bloch, Paw**
Country of Citizenship: **Denmark**	

5	**Full Name of Inventor** (Last Name First): **Hansen, Thomas Kruse**
Country of Citizenship: **Denmark**	

6	**Full Name of Inventor** (Last Name First): **Madsen, Kjeld**
Country of Citizenship: **Denmark**	

7	**Full Name of Inventor** (Last Name First):
Country of Citizenship:	

8	**Full Name of Inventor** (Last Name First):
Country of Citizenship:	

9	**Full Name of Inventor** (Last Name First):
Country of Citizenship:	

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signature of Inventor 1	Signature of Inventor 2	Signature of Inventor 3
Jespe [illegible]	F. Zarragozan	Henrik Stephen
Date 9 oct 2007	Date 12. Oct. 2007	Date 9. oct 2007
Signature of Inventor 4	**Signature of Inventor 5**	**Signature of Inventor 6**
Paw Bloch	[illegible]	[illegible]
Date 9. OKT. 2007	Date 9-oct-2007	Date 12-OKT-2007
Signature of Inventor 7	**Signature of Inventor 8**	**Signature of Inventor 9**
Date	Date	Date

Electronic Acknowledgement Receipt

EFS ID:	2393551
Application Number:	11908834
International Application Number:	
Confirmation Number:	8500
Title of Invention:	Acylated GLP-1 Compounds
First Named Inventor/Applicant Name:	Jesper Lau
Customer Number:	23650
Filer:	Richard W. Bork/Csaba Attila Szakolczai
Filer Authorized By:	Richard W. Bork
Attorney Docket Number:	7140.204-US
Receipt Date:	30-OCT-2007
Filing Date:	
Time Stamp:	15:04:10
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	yes
Payment was successfully received in RAM	$130
RAM confirmation Number	492
Deposit Account	141447
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 1.16 and 1.17	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes) /Message Digest	Multi Part /.zip	Pages (if appl.)
1	Miscellaneous Incoming Letter	DecTrans_7140204US.pdf	70624 c8caf525cb11351796f25ad48831623c2eda3d7b	no	1
Warnings:					
Information:					
2	Oath or Declaration filed	Dec_7140204US.pdf	39283 de52587b60a23707a41cc24bc6fcdb8e8565d99e	no	2
Warnings:					
Information:					
3	Fee Worksheet (PTO-06)	fee-info.pdf	8167 6f2f2d75d19b0b8856e5074430d6cfd9ee543ec6	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	118074		

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.

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A SUBMISSION UNDER 35 U.S.C. 371		ATTORNEY'S DOCKET NUMBER 7140.204-US U.S. APPLICATION NO. (If known, see 37 CFR 1.5) TBD
INTERNATIONAL APPLICATION NO. PCT/EP2006/060855	INTERNATIONAL FILING DATE March 20, 2006	PRIORITY DATE CLAIMED March 18, 2005
TITLE OF INVENTION Acylated GLP-1 Compounds		
APPLICANT(S) FOR DO/EO/US Lau et al.		

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a submission under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a submission under 35 U.S.C. 371
3. [ ] This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9), and (21) indicated below.
4. [ ] The US has been elected (Article 31).
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   - a. [ ] is attached hereto (required only if not communicated by the International Bureau).
   - b. [X] has been communicated by the International Bureau.
   - c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [ ] An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   - a. [ ] is attached hereto
   - b. [ ] has been previously submitted under 35 U.S.C. 154(d)(4).
7. [ ] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   - a. [ ] are attached hereto (required only if not communicated by the International Bureau).
   - b. [ ] have been communicated by the International Bureau
   - c. [ ] have not been made; however, the time limit for making such amendments has NOT expired
   - d. [ ] have not been made and will not be made
8. [ ] An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3))
9. [ ] An oath or declaration of the inventor(s) (35 U.S.C. (c)(4))
10. [ ] An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 11 to 20 below concern document(s) or information included:

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [X] A preliminary amendment.
14. [X] An Application Data Sheet under 37 CFR 1.76.
15. [ ] A substitute specification.
16. [ ] A power of attorney and/or change address letter.
17. [ ] A computer-readable form of the sequence listing in accordance with PCT Rule 13*ter*. 2 and 37 CFR 1.821-1.825
18. [ ] A second copy of the published International Application under 35 U.S.C. 154(d)(4)
19. [ ] A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).

MPI EXHIBIT 1004 PAGE 131

U.S. APPLICATION NO. (if known, see 37 CFR 1.5) TBD	INTERNATIONAL APPLICATION NO. PCT/EP2006/060855	ATTORNEY'S DOCKET NUMBER 7140.204-US

20. Other items or information:

The following fees have been submitted	CALCULATIONS	PTO USE ONLY
21. [X] Basic national fee (37 CFR 1.492(a))……**$300**	$300.00	
22 . [X] Examination fee (37 CFR 1.492(c)) If the written opinion prepared by ISA/US or the international preliminary examination report prepared by IPEA/US indicates all claims satisfy provisions of PCT Article 33	$200.00	
23. [X] Search fee (37 CFR 1.492(b)) If written opinion of the ISA/US or the International preliminary examination report prepared by IPEA/US indicates all claims satisfy provisions of PCT Article 33(1)-(4)……$0 Search fee (37 CFR 1.445(a)(2)) has been paid on the international application to the USPTO as an International Searching Authority……$100 International Search Report prepared by an ISA other than the US and provided to the Office or previously communicated to the US by the IB……$400 All other situations……$500	$400.00	
TOTAL OF 21, 22, and 23 =	$900.00	

[ ] Additional fee for specification and drawings filed in paper over 100 sheets (excluding sequence listing in compliance with 37 CFR 1.821(c) or (e) or computer program listing in an electronic medium) (37 CFR 1.492(j)).
The fee is **$250** for each additional 50 sheets of paper or fraction thereof.

Total Sheets	Extra Sheets	Number of each additional 50 or fraction thereof (round **up** to a whole number)	RATE	
-100 =	/50 =		X **$250**	$

Surcharge of **$130.00** for furnishing any of the search fee, examination fee, or the oath or declaration after the date of commencement of the national stage (37 CFR 1.492(h)).	$	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	$	
Total claims	35- 20 =	15	**X $50**	$750.00	
Independent Claims	2- 3 =	0	X **$200**	$	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ **$360**	$	
			TOTAL OF ABOVE CALCULATIONS=	$1,650.00	
[ ] Applicant claims small entity status. See 37 CFR 1.27. Fees above are reduced by ½.					
			SUBTOTAL =	$1,650.00	
Processing fee of **$130.00** for furnishing of the English translation later than 30 months from the Earliest claimed priority date (37 CFR 1.492(i)). +				$	
			TOTAL NATIONAL FEE =	$1,650.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$	
			TOTAL FEES ENCLOSED =	$	
				Amount to be Refunded:	$
				Amount to be charged	$1,650.00

a. [ ] A check in the amount of $_____ to cover the above fees is enclosed.

b. [X] Please charge my Deposit Account No. 141447 in the amount of $1,650.00 to cover the above fees.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 141447.

d. [ ] Fees are to be charged to a credit. **WARNING**: Information on this form may become public. **Credit card information should not be included on this form**. Provide credit card information and the authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the International Application to pending status.

SEND ALL CORRESPONDENCE TO:

Customer Number 23650

/Richard W. Bork, Reg. No. 36,459/

SIGNATURE

Richard W. Bork

NAME

36,459

REGISTRATION NUMBER

Attorney Docket No.: 7140.204-US PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Lau et al.

Application No.: To be determined Group Art Unit: To be determined
(National Stage application of PCT/EP2006/060855)

Filed: September 17, 2007 Examiner: To be determined
(International Filing Date: March 20, 2006)

For: Acylated GLP-1 Compounds

PRELIMINARY AMENDMENT

MS: PCT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Prior to examination of the above-identified application on the merits, kindly amend the above-captioned patent application as set forth below.

AMENDMENTS TO THE SPECIFICATION begin on page 2 of this paper.

AMENDMENTS TO THE CLAIMS begin on page 3 of this paper.

REMARKS begin on page 17 of this paper.

AMENDMENTS TO THE SPECIFICATION

Kindly amend the specification as follows:

At page 1, after the Title, insert:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a 35 U.S.C. § 371 national stage application of International Patent Application PCT/EP2006/060855 (published as WO 2006/097537), filed March 20, 2006, which claimed priority of European Patent Application 05102171.5, filed March 18, 2005; this application further claims priority under 35 U.S.C. § 119 of U.S. Provisional Application 60/664,497, filed March 23, 2005.

AMENDMENTS TO THE CLAIMS

CLAIM LISTING

1. (Currently Amended) A GLP-1 analog having a modification of at least one non-proteogenic amino acid residue in positions 7 and/or 8 relative to the sequence GLP-1(7-37) (SEQ ID No 1), ~~which~~ where said analog is acylated with a moiety to the lysine residue in position 26, and where said moiety comprises at least two acidic groups, wherein one acidic group is attached terminally.

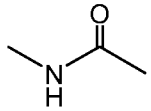
2. (Original) A GLP-1 analog according to claim 1, wherein the moiety attached in position 26 comprises a hydrophilic linker.

3. (Currently Amended) A GLP-1 analog according to claim 2, wherein the hydrophilic linker comprises at least 5 non-hydrogen atoms where 30-50% of ~~these~~ said non-hydrogen atoms are either N or O.

4. (Currently Amended) A GLP-1 analog according to ~~any of the above claims~~ claim 1, wherein the moiety attached in position 26 comprises an albumin binding moiety seperated from the peptide by the hydrophilic linker.

5. (Original) A GLP-1 analog according to claim 4 wherein the albumin binding moiety is a linear or branched lipophilic moiety containing 4-40 carbon atoms having a distal acidic group.

6. (Currently Amended) A GLP-1 analog according to ~~any of the above claims~~ claim 1, wherein the acylated moiety is B-U',
where U' is selected from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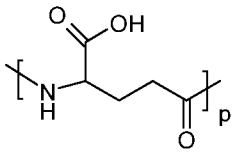
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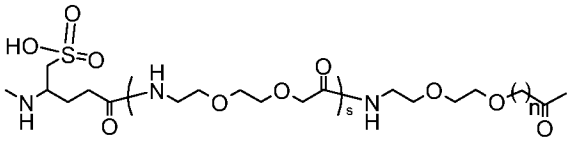

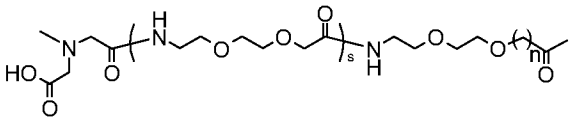

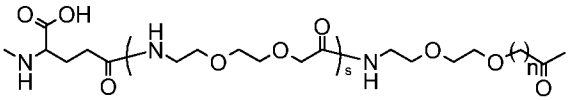
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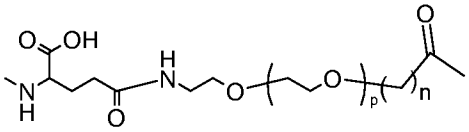

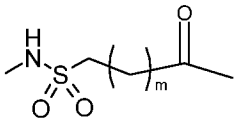
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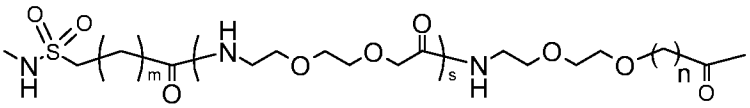
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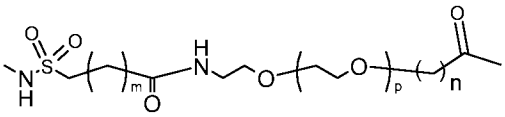

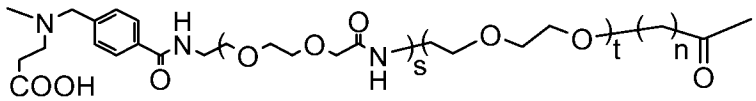
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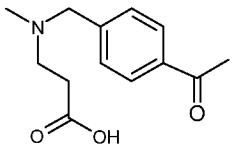

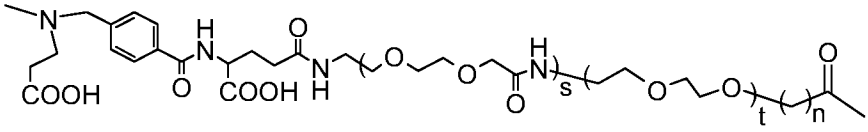
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m is 0, 1, 2, 3, 4, 5, or 6,

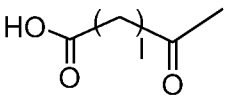
n is 1, 2 or 3

s is 0, 1, 2, or 3,

t is 0, 1, 2, 3, or 4

p is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17,18, 19, 20, 21, 22, or 23;

and where B is an acidic group selected from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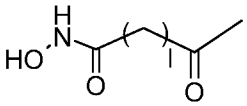
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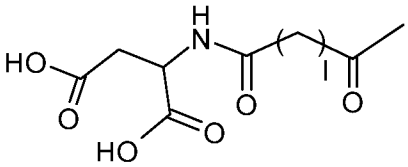
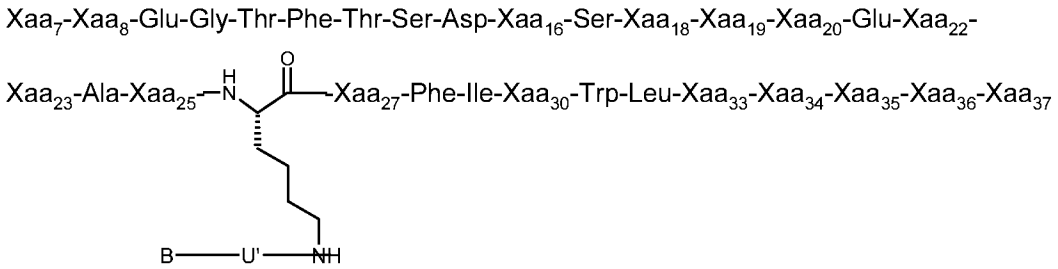


where l is 12, 13, 14, 15, 16, 17, 18, 19 or 20

7. (Original) A GLP-1 analog which is a compound of formula I (SEQ ID No. 2):



Formula I

wherein

Xaa_7 is L-histidine, imidazopropionyl, α-hydroxy-histidine, D-histidine, desamino-histidine, 2-amino-histidine, β-hydroxy-histidine, homohistidine, N^{α}-acetyl-histidine, N^{α}-formyl-histidine, α-fluoromethyl-histidine, α-methyl-histidine, 3-pyridylalanine, 2-pyridylalanine or 4-pyridylalanine

Xaa_8 is Ala, Gly, Val, Leu, Ile, Thr, Ser, Lys, Aib, (1-aminocyclopropyl) carboxylic acid, (1-aminocyclobutyl) carboxylic acid, (1-aminocyclopentyl) carboxylic acid, (1-aminocyclohexyl) carboxylic acid, (1-aminocycloheptyl) carboxylic acid, or (1-aminocyclooctyl) carboxylic acid;

Xaa_{16} is Val or Leu;

Xaa_{18} is Ser, Lys or Arg;

Xaa_{19} is Tyr or Gln;

Xaa_{20} is Leu or Met;

Xaa_{22} is Gly, Glu or Aib;

Xaa_{23} is Gln, Glu, Lys or Arg;

Xaa_{25} is Ala or Val;

Xaa_{27} is Glu or Leu;

Xaa_{30} is Ala, Glu or Arg;

Xaa_{33} is Val or Lys;

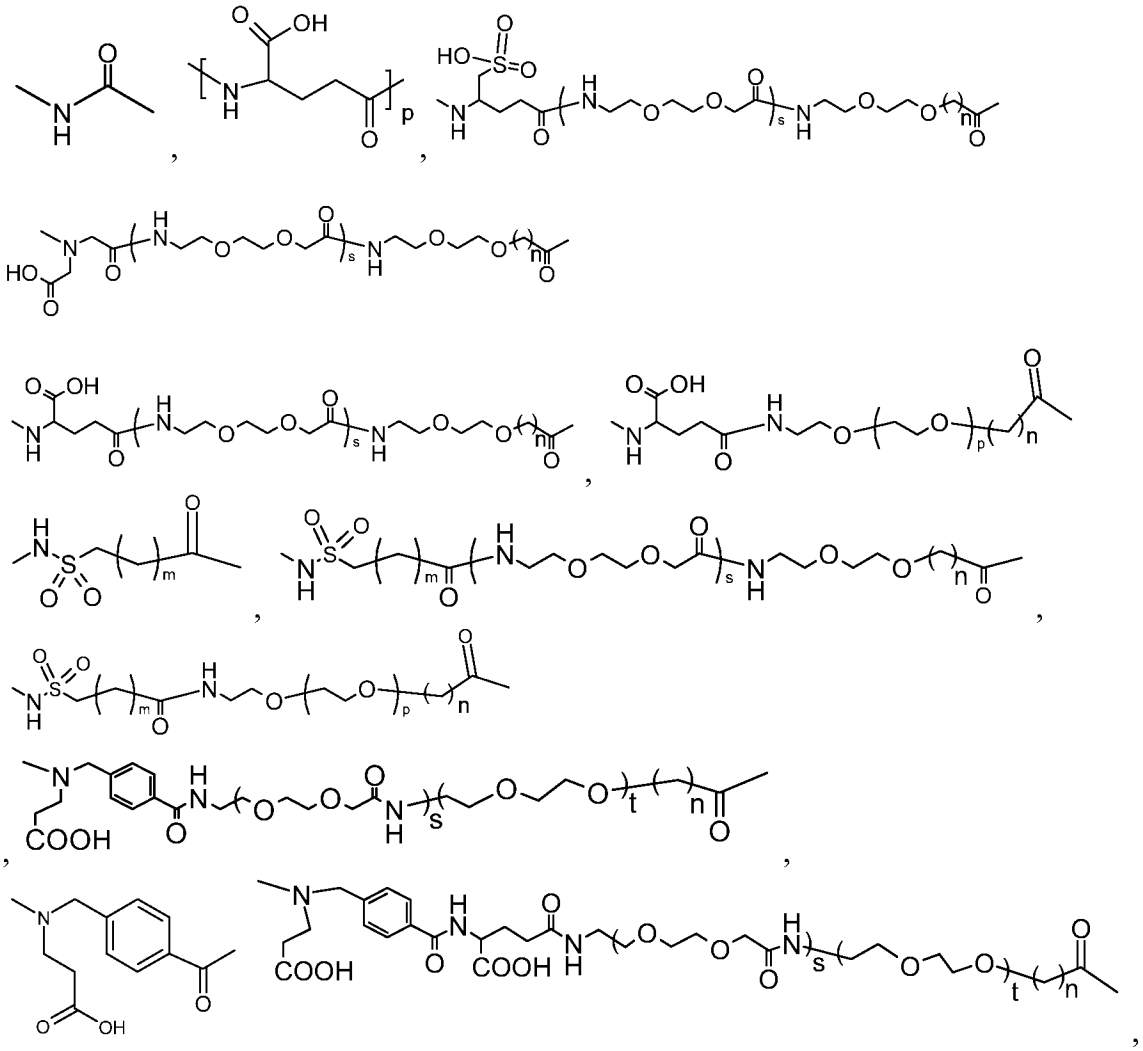
Xaa_{34} is Lys, Glu, Asn or Arg;

Xaa_{35} is Gly or Aib;

Xaa_{36} is Arg, Gly or Lys, or is absent;

Xaa_{37} is Gly, Ala, Glu, Pro, Lys, or is absent;

and B and U' together is the acylated moiety, where U' is selected from



m is 0, 1, 2, 3, 4, 5, or 6,

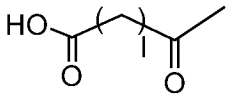
n is 1, 2 or 3

s is 0, 1, 2, or 3,

t is 0, 1, 2, 3, or 4

p is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17,18, 19, 20, 21, 22, or 23;

and where B is an acidic group selected from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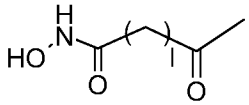
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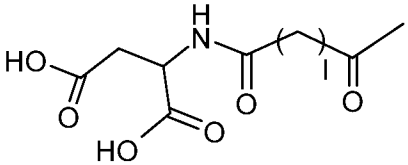
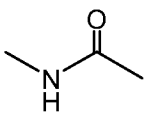


where l is 12, 13, 14, 15, 16, 17, 18, 19 or 20;

8. (Currently Amended) A GLP-1 analog according to ~~claims 6-7~~ claim 6, wherein U' is selected from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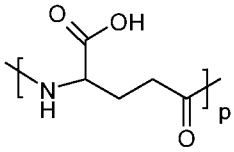
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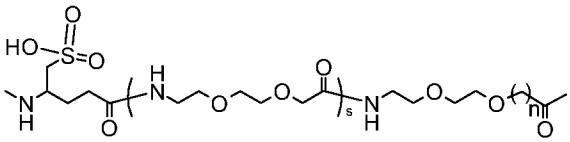

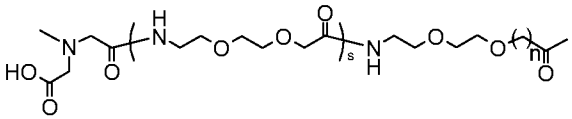

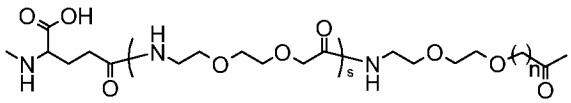
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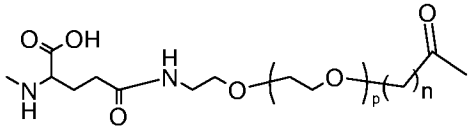

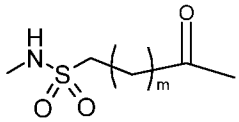
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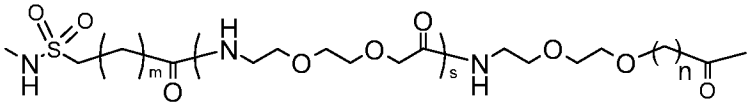
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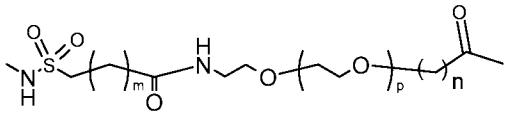

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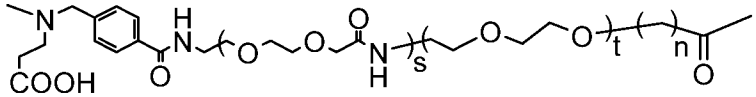
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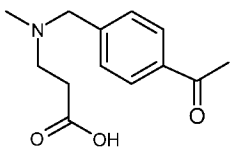
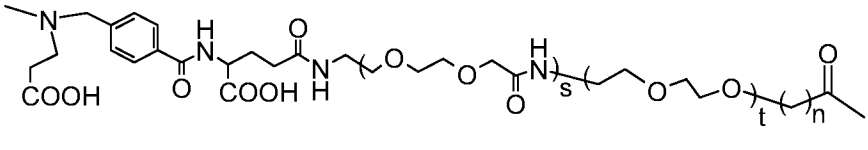


,

m is 2, 3, 4 or 5,

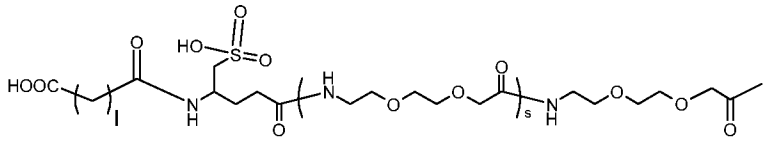
n is 1 or 2

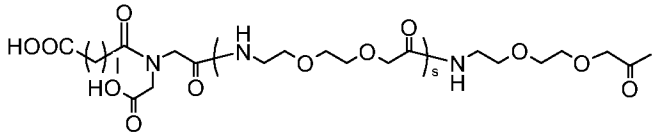
s is 0, 1, or 2,

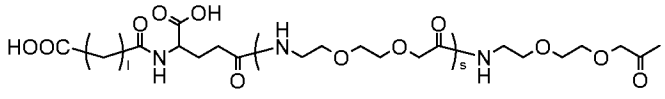
t is 0, 1, 2, or 3

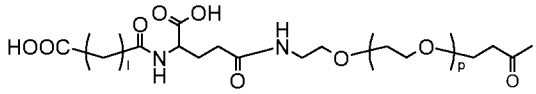
p is 1, 2, 3, 4, 7, 11 or 23

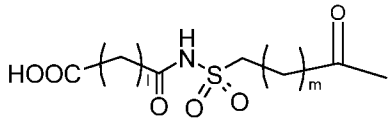
9. (Currently Amended) A GLP-1 analog according to ~~claims 6-8~~ claim 6, wherein B-U'- is

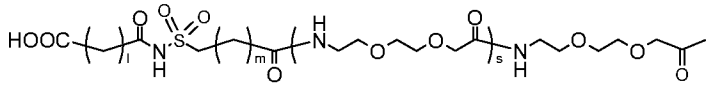


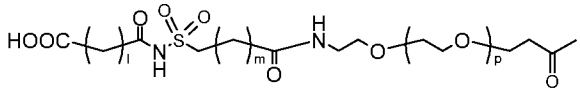


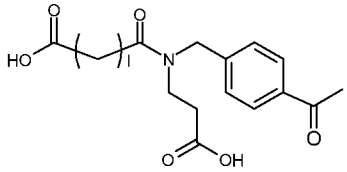


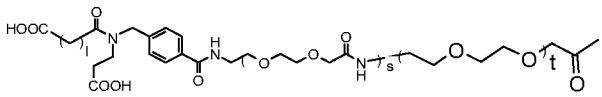


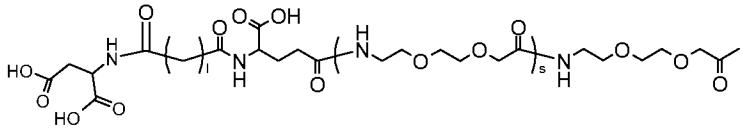


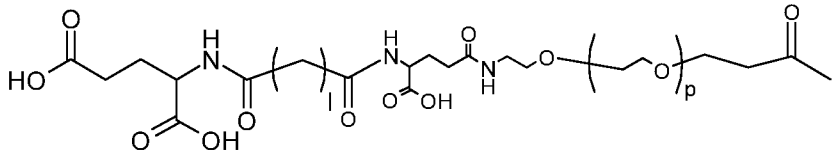












where l is 14, 15, 16, 17, 18, 19 or 20;

p is 1, 2, 3, 4, 7, 8 , 9, 10, 11 or 12.

s is 0, 1 or 2

t is 0 or 1;

m is 2, 3 or 4;

10. (Original) A GLP-1 analog according to claim 9, wherein

where l is 14, 15, 16, 17 or 18

p is 1, 2, 3, 4 or 11;

s is 0, 1 or 2;

t is 0 or 1;

11. (Currently Amended) A GLP-1 analog according to ~~any of the claims 6-10~~ claim 6, wherein s is 1.

12. (Currently Amended) A GLP-1 analog according to ~~any of the claims 6-10~~ claim 6, wherein l is 16

13. (Currently Amended) A GLP-1 analog according to ~~any of the claims 6-10~~ claim 6, wherein p is 3 or 4.

14. (Currently Amended) A GLP-1 analog according to ~~any of the claims 6-10~~ claim 6, wherein n is 1.

15. (Currently Amended) A GLP-1 analog according to ~~any of the claims 7-14~~ claim 7,

wherein

Xaa_7 is His or desamino-histidine;

Xaa_8 is Ala, Gly, Val, Leu, Ile, Lys or Aib;

Xaa_{16} is Val;

Xaa_{18} is Ser;

Xaa_{19} is Tyr;

Xaa_{20} is Leu;

Xaa_{22} is Gly, Glu or Aib;

Xaa_{23} is Gln or Glu;

Xaa_{25} is Ala;

Xaa_{27} is Glu;

Xaa_{30} is Ala or Glu;

Xaa_{33} is Val;

Xaa_{34} is Lys or Arg;

Xaa_{35} is Gly or Aib;

Xaa_{36} is Arg or Lys

Xaa_{37} is Gly, amide or is absent;

16. (Original) A GLP-1 analog according to claim 15, wherein

Xaa_7 is His

Xaa_8 is Gly, or Aib;

Xaa_{16} is Val;

Xaa_{18} is Ser;

Xaa_{19} is Tyr;

Xaa_{20} is Leu;

Xaa_{22} is Glu or Aib;

Xaa_{23} is Gln;

Xaa_{25} is Ala;

Xaa_{27} is Glu;

Xaa_{30} is Ala;

Xaa_{33} is Val;

Xaa_{34} is Lys or Arg;

Xaa_{35} is Gly or Aib;

Xaa_{36} is Arg

Xaa_{37} is Gly

17. (Currently Amended) A GLP-1 analog according to ~~any one of the claims 1-15~~ claim 1, wherein said GLP-1 analog comprises a modification of the N-terminal L-histidine in position 7 of the GLP-1(7-37) sequence.

18. (Original) A GLP-1 analog according to claim 17, wherein said GLP-1 analog comprises imidazopropionyl^7, α-hydroxy-histidine^7 or N-methyl-histidine^7, D-histidine^7, desamino-histidine^7, 2-amino-histidine^7, β-hydroxy-histidine^7, homohistidine^7, N^{α}-acetyl-histidine^7, α-fluoromethyl-histidine^7, α-methyl-histidine^7, 3-pyridylalanine^7, 2-pyridylalanine^7 or 4-pyridylalanine^7.

19. (Currently Amended) A GLP-1 analog according to ~~any one of the claims 1-18~~ claim 1, wherein said GLP-1 analog comprises a substitution of the L-alanine in position 8 of the GLP-1(7-37) sequence for another amino acid residue.

20. (Original) A GLP-1 analog according to claim 19, wherein said GLP-1 analog comprises Aib^8, Gly^8, Val^8, Ile^8, Leu^8, Ser^8, Thr^8, (1-aminocyclopropyl) carboxylic acid, (1-aminocyclobutyl) carboxylic acid, (1-aminocyclopentyl) carboxylic acid, (1-aminocyclohexyl) carboxylic acid, (1-aminocycloheptyl) carboxylic acid, or (1-aminocyclooctyl) carboxylic acid.

21. (Original) A GLP-1 analog according to claim 20, wherein said GLP-1 analog comprises Aib^8;

22. (Currently Amended) A GLP-1 analog according to ~~any one of the preceding claims~~ claim 1, wherein said GLP-1 analog comprises no more than fifteen amino acid residues which have been exchanged, added or deleted as compared to GLP-1(7-37) (SEQ ID No. 1),

23. (Original) A GLP-1 analog according to claim 22, wherein no more than ten amino acid residues which have been exchanged, added or deleted as compared to GLP-1(7-37) (SEQ ID No. 1).

24. (Original) A GLP-1 analog according to claim 23, wherein said GLP-1 analog comprises no more than six amino acid residues which have been exchanged, added or deleted as compared to GLP-1(7-37) (SEQ ID No. 1).

25. (Currently Amended) A GLP-1 analog according to ~~any of the above claims~~ claim 1, wherein said GLP-1 analog comprises no more than 3 amino acid residues which are not encoded by the genetic code.

26. (Currently Amended) A GLP-1 analog according to ~~any one of the preceding claims~~ claim 1, wherein said GLP-1 analog comprises only one lysine residue.

27. (Currently Amended) A GLP-1 analog according to ~~any of the above claims~~ claim 1, which is

Aib^{8},Arg^{34}-GLP-1(7-37)

Aib^{8,22},Arg^{34}-GLP-1(7-37).

Arg^{34}-GLP-1(7-37).

[3-(4-Imidazolyl)Propionyl7,Arg34]GLP-1-(7-37)peptide

Gly^{8},Arg^{34}-GLP-1(7-37)

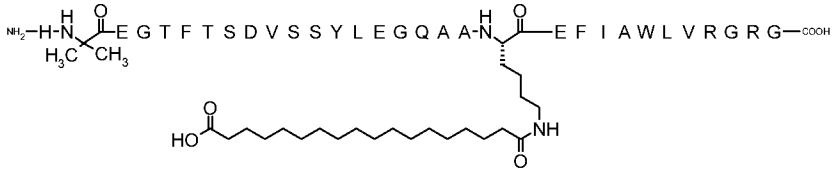
Aib^{8},Arg^{34}, Pro^{37}-GLP-1(7-37)

Aib^{8,22,27,30,35},Arg^{34},Pro^{37}- GLP-1 (7-37)amide,

all of which are substituted by B-U' in position 26.

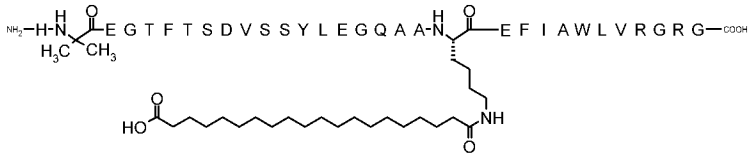
28. (Currently Amended) A compound according to ~~any one of the preceding claims~~ claim 1, which is selected from

(NNC 0113-0093, PaBl)



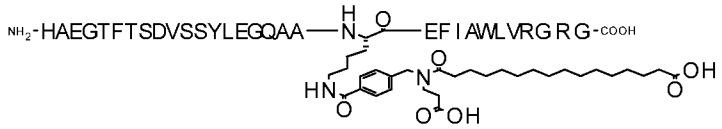
N-ε^{26}--(17-carboxyheptadecanoyl)-[Aib8,Arg34]GLP-1-(7-37)-peptide,

(NNC 0113-0094, PaBl)

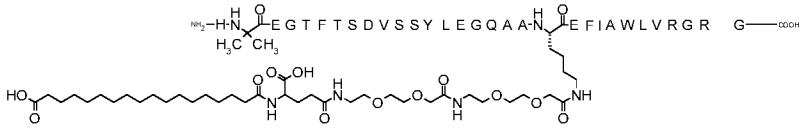


N-ε^{26}-(19-carboxynonadecanoyl)-[Aib8,Arg34]GLP-1-(7-37)-peptide,

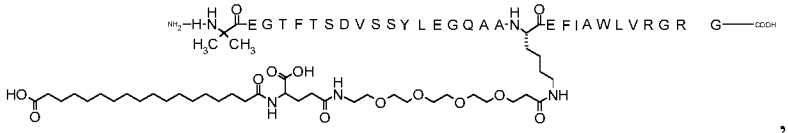
(NNC 0113-0105, HSt)



N-ε^{26}- (4-{[N-(2-carboxyethyl)-N-(15-carboxypentadecanoyl)amino]methyl}benzoyl)[Arg34]GLP-1-(7-37), (NNC 0113-0217, PaBl)



N-ε^{26}-[2-(2-[2-(2-[2-(2-[4-(17-Carboxyheptadecanoylamino)-4(S)-carboxybutyrylamino]ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][Aib8,Arg34]GLP-1-(7-37)peptide,



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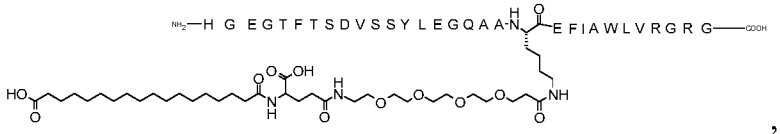

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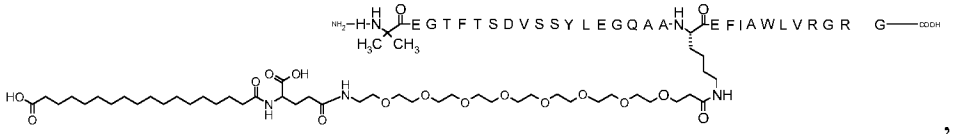

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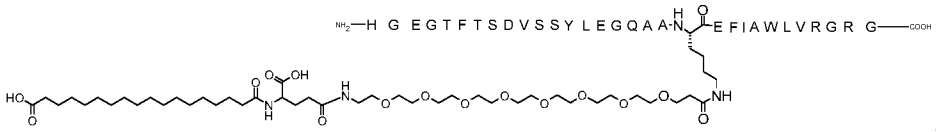

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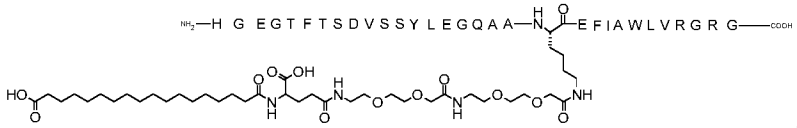

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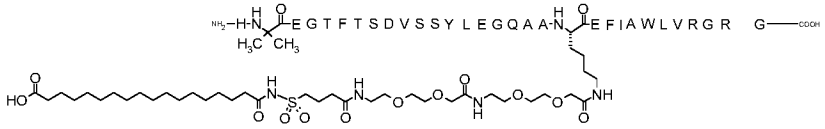

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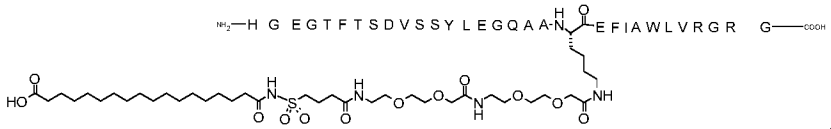

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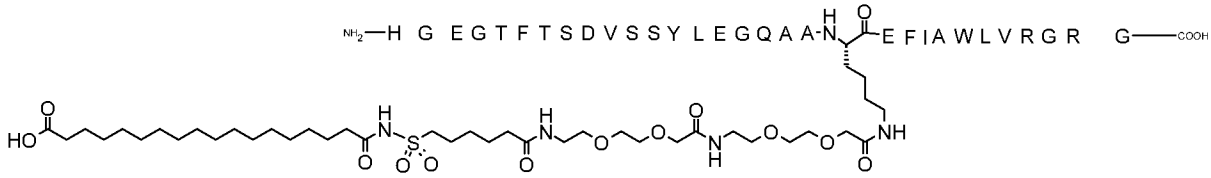

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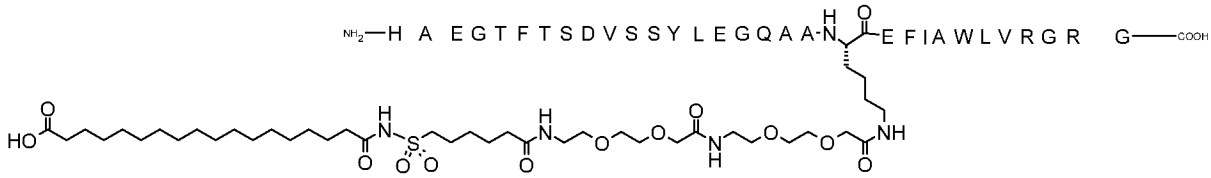

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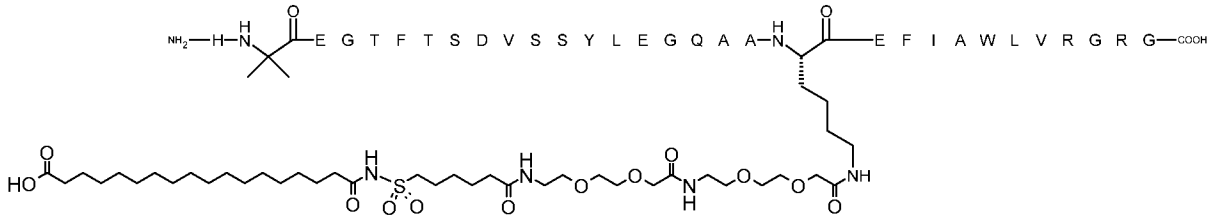

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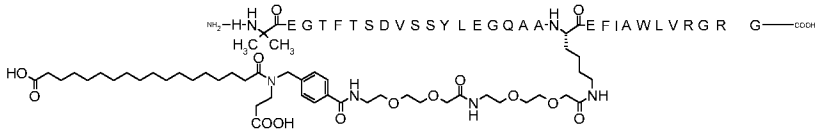

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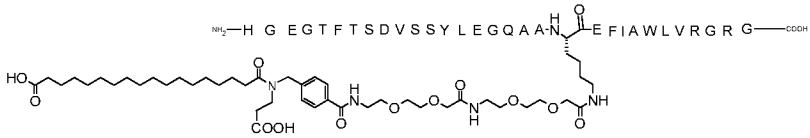

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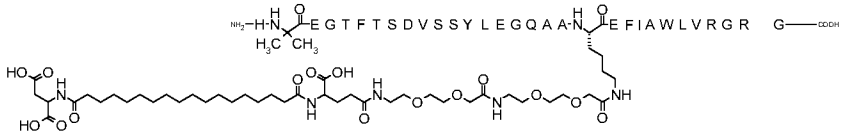

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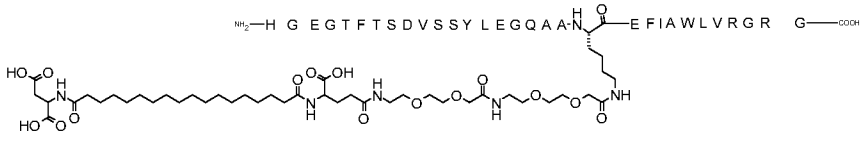

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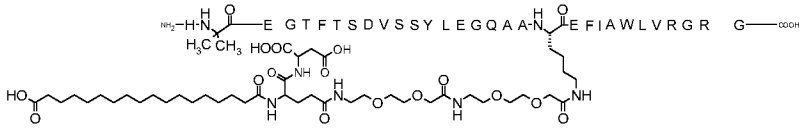

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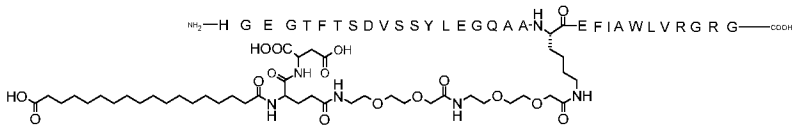

29. (Currently Amended) A method for increasing the time of action in a patient of a GLP-1 analog, ~~characterised in~~ said method comprising acylating said GLP-1 analog with a moiety B-U' as disclosed in ~~any of the preceding claims~~ claim 6, on the lysine residue in position 26 of said GLP-1 analog.

30. (Currently Amended) A method for increasing the time of action in a patient of a GLP-1 analog to more than about 40 hours, ~~characterised in~~ said method comprising modifying at least one of the amino acid residues in positions 7 and 8 of a GLP-1(7-37) peptide or an analog thereof, and acylating said GLP-1 analog with a moiety B-U'- as disclosed in ~~any of the preceding claims~~ claim 6 on the lysine residue in position 26 of said GLP-1 analog.

31. (Currently Amended) A pharmaceutical composition comprising a compound according to ~~any one of claims 1-28~~ claim 1, and a pharmaceutically acceptable excipient.

32. (Original) The pharmaceutical composition according to claim 31, which is suited for parenteral administration.

33-36 (Cancelled)

37. (New) A method for treating hyperglycemia, type 2 diabetes, impaired glucose tolerance, type 1 diabetes, obesity, hypertension, syndrome X, dyslipidemia, cognitive disorders, atheroschlerosis, myocardial infarction, coronary heart disease and other cardiovascular disorders, stroke, inflammatory bowel syndrome, dyspepsia and/or gastric ulcers in a subject, said method comprising administering to a subject in need of such treatment an effective amount of a GLP-1 analog according to claim 1.

38. (New) A method for delaying or preventing disease progression in type 2 diabetes in a subject, said method comprising administering to a subject in need of such treatment an effective amount of a GLP-1 analog according to claim 1.

39. (New) A method for decreasing food intake, decreasing β-cell apoptosis, increasing β-cell function and β-cell mass, and/or for restoring glucose sensitivity to β-cells in a subject, said method comprising administering to a subject in need of such treatment an effective amount of a GLP-1 analog according to claim 1.

REMARKS

Claims 1-32 and 37-39 are pending following entry of this Amendment.

Applicants enclose herewith the Sequence Listing for the above-captioned application. The information contained in the attached "SEQUENCE LISTING" is identical to the information in the specification as originally filed. No new matter is added.

It is believed that the claims are in condition for allowance, and a determination to that effect is earnestly solicited.

Respectfully submitted,

Date: September 17, 2007

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Use the following customer number for all correspondence regarding this application.

23650

PATENT TRADEMARK OFFICE

APPLICATION DATA SHEET

Application Information	
Application Type::	*A 371 National Stage Filing of International Application PCT/EP2006/060855 filed March 20, 2006*
Subject Matter::	Utility
Sequence submission?::	*Electronic*
Title::	Acylated GLP-1 Compounds
Attorney Docket Number::	7140.204-US
Request for Early Publication?::	No
Small Entity?::	No
Request for Non-Publication?::	No

Applicant Information

Applicant Authority Type::	*Inventor*
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Family Name::	Lau
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Country of Residence::	Denmark
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City of mailing address::	Bagsværd
Country of mailing address::	Denmark
Postal or Zip Code of mailing address::	DK-2880

Applicant Authority Type::	*Inventor*
Primary Citizenship Country::	Germany
Status::	*Full Capacity*
Given Name::	Florencio
Middle Name::	Zaragoza
Family Name::	Dorwald
City of Residence::	Smorum
Country of Residence::	Denmark
Street of mailing address::	Novo Nordisk A/S – Novo Allé
City of mailing address::	Bagsværd
Country of mailing address::	Denmark
Postal or Zip Code of mailing address::	DK-2880
Applicant Authority Type::	*Inventor*
Primary Citizenship Country::	Denmark
Status::	*Full Capacity*
Given Name::	Henrik
Family Name::	Stephensen
City of Residence::	Farum
Country of Residence::	Denmark
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(54) Title: ACYLATED GLP-1 COMPOUNDS

(57) Abstract: Protracted GLP-1 compounds and therapeutic uses thereof.

ACYLATED GLP-1 COMPOUNDS

FIELD OF THE INVENTION

This invention relates to the field of therapeutic peptides, i.e. to new protracted GLP-1 compounds.

BACKGROUND OF THE INVENTION

A range of different approaches have been used for modifying the structure of glucagon-like peptide 1 (GLP-1) compounds in order to provide a longer duration of action in vivo.
WO 96/29342 discloses peptide hormone derivatives wherein the parent peptide hormone has been modified by introducing a lipophilic substituent in the C-terminal amino acid residue or in the N-terminal amino acid residue.
WO 98/08871 discloses GLP-1 derivatives wherein at least one amino acid residue of the parent peptide has a lipophilic substituent attached.
WO 99/43708 discloses GLP-1(7-35) and GLP-1(7-36) derivatives which have a lipophilic substituent attached to the C-terminal amino acid residue.
WO 00/34331 discloses acylated GLP-1 analogs.
WO 00/69911 discloses activated insulinotropic peptides to be injected into patients where they are supposed to react with blood components to form conjugates and thereby alledgedly providing longer duration of action in vivo.
WO 02/46227 discloses GLP-1 and exendin-4 analogs fused to human serum albumin in order to extend in vivo half-life.

Many diabetes patients particularly in the type 2 diabetes segment are subject to so-called “needle-phobia”, i.e. a substantial fear of injecting themselves. In the type 2 diabetes segment most patients are treated with oral hypoglycaemic agents, and since GLP-1 compounds are expected to be the first injectable product these patients will be administered, the fear of injections may become a serious obstacle for the widespread use of the clinically very promising GLP-1 compounds. Thus, there is a need to develop new GLP-1 compounds which can be administered less than once daily, e.g. once every second or third day preferably once weekly, while retaining an acceptable clinical profile.

SUMMARY OF THE INVENTION

The invention provides a GLP-1 analog having a modification of at least one non-proteogenic amino acid residue in positions 7 and/or 8 relative to the sequence GLP-1(7-37) (SEQ ID No 1), which is acylated with a moiety to the lysine residue in position 26, and where said moiety comprises at least two acidic groups, wherein one acidic group is attached terminally.

The present invention also provides pharmaceutical compositions comprising a compound according to the present invention and the use of compounds according to the present invention for preparing medicaments for treating disease.

The invention provides a method for increasing the time of action in a patient of a GLP-1 analog, characterised in acylating said GLP-1 analog with a moiety B-U' as disclosed in any of the preceding claims, on the lysine residue in position 26 of said GLP-1 analog.

DESCRIPTION OF THE INVENTION

In the present specification, the following terms have the indicated meaning :

The term "polypeptide" and "peptide" as used herein means a compound composed of at least five constituent amino acids connected by peptide bonds. The constituent amino acids may be from the group of the amino acids encoded by the genetic code and they may be natural amino acids which are not encoded by the genetic code, as well as synthetic amino acids. Natural amino acids which are not encoded by the genetic code are e.g., γ-carboxyglutamate, ornithine, phosphoserine, D-alanine and D-glutamine. Synthetic amino acids comprise amino acids manufactured by chemical synthesis, i.e. D-isomers of the amino acids encoded by the genetic code such as D-alanine and D-leucine, Aib (α-aminoisobutyric acid), Abu (α-aminobutyric acid), Tle (tert-butylglycine), β-alanine, 3-aminomethyl benzoic acid, anthranilic acid.

The 22 proteogenic amino acids are:

Alanine, Arginine, Asparagine, Aspartic acid, Cysteine, Cystine, Glutamine, Glutamic acid, Glycine, Histidine, Hydroxyproline, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Proline, Serine, Threonine, Tryptophan, Tyrosine, Valine.

Thus a non-proteogenic amino acid is a moiety which can be incorporated into a peptide via peptide bonds but is not a proteogenic amino acid. Examples are γ-carboxyglutamate, ornithine, phosphoserine, the D-amino acids such as D-alanine and D-glutamine, Synthetic non-proteogenic amino acids comprise amino acids manufactured by chemical synthesis, i.e. D-isomers of the amino acids encoded by the genetic code such as D-alanine and D-leucine,

Aib (α-aminoisobutyric acid), Abu (α-aminobutyric acid), Tle (tert-butylglycine), 3-aminomethyl benzoic acid, anthranilic acid, des-amino-Histidine, the beta analogs of amino acids such as β-alanine etc. D-histidine, desamino-histidine, 2-amino-histidine, β-hydroxy-histidine, homohistidine, N^{α}-acetyl-histidine, α-fluoromethyl-histidine, α-methyl-histidine, 3-pyridylalanine, 2-pyridylalanine or 4-pyridylalanine, (1-aminocyclopropyl) carboxylic acid, (1-aminocyclobutyl) carboxylic acid, (1-aminocyclopentyl) carboxylic acid, (1-aminocyclohexyl) carboxylic acid, (1-aminocycloheptyl) carboxylic acid, or (1-aminocyclooctyl) carboxylic acid;

The term "analogue" as used herein referring to a polypeptide means a modified peptide wherein one or more amino acid residues of the peptide have been substituted by other amino acid residues and/or wherein one or more amino acid residues have been deleted from the peptide and/or wherein one or more amino acid residues have been deleted from the peptide and or wherein one or more amino acid residues have been added to the peptide. Such addition or deletion of amino acid residues can take place at the N-terminal of the peptide and/or at the C-terminal of the peptide. A simple system is often used to describe analogues : For example [Arg^{34}]GLP-1(7-37)Lys designates a GLP-1(7-37) analogue wherein the naturally occurring lysine at position 34 has been substituted with arginine and wherein a lysine has been added to the terminal amino acid residue, i.e. to the Gly^{37}. All amino acids for which the optical isomer is not stated is to be understood to mean the L-isomer. In embodiments of the invention a maximum of 17 amino acids have been modified. In embodiments of the invention a maximum of 15 amino acids have been modified. In embodiments of the invention a maximum of 10 amino acids have been modified. In embodiments of the invention a maximum of 8 amino acids have been modified. In embodiments of the invention a maximum of 7 amino acids have been modified. In embodiments of the invention a maximum of 6 amino acids have been modified. In embodiments of the invention a maximum of 5 amino acids have been modified. In embodiments of the invention a maximum of 4 amino acids have been modified. In embodiments of the invention a maximum of 3 amino acids have been modified. In embodiments of the invention a maximum of 2 amino acids have been modified. In embodiments of the invention 1 amino acid has been modified.

The term "derivative" as used herein in relation to a peptide means a chemically modified peptide or an analogue thereof, wherein at least one substituent is not present in the unmodified peptide or an analogue thereof, i.e. a peptide which has been covalently modified. Typical modifications are amides, carbohydrates, alkyl groups, acyl groups, esters and the like. An example of a derivative of GLP-1(7-37) is $N^{\varepsilon 26}$-((4S)-4-(hexadecanoylamino)-carboxy-butanoyl)[Arg^{34}, Lys^{26}]GLP-1-(7-37).

The term "GLP-1 peptide" as used herein means GLP-1(7-37) (SEQ ID No 1), a GLP-1(7-37) analogue, a GLP-1(7-37) derivative or a derivative of a GLP-1(7-37) analogue. In one embodiment the GLP-1 peptide is an insulinotropic agent.

The term "insulinotropic agent" as used herein means a compound which is an agonist of the human GLP-1 receptor, i.e. a compound which stimulates the formation of cAMP in a suitable medium containing the human GLP-1 receptor (one such medium disclosed below). The potency of an insulinotropic agent is determined by calculating the EC_{50} value from the dose-response curve as described below.

Baby hamster kidney (BHK) cells expressing the cloned human GLP-1 receptor (BHK- 467-12A) were grown in DMEM media with the addition of 100 IU/mL penicillin, 100 µg/mL streptomycin, 5% fetal calf serum and 0.5 mg/mL Geneticin G-418 (Life Technologies). The cells were washed twice in phosphate buffered saline and harvested with Versene. Plasma membranes were prepared from the cells by homogenisation with an Ultraturrax in buffer 1 (20 mM HEPES-Na, 10 mM EDTA, pH 7.4). The homogenate was centrifuged at 48,000 x g for 15 min at 4°C. The pellet was suspended by homogenization in buffer 2 (20 mM HEPES-Na, 0.1 mM EDTA, pH 7.4), then centrifuged at 48,000 x g for 15 min at 4°C. The washing procedure was repeated one more time. The final pellet was suspended in buffer 2 and used immediately for assays or stored at -80°C.

The functional receptor assay was carried out by measuring cyclic AMP (cAMP) as a response to stimulation by the insulinotropic agent. cAMP formed was quantified by the AlphaScreen™ cAMP Kit (Perkin Elmer Life Sciences). Incubations were carried out in half-area 96-well microtiter plates in a total volume of 50 µL buffer 3 (50 mM Tris-HCl, 5 mM HEPES, 10 mM $MgCl_2$, pH 7.4) and with the following addiditions: 1 mM ATP, 1 µM GTP, 0.5 mM 3-isobutyl-1-methylxanthine (IBMX), 0.01 % Tween-20, 0.1% BSA, 6 µg membrane preparation, 15 µg/mL acceptor beads, 20µg/mL donor beads preincubated with 6 nM biotinyl-cAMP. Compounds to be tested for agonist activity were dissolved and diluted in buffer 3. GTP was freshly prepared for each experiment. The plate was incubated in the dark with slow agitation for three hours at room temperature followed by counting in the Fusion™ instrument (Perkin Elmer Life Sciences). Concentration-response curves were plotted for the individual compounds and EC_{50} values estimated using a four-parameter logistic model with Prism v. 4.0 (GraphPad, Carlsbad, CA).

The term "DPP-IV protected" as used herein referring to a polypeptide means a polypeptide which has been chemically modified in order to render said compound resistant to the plasma peptidase dipeptidyl aminopeptidase-4 (DPP-IV). The DPP-IV enzyme in plasma is known to be involved in the degradation of several peptide hormones, e.g. GLP-1, GLP-2, Exendin-4 etc. Thus, a considerable effort is being made to develop analogues and derivatives

of the polypeptides susceptible to DPP-IV mediated hydrolysis in order to reduce the rate of degradation by DPP-IV. In one embodiment a DPP-IV protected peptide is more resistant to DPP-IV than GLP-1(7-37) or Exendin-4(1-39).

Resistance of a peptide to degradation by dipeptidyl aminopeptidase IV is determined by the following degradation assay :

Aliquots of the peptide (5 nmol) are incubated at 37 ºC with 1 µL of purified dipeptidyl aminopeptidase IV corresponding to an enzymatic activity of 5 mU for 10-180 minutes in 100 µL of 0.1 M triethylamine-HCl buffer, pH 7.4. Enzymatic reactions are terminated by the addition of 5 µL of 10% trifluoroacetic acid, and the peptide degradation products are separated and quantified using HPLC analysis. One method for performing this analysis is : The mixtures are applied onto a Vydac C18 widepore (30 nm pores, 5 µm particles) 250 x 4.6 mm column and eluted at a flow rate of 1 ml/min with linear stepwise gradients of acetonitrile in 0.1% trifluoroacetic acid (0% acetonitrile for 3 min, 0-24% acetonitrile for 17 min, 24-48% acetonitrile for 1 min) according to Siegel et al., Regul. Pept. 1999;79:93-102 and Mentlein et al. Eur. J. Biochem. 1993;214:829-35. Peptides and their degradation products may be monitored by their absorbance at 220 nm (peptide bonds) or 280 nm (aromatic amino acids), and are quantified by integration of their peak areas related to those of standards. The rate of hydrolysis of a peptide by dipeptidyl aminopeptidase IV is estimated at incubation times which result in less than 10% of the peptide being hydrolysed.

The term "C_{1-6}-alkyl" as used herein means a saturated, branched, straight or cyclic hydrocarbon group having from 1 to 6 carbon atoms. Representative examples include, but are not limited to, methyl, ethyl, n-propyl, isopropyl, butyl, isobutyl, *sec*-butyl, *tert*-butyl, n-pentyl, isopentyl, neopentyl, *tert*-pentyl, n-hexyl, isohexyl, cyclohexane and the like.

The term "pharmaceutically acceptable" as used herein means suited for normal pharmaceutical applications, i.e. giving rise to no adverse events in patients etc.

The term "excipient" as used herein means the chemical compounds which are normally added to pharmaceutical compositions, e.g. buffers, tonicity agents, preservatives and the like.

The term "effective amount" as used herein means a dosage which is sufficient to be effective for the treatment of the patient compared with no treatment.

The term "pharmaceutical composition" as used herein means a product comprising an active compound or a salt thereof together with pharmaceutical excipients such as buffer, preservative, and optionally a tonicity modifier and/or a stabilizer. Thus a pharmaceutical composition is also known in the art as a pharmaceutical formulation.

The term "treatment of a disease" as used herein means the management and care of a patient having developed the disease, condition or disorder. The purpose of treatment is to combat the disease, condition or disorder. Treatment includes the administration of the active compounds to eliminate or control the disease, condition or disorder as well as to alleviate the symptoms or complications associated with the disease, condition or disorder.

In another aspect the present invention relates to an acylated GLP-1 analogue that can bind to albumin and the GLP-1 receptor simultaneously.

In another aspect the present invention relates to an acylated GLP-1 analogue that bind to the GLP-1 receptor with an affinity below 100nM, preferable below 30 nM in the presence of 2% albumin.

In another aspect the present invention relates to an acylated GLP-1 analogue which affinity to the GLP-1 receptor is only partly decreased when comparing the affinity in the presence of very low concentration (e.g. 0.005% to 0.2%) of human albumin to the affinity in the presence of 2% human albumin. The shift in binding affinity under these conditions is less than 50 fold, preferable below 30 fold and more preferable below 10 fold.

The term "albumin binding moiety" as used herein means a residue which binds non-covalently to human serum albumin. The albumin binding residue attached to the therapeutic polypeptide typically has an affinity below 10 µM to human serum albumin and preferably below 1 µM. A range of albumin binding residues are known among linear and branched lipohophillic moieties containing 4-40 carbon atoms having a distal acidic group.

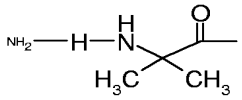
The term "hydrophilic linker" as used herein means a spacer that separates a peptide and an albumin binding residue with a chemical moiety which comprises at least 5 non-hydrogen atoms where 30-50% of these are either N or O.

The term "acidic groups" as used herein means organic chemical groups which are fully or partly negatively charged at physiological pH. The pKa value of such groups is below 7, preferable below 5. This includes but is not limited to carboxylic acids, sulphonic acids, phosphoric acids or heterocyclic ring systems which are fully or partly negatively charged at physiological pH.

In the below structural formula II the moiety U is a di-radical may be attached to the terminal groups B and the aminogroup of the lysine amino acid in the peptide in two different ways. In embodiments of the invention the U in formula II is attached with the group B attached at the end of the alkyl chain and the peptide at the other end.

In the formulas below the terminal bonds from the attached groups are to be regarded as attachment bonds and not ending in methylene groups unless stated.

In the formulas below


means the H_2N-His-Aib- N-terminal of the GLP-1 analogue.

In an embodiment the invention provides a GLP-1 analog acylated with a lipophillic albumin binding moiety containing at least two free acidic chemical groups attached via a non natural amino acid linker to the lysine residue in position 26.

In an embodiment, the term free acidic chemical groups is to be understood as having the same meaning as "acidic groups" as used herein.

In an embodiment the invention provides an acylated GLP-1 analog where said GLP-1 analog is stabilised against DPP-IV by modification of at least one amino acid residue in positions 7 and 8 relative to the sequence GLP-1(7-37) (SEQ ID No 1), and where said acylation is a diacid attached to the lysine residue in position 26 optionally via a non natural amino acid hydrophilic linker.

In an embodiment of the invention a GLP-1 analog having a modification of at least one non-proteogenic amino acid residue in positions 7 and/or 8 relative to the sequence GLP-1(7-37) (SEQ ID No 1), which is acylated with a moiety to the lysine residue in position 26, and where said moiety comprises at least two acidic groups, wherein one acidic group is attached terminally.

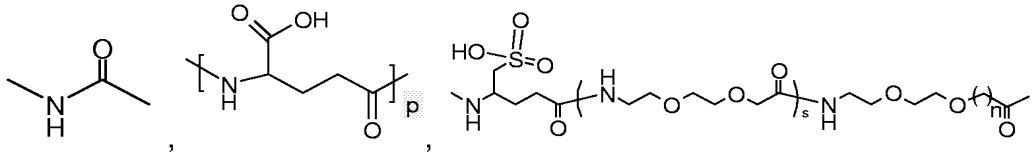
An embodiment provides a GLP-1 analog according to the above embodiment, wherein the moiety attached in position 26 comprises a hydrophilic linker.

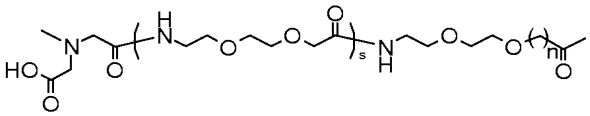
An embodiment provides a GLP-1 analog according to the above embodiments, wherein the hydrophilic linker comprises at least 5 non-hydrogen atoms where 30-50% of these are either N or O.

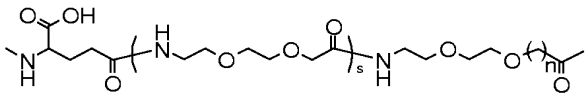
An embodiment provides a GLP-1 analog according to any of the above embodiments, wherein the moiety attached in position 26 comprises an albumin binding moiety seperated from the peptide by the hydrophilic linker.

An embodiment provides a GLP-1 analog according to the above embodiment, wherein the albumin binding moiety is a linear or branched lipophilic moiety containing 4-40 carbon atoms having a distal acidic group.

An embodiment provides a GLP-1 analog according to any of the above embodiments, wherein the acylated moiety is B-U', where U' is selected from







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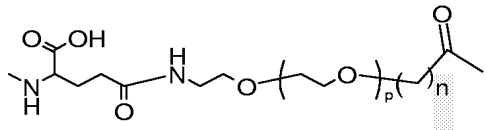

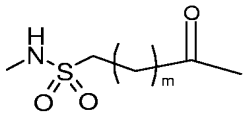

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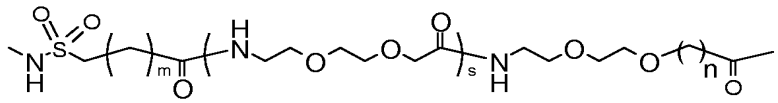

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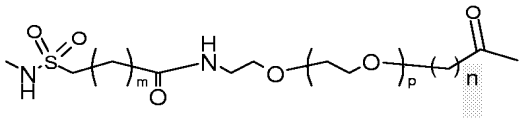

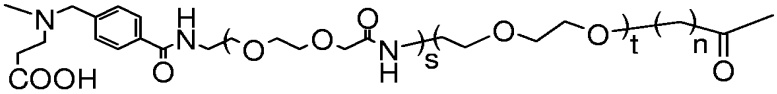

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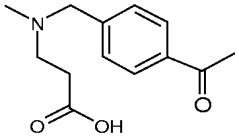

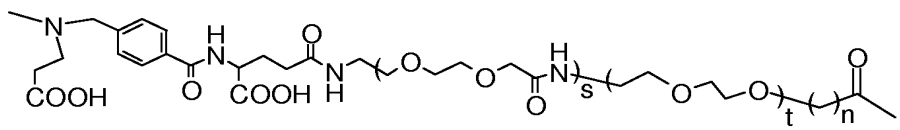

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m is 0, 1, 2, 3, 4, 5, or 6,

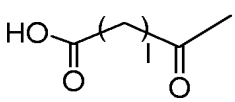
n is 1, 2 or 3

s is 0, 1, 2, or 3,

t is 0, 1, 2, 3, or 4

p is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17,18, 19, 20, 21, 22, or 23;

and where B is an acidic group selected from



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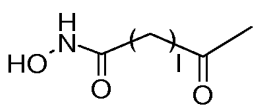
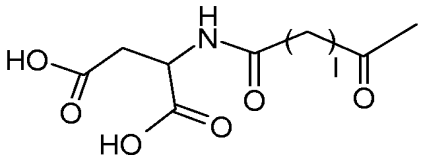
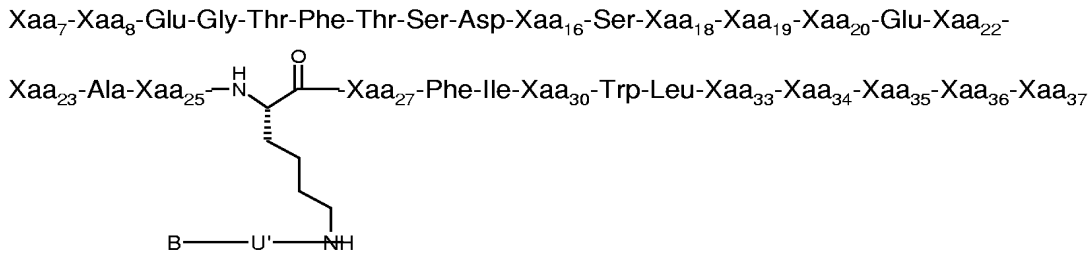


and



where l is 12, 13, 14, 15, 16, 17, 18, 19 or 20;

An embodiment provides a GLP-1 analog according to any of the above embodiments, which is a compound of formula I (SEQ ID No. 2) :



Formula I

wherein

Xaa_7 is L-histidine, imidazopropionyl, α-hydroxy-histidine, D-histidine, desamino-histidine, 2-amino-histidine, β-hydroxy-histidine, homohistidine, N^{α}-acetyl-histidine, N^{α}-formyl-histidine, α-fluoromethyl-histidine, α-methyl-histidine, 3-pyridylalanine, 2-pyridylalanine or 4-pyridylalanine

Xaa_8 is Ala, Gly, Val, Leu, Ile, Thr, Ser, Lys, Aib, (1-aminocyclopropyl) carboxylic acid, (1-aminocyclobutyl) carboxylic acid, (1-aminocyclopentyl) carboxylic acid, (1-aminocyclohexyl) carboxylic acid, (1-aminocycloheptyl) carboxylic acid, or (1-aminocyclooctyl) carboxylic acid;

Xaa_{16} is Val or Leu;

Xaa_{18} is Ser, Lys or Arg;

Xaa_{19} is Tyr or Gln;

Xaa_{20} is Leu or Met;

Xaa_{22} is Gly, Glu or Aib;

Xaa_{23} is Gln, Glu, Lys or Arg;

Xaa_{25} is Ala or Val;

Xaa_{27} is Glu or Leu;

Xaa_{30} is Ala, Glu or Arg;

Xaa_{33} is Val or Lys;

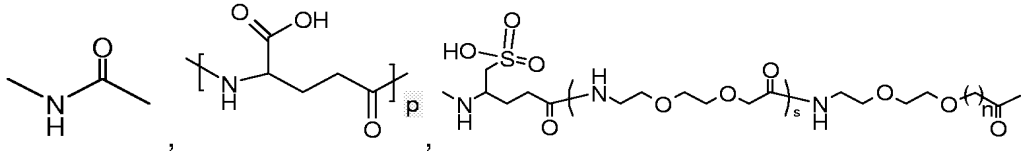
Xaa_{34} is Lys, Glu, Asn or Arg;

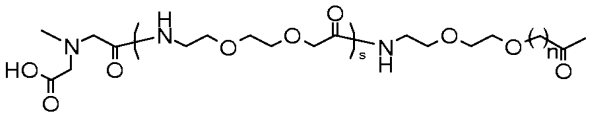
Xaa_{35} is Gly or Aib;

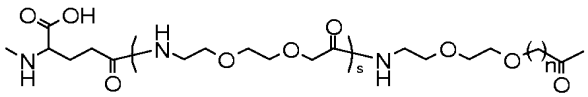
Xaa_{36} is Arg, Gly or Lys, or is absent;

Xaa_{37} is Gly, Ala, Glu, Pro, Lys, or is absent;

and B and U' together is the acylated moiety, where U' is selected from







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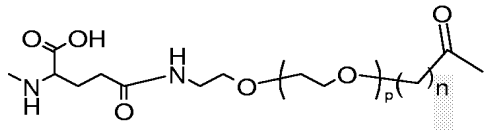

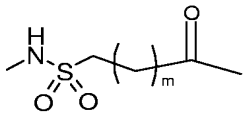

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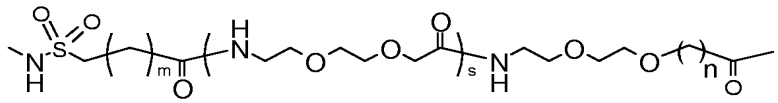

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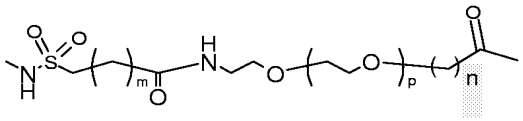

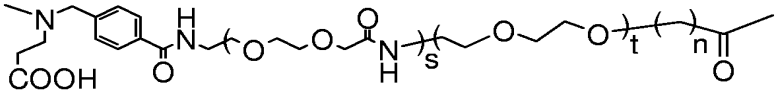

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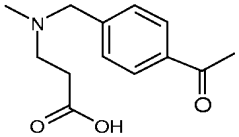
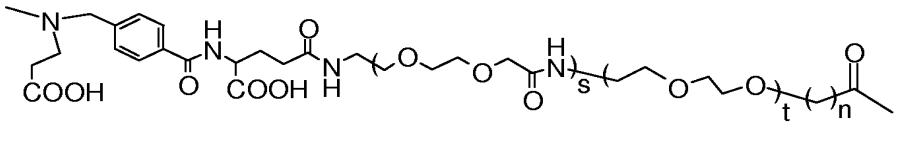




,

m is 0, 1, 2, 3, 4, 5, or 6,

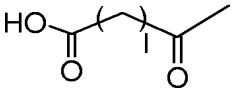
n is 1, 2 or 3

s is 0, 1, 2, or 3,

t is 0, 1, 2, 3, or 4

p is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17,18, 19, 20, 21, 22, or 23;

and where B is an acidic group selected from



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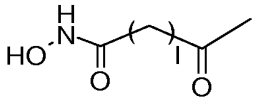

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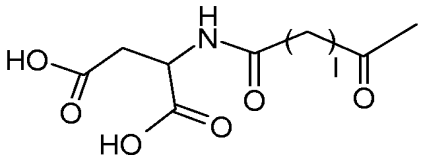
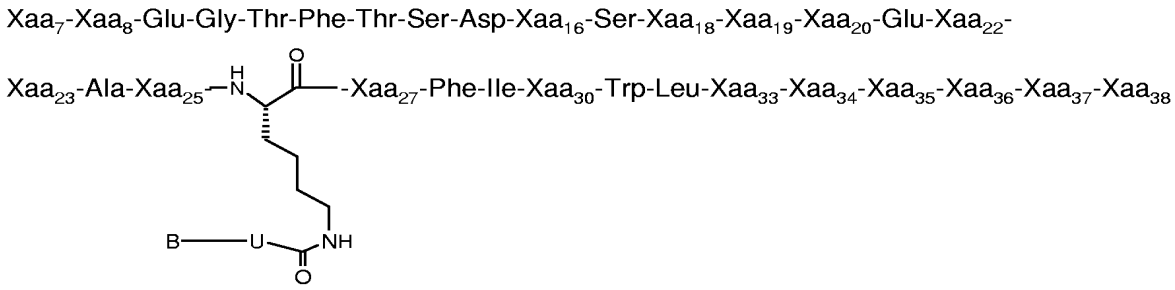


where l is 12, 13, 14, 15, 16, 17, 18, 19 or 20;

In an embodiment the invention provides a compound which is a compound of formula II (SEQ ID No. 3) :



Formula II

The formula II is identical to formula I as stated in an embodiment above, where the moiety B-U is replaced by B-U'. The difference being only the incorporation of the carboxy group in the U' relative to U, which is without the attaching carboxy group.

In formula II each of the Xaa's has the following meaning:

Xaa_7 is L-histidine, D-histidine, desamino-histidine, 2-amino-histidine, β-hydroxy-histidine, homohistidine, N^{α}-acetyl-histidine, α-fluoromethyl-histidine, α-methyl-histidine, 3-pyridylalanine, 2-pyridylalanine or 4-pyridylalanine;

Xaa_8 is Ala, Gly, Val, Leu, Ile, Lys, Aib, (1-aminocyclopropyl) carboxylic acid, (1-aminocyclobutyl) carboxylic acid, (1-aminocyclopentyl) carboxylic acid, (1-aminocyclohexyl) carboxylic acid, (1-aminocycloheptyl) carboxylic acid, or (1-aminocyclooctyl) carboxylic acid;

Xaa_{16} is Val or Leu;

Xaa_{18} is Ser, Lys or Arg;

Xaa_{19} is Tyr or Gln;

Xaa_{20} is Leu or Met;

Xaa_{22} is Gly, Glu or Aib;

Xaa_{23} is Gln, Glu, Lys or Arg;

Xaa_{25} is Ala or Val;

Xaa_{27} is Glu or Leu;

Xaa_{30} is Ala, Glu or Arg;

Xaa_{33} is Val or Lys;

Xaa_{34} is Lys, Glu, Asn or Arg;

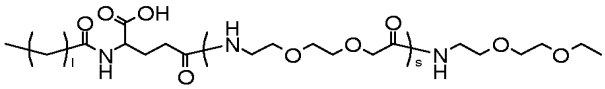
Xaa_{35} is Gly or Aib;

Xaa_{36} is Arg, Gly or Lys, or is absent;

Xaa_{37} is Gly, Ala, Glu, Pro, Lys, or is absent;

Xaa_{38} is Lys, Ser, amide or is absent;

and where U is a spacer selected from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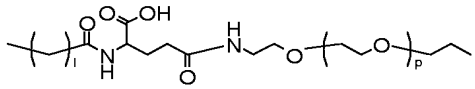

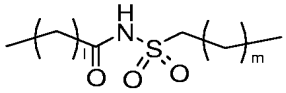
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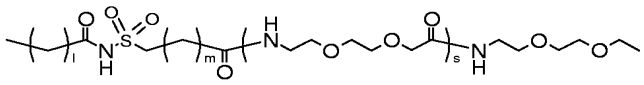
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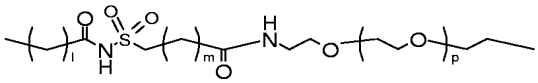
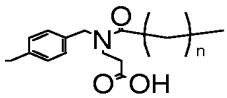



where n is 12, 13, 14, 15, 16, 17 or 18

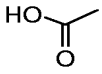
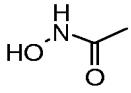
l is 12, 13, 14, 15, 16, 17 or 18,

m is 0, 1, 2, 3, 4, 5, or 6,

s is 0, 1, 2, or 3,

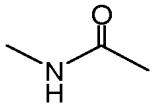
p is 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17,18, 19, 20, 21, 22, or 23;

and where B is an acidic group selected from

 and  .

In the embodiments below when referring to U' in formula I it is to be understood as also referring to formula II and U, with the only difference being the carboxy group.

An embodiment provides a GLP-1 analog according to the embodiments above, wherein U' is selected from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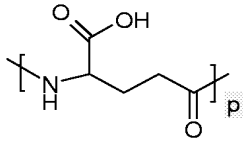
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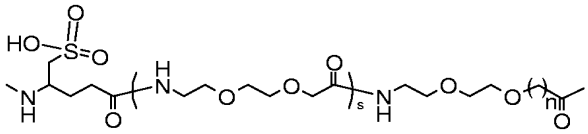

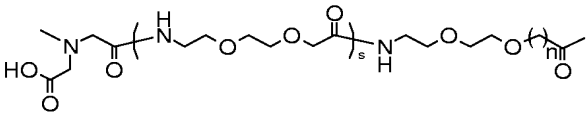

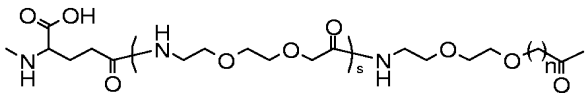
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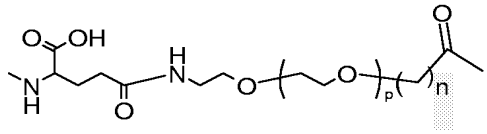

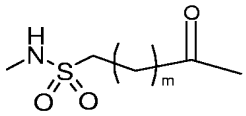
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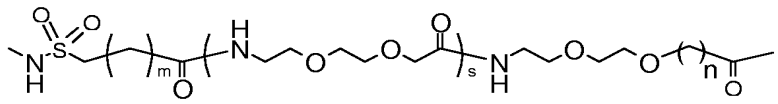
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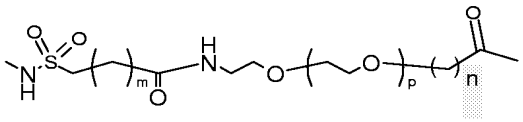

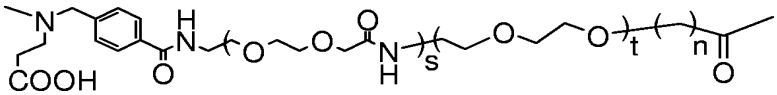
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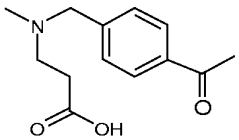
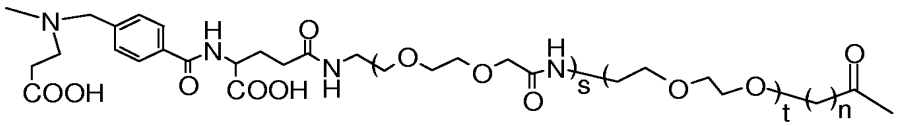
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m is 2, 3, 4 or 5,

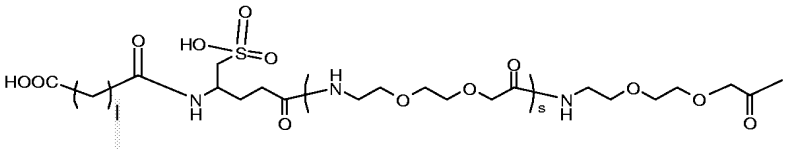
n is 1 or 2

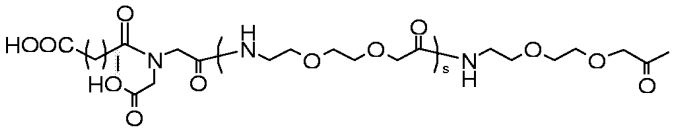
s is 0, 1, or 2,

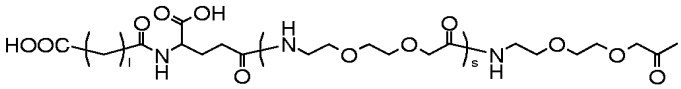
t is 0, 1, 2, or 3

p is 1, 2, 3, 4, 7, 11 or 23

An embodiment provides a GLP-1 analog according to the embodiments above, wherein B-U'- is



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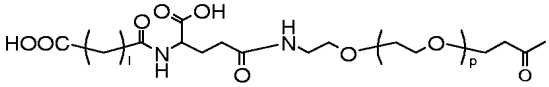

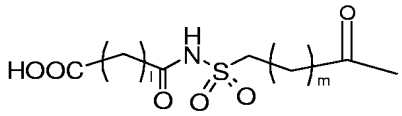
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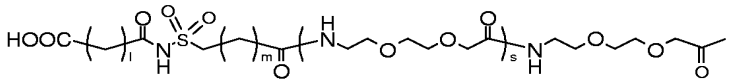
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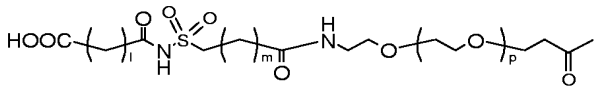
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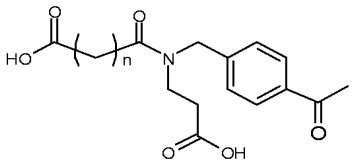
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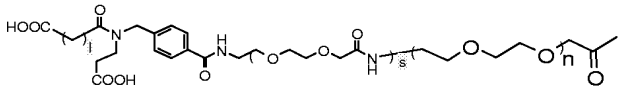
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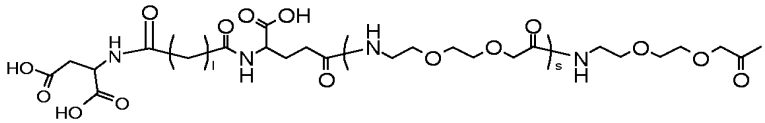
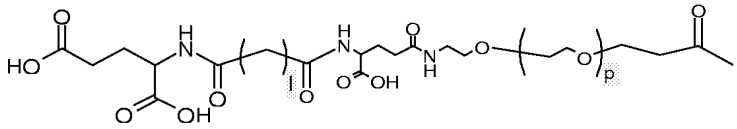



where l is 14, 15, 16, 17, 18, 19 or 20;

p is 1, 2, 3, 4, 7, 8 , 9, 10, 11 or 12.

s is 0, 1 or 2

t is 0 or 1;

An embodiment according to the above wherein

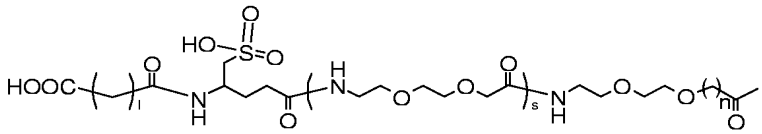
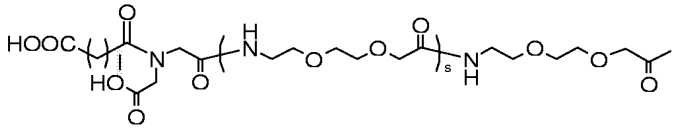
where l is 14, 15, 16, 17 or 18

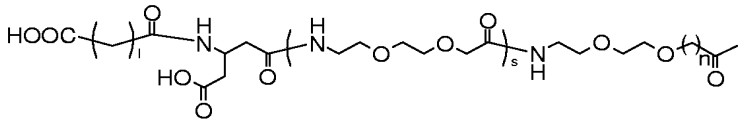
p is 1, 2, 3, 4 or 11;

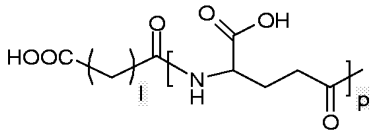
s is 0, 1 or 2;

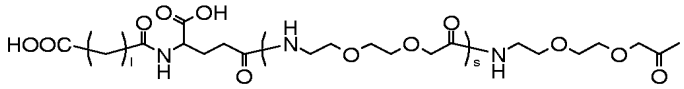
t is 0 or 1;

An embodiment provides a GLP-1 analog according to the embodiment above, wherein B-U' is







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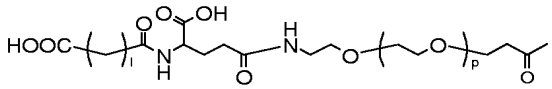

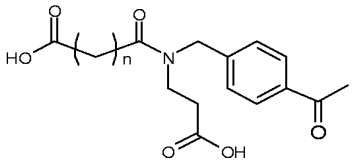

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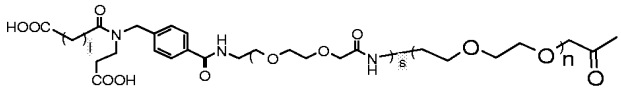


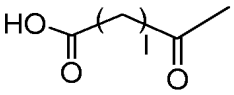
where l is 14, 15, 16, 17, 18, 19 or 20;

p is 1, 2, 3, or 4.

s is 0, 1 or 2

n is 0, 1 or 2

An embodiment according to any of the above embodiments is wherein B is



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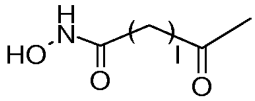

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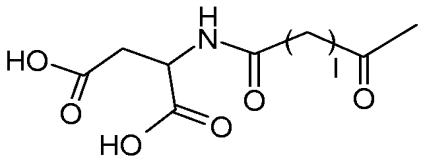
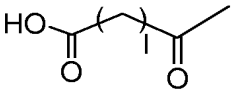


and l is 14,16, 18 or 20;

An embodiment provides a GLP-1 analog according to any of the embodiments above, wherein B is



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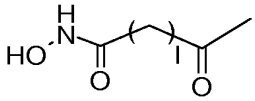

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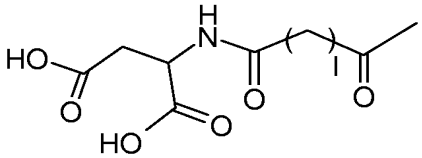


where l is 14, 15, or 16.

An embodiment provides a GLP-1 analog according to any of the embodiments above, wherein s is 1.

An embodiment provides a GLP-1 analog according to any of the embodiments above, wherein n is 1.

An embodiment provides a GLP-1 analog according any of the embodiments above, wherein l is 14, 15 or 16; In embodiments l is 17, 18, 19 or 20. In embodiments l is 15, 16 or 17. In embodiments l is 18, 19 or 20. In embodiments l is 14. In embodiments l is 16. In embodiments l is 18. In embodiments l is 20.

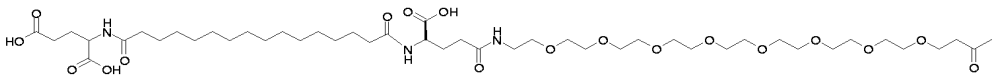
An embodiment provides a GLP-1 analog according to any of the embodiments above, wherein p is 1.

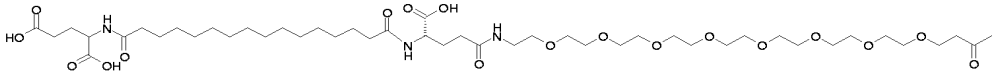
An embodiment provides a GLP-1 analog according to any of the embodiments above, wherein p is 2.

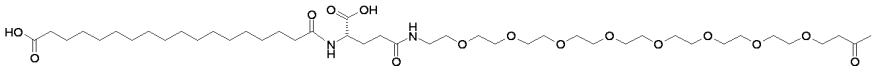
An embodiment provides a GLP-1 analog according to any of the embodiments above, wherein p is 3.

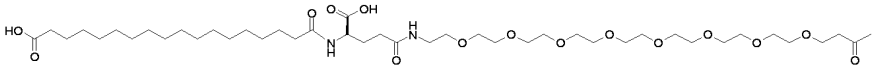
An embodiment provides a GLP-1 analog according to any of the embodiments above, wherein p is 4.

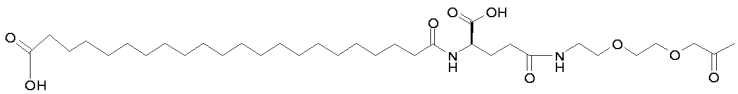
An embodiment provides a GLP-1 analog according to any of the embodiments above, wherein B-U' is

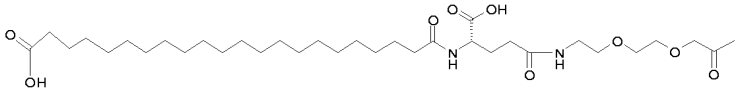


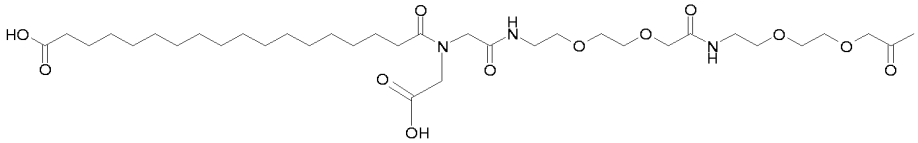




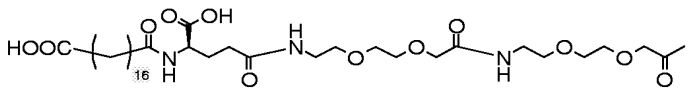




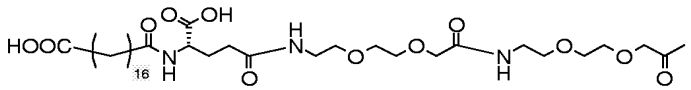




An embodiment provides a GLP-1 analog according to any of the embodiments above, wherein B-U' is



An embodiment provides a GLP-1 analog according to any of the embodiments above, wherein B-U' is



An embodiment provides a GLP-1 analog according to formula I above, wherein
Xaa_7 is His or desamino-histidine;
Xaa_8 is Ala, Gly, Val, Leu, Ile, Lys or Aib;
Xaa_{16} is Val;
Xaa_{18} is Ser;
Xaa_{19} is Tyr;
Xaa_{20} is Leu;
Xaa_{22} is Gly, Glu or Aib;
Xaa_{23} is Gln or Glu;
Xaa_{25} is Ala;
Xaa_{27} is Glu;
Xaa_{30} is Ala or Glu;
Xaa_{33} is Val;
Xaa_{34} is Lys or Arg;
Xaa_{35} is Gly or Aib;
Xaa_{36} is Arg or Lys
Xaa_{37} is Gly, amide or is absent;

An embodiment provides a GLP-1 analog according to formula I above, wherein
Xaa_7 is His
Xaa_8 is Gly, or Aib;
Xaa_{16} is Val;
Xaa_{18} is Ser;

Xaa_{19} is Tyr;
Xaa_{20} is Leu;
Xaa_{22} is Glu or Aib;
Xaa_{23} is Gln;
Xaa_{25} is Ala;
Xaa_{27} is Glu;
Xaa_{30} is Ala;
Xaa_{33} is Val;
Xaa_{34} is Lys or Arg;
Xaa_{35} is Gly or Aib;
Xaa_{36} is Arg
Xaa_{37} is Gly

An embodiment provides a GLP-1 analog according to any one of the above embodiments, wherein said GLP-1 analog comprises a modification of the N-terminal L-histidine in position 7 of the GLP-1(7-37) sequence.

An embodiment provides a GLP-1 analog according to the embodiment above, wherein said GLP-1 analog comprises imidazopropionyl^7, α-hydroxy-histidine^7 or N-methyl-histidine^7, D-histidine^7, desamino-histidine^7, 2-amino-histidine^7, β-hydroxy-histidine^7, homohistidine^7, N^{α}-acetyl-histidine^7, α-fluoromethyl-histidine^7, α-methyl-histidine^7, 3-pyridylalanine^7, 2-pyridylalanine^7 or 4-pyridylalanine^7.

An embodiment provides a GLP-1 analog according to any one of the embodiments above, wherein said GLP-1 analog comprises a substitution of the L-alanine in position 8 of the GLP-1(7-37) sequence for another amino acid residue.

An embodiment provides a GLP-1 analog according to the embodiment above, wherein said GLP-1 analog comprises Aib^8, Gly^8, Val^8, Ile^8, Leu^8, Ser^8, Thr^8, (1-aminocyclopropyl) carboxylic acid, (1-aminocyclobutyl) carboxylic acid, (1-aminocyclopentyl) carboxylic acid, (1-aminocyclohexyl) carboxylic acid, (1-aminocycloheptyl) carboxylic acid, or (1-aminocyclooctyl) carboxylic acid.

An embodiment provides a GLP-1 analog according to any of the the embodiment above, wherein said GLP-1 analog comprises Aib^8;

In one embodiment of the invention said GLP-1 analog is Aib^{8},Arg^{34}-GLP-1(7-37) or $Aib^{8,22}$,Arg^{34}-GLP-1(7-37).

An embodiment provides a GLP-1 analog according to any of the above embodiments, wherein said GLP-1 analog comprises no more than fifteen amino acid residues which have been exchanged, added or deleted as compared to GLP-1(7-37) (SEQ ID No. 1),

An embodiment provides a GLP-1 analog according to the embodiment above, wherein no more than ten amino acid residues which have been exchanged, added or deleted as compared to GLP-1(7-37) (SEQ ID No. 1).

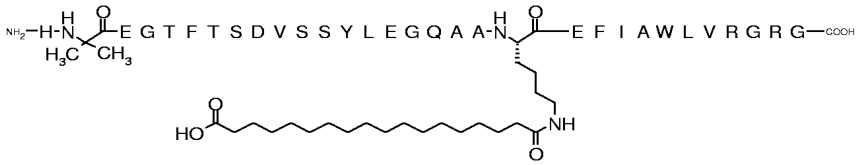
An embodiment provides a GLP-1 analog according to the embodiment above, wherein said GLP-1 analog comprises no more than six amino acid residues which have been exchanged, added or deleted as compared to GLP-1(7-37) (SEQ ID No. 1).

An embodiment provides a GLP-1 analog according to any of the above embodiments, wherein said GLP-1 analog comprises no more than 3 amino acid residues which are not encoded by the genetic code.

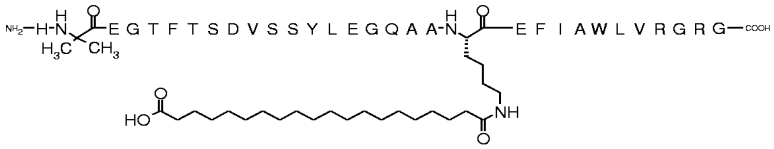
An embodiment provides a GLP-1 analog according to any of the above embodiments, wherein said GLP-1 analog comprises only one lysine residue.

An embodiment provides a GLP-1 analog according to any of the above embodiments, which is
Aib^{8},Arg^{34}-GLP-1(7-37)
$Aib^{8,22}$,Arg^{34}-GLP-1(7-37).
Arg^{34}-GLP-1(7-37).
[3-(4-Imidazolyl)Propionyl7,Arg34]GLP-1-(7-37)peptide
Gly^{8},Arg^{34}-GLP-1(7-37)
Aib^{8},Arg^{34}, Pro^{37}-GLP-1(7-37)
$Aib^{8,22,27,30,35}$,Arg^{34},Pro^{37}- GLP-1 (7-37)amide,
all of which are substituted by B-U' in position 26.

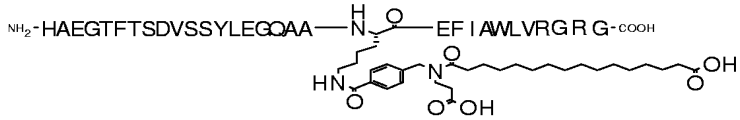
An embodiment provides a GLP-1 analog according to any one of the preceding embodiments, which is selected from



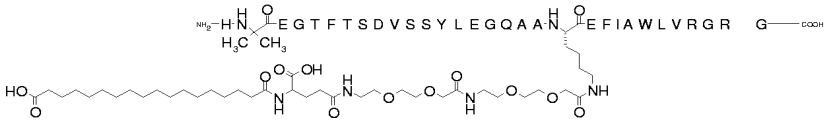
N-ε^{26}-(17-carboxyheptadecanoyl)-[Aib8,Arg34]GLP-1-(7-37)-peptide,



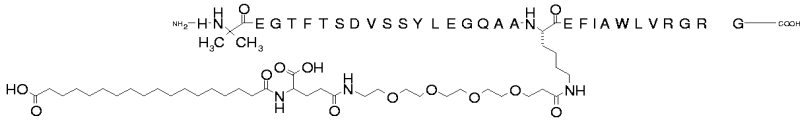
N-ε^{26}-(19-carboxynonadecanoyl)-[Aib8,Arg34]GLP-1-(7-37)-peptide,



N-ε^{26}-(4-{[N-(2-carboxyethyl)-N-(15-carboxypentadecanoyl)amino]methyl}benzoyl)[Arg34]GLP-1-(7-37),



N-ε^{26}-[2-(2-[2-(2-[2-(2-[4-(17-Carboxyheptadecanoylamino)-4(S)-carboxybutyrylamino]ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][Aib8,Arg34]GLP-1-(7-37)peptide,



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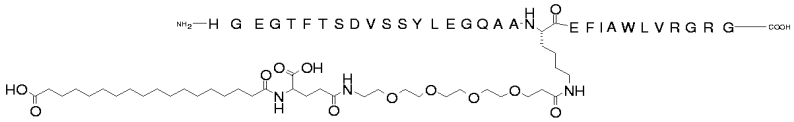

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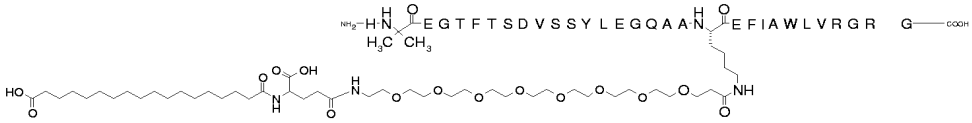

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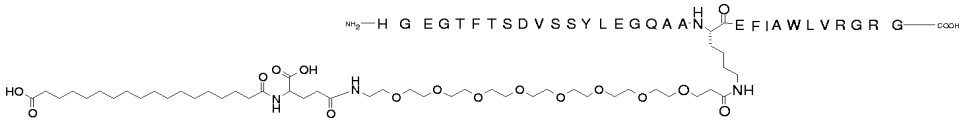

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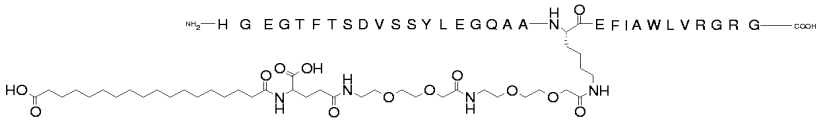

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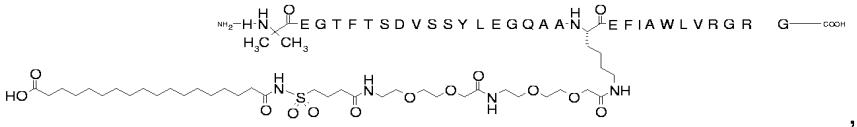

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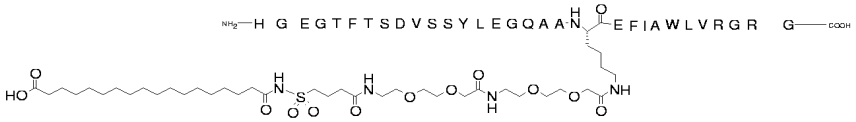

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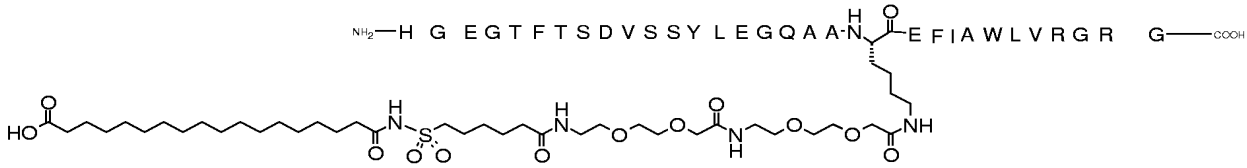

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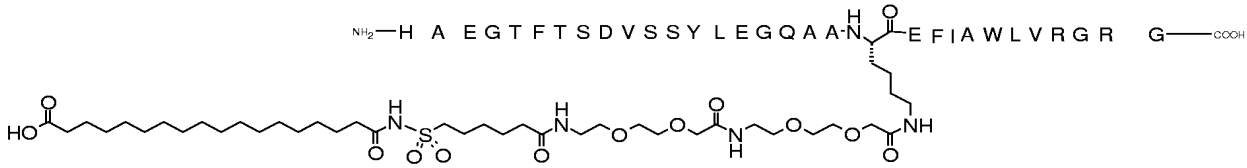

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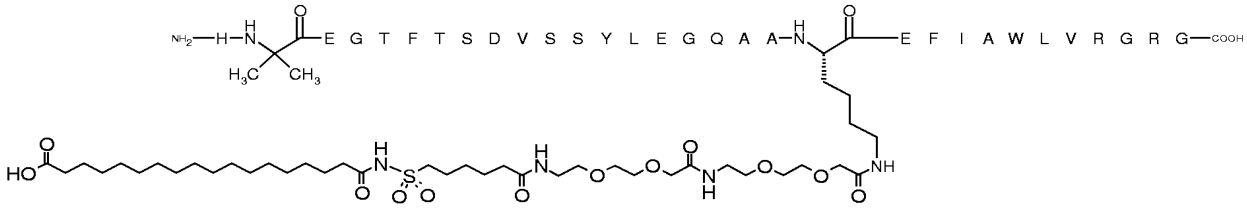

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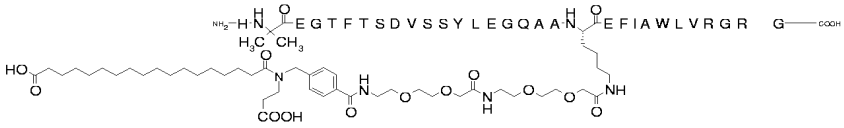

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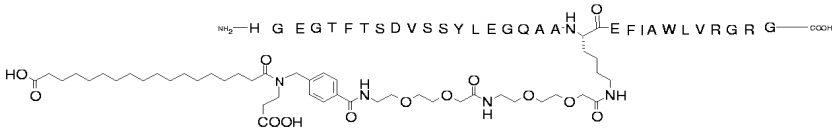

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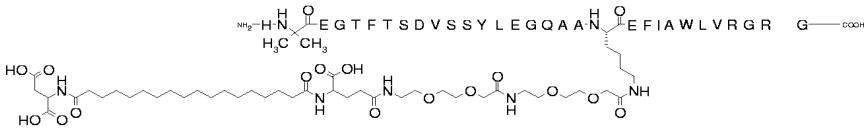

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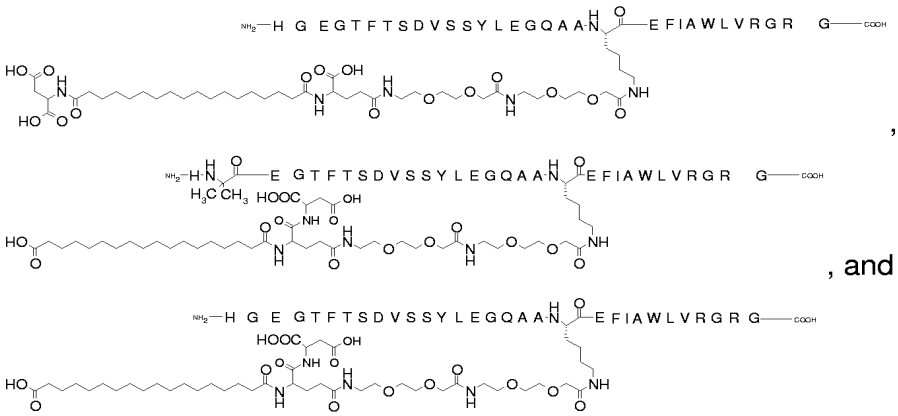

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, and

An embodiment provides a method for increasing the time of action in a patient of a GLP-1 analog, characterised in acylating said GLP-1 analog with a moiety B-U as disclosed in any of the preceding embodiments, on the lysine residue in position 26 of said GLP-1 analog.

An embodiment provides a method for increasing the time of action in a patient of a GLP-1 analog to more than about 40 hours, characterised in modifying at least one of the amino acid residues in positions 7 and 8 of a GLP-1(7-37) peptide or an analog thereof, and acylating said GLP-1 analog with a moiety B-U'- as disclosed in any of the preceding embodiments on the lysine residue in position 26 of said GLP-1 analog.

An embodiment provides a pharmaceutical composition comprising a compound according to any one the embodiments above, and a pharmaceutically acceptable excipient.

An embodiment provides a pharmaceutical composition according to the embodiment above, which is suited for parenteral administration.

An embodiment provides the use of a compound according to any one of the embodiments above for the preparation of a medicament.

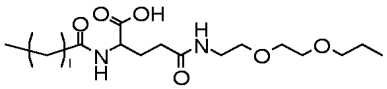
An embodiment provides the use of a compound according to any one of the embodiments above for the preparation of a medicament for the treatment or prevention of hyperglycemia, type 2 diabetes, impaired glucose tolerance, type 1 diabetes, obesity, hypertension, syndrome X, dyslipidemia, cognitive disorders, atheroschlerosis, myocardial infarction, coronary heart disease and other cardiovascular disorders, stroke, inflammatory bowel syndrome, dyspepsia and gastric ulcers.

An embodiment provides the use of a compound according to any one of the embodiments above for the preparation of a medicament for delaying or preventing disease progression in type 2 diabetes.

An embodiment provides the use of a compound according to any one of the embodiments above for the preparation of a medicament for decreasing food intake, decreasing β-cell apoptosis, increasing β-cell function and β-cell mass, and/or for restoring glucose sensitivity to β-cells.

In an embodiment the invention provides a compound according to the embodiments above, wherein said GLP-1 analog is Aib^{8},Arg^{34}-GLP-1(7-37) or $Aib^{8,22}$,Arg^{34}-GLP-1(7-37) attached to a linker B-U';

In an embodiment of Formula II, B-U represents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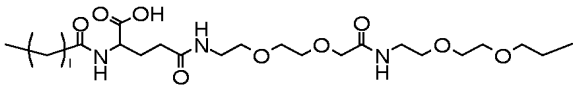
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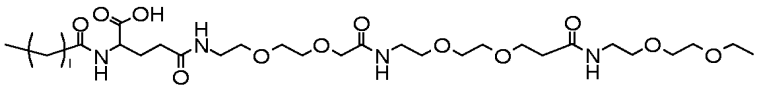
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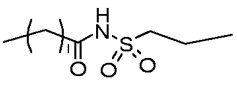
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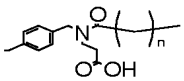
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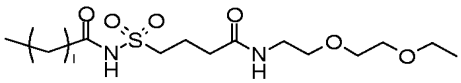
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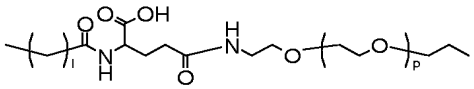
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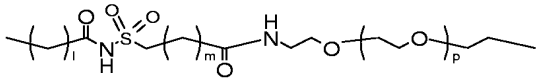


where l is 14, 15 or 16;
n is 15, 16, 17 or 18;
p is 3, 7, 11 or 24.

In embodiments the invention provides a compound according to any one of the embodiments above, wherein said diacid comprises a dicarboxylic acid.

In embodiments the invention provides a compound according to any one of the embodiments above, wherein the acylation group comprises a straight-chain or branched alkane α,ω-dicarboxylic acid.

In embodiments the invention provides compound according to the embodiment above, wherein the acylation group comprises the structure HOOC-$(CH_2)_n$CO-, wherein n is 12 to 20.

In embodiments the invention provides a compound according to the embodiment above, wherein the acylation group comprises a structure selected from HOOC-$(CH_2)_{14}$CO-, HOOC-$(CH_2)_{15}$CO-, HOOC-$(CH_2)_{16}$CO-, HOOC-$(CH_2)_{17}$CO-, and HOOC-$(CH_2)_{18}$CO-.

In embodiments the invention provides a compound according to the embodiment above, wherein the acylation group comprises the structure HOOC-$(CH_2)_{16}$CO-.

Another object of the present invention is to provide a pharmaceutical formulation comprising a compound according to the present invention which is present in a concentration from 0.1 mg/ml to 25 mg/ml, and wherein said formulation has a pH from 3.0 to 9.0. The formulation may further comprise a buffer system, preservative(s), tonicity agent(s), chelating agent(s), stabilizers and surfactants. In one embodiment of the invention the pharmaceutical formulation is an aqueous formulation, i.e. formulation comprising water. Such formulation is typically a solution or a suspension. In a further embodiment of the invention the pharmaceutical formulation is an aqueous solution. The term “aqueous formulation” is defined as a formulation comprising at least 50 %w/w water. Likewise, the term “aqueous solution” is defined as a solution comprising at least 50 %w/w water, and the term “aqueous suspension” is defined as a suspension comprising at least 50 %w/w water.

In another embodiment the pharmaceutical formulation is a freeze-dried formulation, whereto the physician or the patient adds solvents and/or diluents prior to use.

In another embodiment the pharmaceutical formulation is a dried formulation (e.g. freeze-dried or spray-dried) ready for use without any prior dissolution.

In a further aspect the invention relates to a pharmaceutical formulation comprising an aqueous solution of a compound according to the present invention, and a buffer, wherein said compound is present in a concentration from 0.1 mg/ml or above, and wherein said formulation has a pH from about 3.0 to about 9.0.

In another embodiment of the invention the pH of the formulation is from about 7.0 to about 9.5. In another embodiment of the invention the pH of the formulation is from about 3.0 to about 7.0. In another embodiment of the invention the pH of the formulation is from about 5.0 to about 7.5. In another embodiment of the invention the pH of the formulation is from about 7.5 to about 9.0. In another embodiment of the invention the pH of the formulation is from about 7.5 to about 8.5. In another embodiment of the invention the pH of the formulation is from about 6.0 to about 7.5. In another embodiment of the invention the pH of the formulation

is from about 6.0 to about 7.0. In another embodiment the pharmaceutical formulation is from 8.0 to 8.5.

In a further embodiment of the invention the buffer is selected from the group consisting of sodium acetate, sodium carbonate, citrate, glycylglycine, histidine, glycine, lysine, arginine, sodium dihydrogen phosphate, disodium hydrogen phosphate, sodium phosphate, and tris(hydroxymethyl)-aminomethan, bicine, tricine, malic acid, succinate, maleic acid, fumaric acid, tartaric acid, aspartic acid or mixtures thereof. Each one of these specific buffers constitutes an alternative embodiment of the invention.

In a further embodiment of the invention the formulation further comprises a pharmaceutically acceptable preservative. In a further embodiment of the invention the preservative is selected from the group consisting of phenol, o-cresol, m-cresol, p-cresol, methyl p-hydroxybenzoate, propyl p-hydroxybenzoate, 2-phenoxyethanol, butyl p-hydroxybenzoate, 2-phenylethanol, benzyl alcohol, chlorobutanol, and thiomerosal, bronopol, benzoic acid, imidurea, chlorohexidine, sodium dehydroacetate, chlorocresol, ethyl p-hydroxybenzoate, benzethonium chloride, chlorphenesine (3p-chlorphenoxypropane-1,2-diol) or mixtures thereof. In an embodiment the preservative is phenol or m-cresol. In a further embodiment of the invention the preservative is present in a concentration from 0.1 mg/ml to 20 mg/ml. In a further embodiment of the invention the preservative is present in a concentration from 0.1 mg/ml to 5 mg/ml. In a further embodiment of the invention the preservative is present in a concentration from 5 mg/ml to 10 mg/ml. In a further embodiment of the invention the preservative is present in a concentration from 10 mg/ml to 20 mg/ml. Each one of these specific preservatives constitutes an alternative embodiment of the invention. The use of a preservative in pharmaceutical compositions is well-known to the skilled person. For convenience reference is made to Remington: *The Science and Practice of Pharmacy*, 19th edition, 1995.

In a further embodiment of the invention the formulation further comprises an isotonic agent. In a further embodiment of the invention the isotonic agent is selected from the group consisting of a salt (e.g. sodium chloride), a sugar or sugar alcohol, an amino acid (e.g. L-glycine, L-histidine, arginine, lysine, isoleucine, aspartic acid, tryptophan, threonine), an alditol (e.g. glycerol (glycerine), 1,2-propanediol (propyleneglycol), 1,3-propanediol, 1,3-butanediol) polyethyleneglycol (e.g. PEG400), or mixtures thereof. In an embodiment the isotoncity agent is propyleneglycol. Any sugar such as mono-, di-, or polysaccharides, or water-soluble glucans, including for example fructose, glucose, mannose, sorbose, xylose,

maltose, lactose, sucrose, trehalose, dextran, pullulan, dextrin, cyclodextrin, soluble starch, hydroxyethyl starch and carboxymethylcellulose-Na may be used. In one embodiment the sugar additive is sucrose. Sugar alcohol is defined as a C4-C8 hydrocarbon having at least one -OH group and includes, for example, mannitol, sorbitol, inositol, galactitol, dulcitol, xylitol, and arabitol. In one embodiment the sugar alcohol additive is mannitol. The sugars or sugar alcohols mentioned above may be used individually or in combination. There is no fixed limit to the amount used, as long as the sugar or sugar alcohol is soluble in the liquid preparation and does not adversely effect the stabilizing effects achieved using the methods of the invention. In one embodiment, the sugar or sugar alcohol concentration is between about 1 mg/ml and about 150 mg/ml. In a further embodiment of the invention the isotonic agent is present in a concentration from 1 mg/ml to 50 mg/ml. In a further embodiment of the invention the isotonic agent is present in a concentration from 1 mg/ml to 7 mg/ml. In an embodiment of the invention the isotonic agent is present in a concentration from 5 mg/ml to 7 mg/ml. In a further embodiment of the invention the isotonic agent is present in a concentration from 8 mg/ml to 24 mg/ml. In a further embodiment of the invention the isotonic agent is present in a concentration from 25 mg/ml to 50 mg/ml. Each one of these specific isotonic agents constitutes an alternative embodiment of the invention. The use of an isotonic agent in pharmaceutical compositions is well-known to the skilled person. For convenience reference is made to Remington: *The Science and Practice of Pharmacy*, 19th edition, 1995.

In a further embodiment of the invention the formulation further comprises a chelating agent. In a further embodiment of the invention the chelating agent is selected from salts of ethylenediaminetetraacetic acid (EDTA), citric acid, and aspartic acid, and mixtures thereof. In a further embodiment of the invention the chelating agent is present in a concentration from 0.1mg/ml to 5mg/ml. In a further embodiment of the invention the chelating agent is present in a concentration from 0.1mg/ml to 2mg/ml. In a further embodiment of the invention the chelating agent is present in a concentration from 2mg/ml to 5mg/ml. Each one of these specific chelating agents constitutes an alternative embodiment of the invention. The use of a chelating agent in pharmaceutical compositions is well-known to the skilled person. For convenience reference is made to Remington: *The Science and Practice of Pharmacy*, 19th edition, 1995.

In a further embodiment of the invention the formulation further comprises a stabilizer. The use of a stabilizer in pharmaceutical compositions is well-known to the skilled person. For convenience reference is made to Remington: *The Science and Practice of Pharmacy*, 19th edition, 1995.

More particularly, compositions of the invention are stabilized liquid pharmaceutical compositions whose therapeutically active components include a polypeptide that possibly exhibits aggregate formation during storage in liquid pharmaceutical formulations. By "aggregate formation" is intended a physical interaction between the polypeptide molecules that results in formation of oligomers, which may remain soluble, or large visible aggregates that precipitate from the solution. By "during storage" is intended a liquid pharmaceutical composition or formulation once prepared, is not immediately administered to a subject. Rather, following preparation, it is packaged for storage, either in a liquid form, in a frozen state, or in a dried form for later reconstitution into a liquid form or other form suitable for administration to a subject. By "dried form" is intended the liquid pharmaceutical composition or formulation is dried either by freeze drying (i.e., lyophilization; see, for example, Williams and Polli (1984) J. Parenteral Sci. Technol. 38:48-59), spray drying (see Masters (1991) in Spray-Drying Handbook (5th ed; Longman Scientific and Technical, Essez, U.K.), pp. 491-676; Broadhead et al. (1992) Drug Devel. Ind. Pharm. 18:1169-1206; and Mumenthaler et al. (1994) Pharm. Res. 11:12-20), or air drying (Carpenter and Crowe (1988) Cryobiology 25:459-470; and Roser (1991) Biopharm. 4:47-53). Aggregate formation by a polypeptide during storage of a liquid pharmaceutical composition can adversely affect biological activity of that polypeptide, resulting in loss of therapeutic efficacy of the pharmaceutical composition. Furthermore, aggregate formation may cause other problems such as blockage of tubing, membranes, or pumps when the polypeptide-containing pharmaceutical composition is administered using an infusion system.

The pharmaceutical compositions of the invention may further comprise an amount of an amino acid base sufficient to decrease aggregate formation by the polypeptide during storage of the composition. By "amino acid base" is intended an amino acid or a combination of amino acids, where any given amino acid is present either in its free base form or in its salt form. Where a combination of amino acids is used, all of the amino acids may be present in their free base forms, all may be present in their salt forms, or some may be present in their free base forms while others are present in their salt forms. In one embodiment, amino acids to use in preparing the compositions of the invention are those carrying a charged side chain, such as arginine, lysine, aspartic acid, and glutamic acid. Any stereoisomer (i.e., L, D, or a mixture thereof) of a particular amino acid (e.g. methionine, histidine, imidazole, arginine, lysine, isoleucine, aspartic acid, tryptophan, threonine and mixtures thereof) or combinations of these stereoisomers, may be present in the pharmaceutical compositions of the invention so long as the particular amino acid is present either in its free base form or its salt form. In one embodiment the L-stereoisomer is used. Compositions of the invention may also be formulated with analogues of these amino acids. By "amino acid analogue" is intended a

derivative of the naturally occurring amino acid that brings about the desired effect of decreasing aggregate formation by the polypeptide during storage of the liquid pharmaceutical compositions of the invention. Suitable arginine analogues include, for example, aminoguanidine, ornithine and N-monoethyl L-arginine, suitable methionine analogues include ethionine and buthionine and suitable cysteine analogues include S-methyl-L cysteine. As with the other amino acids, the amino acid analogues are incorporated into the compositions in either their free base form or their salt form. In a further embodiment of the invention the amino acids or amino acid analogues are used in a concentration, which is sufficient to prevent or delay aggregation of the protein.

In a further embodiment of the invention methionine (or other sulphuric amino acids or amino acid analogous) may be added to inhibit oxidation of methionine residues to methionine sulfoxide when the polypeptide acting as the therapeutic agent is a polypeptide comprising at least one methionine residue susceptible to such oxidation. By "inhibit" is intended minimal accumulation of methionine oxidized species over time. Inhibiting methionine oxidation results in greater retention of the polypeptide in its proper molecular form. Any stereoisomer of methionine (L or D) or combinations thereof can be used. The amount to be added should be an amount sufficient to inhibit oxidation of the methionine residues such that the amount of methionine sulfoxide is acceptable to regulatory agencies. Typically, this means that the composition contains no more than about 10% to about 30% methionine sulfoxide. Generally, this can be achieved by adding methionine such that the ratio of methionine added to methionine residues ranges from about 1:1 to about 1000:1, such as 10:1 to about 100:1.

In a further embodiment of the invention the formulation further comprises a stabilizer selected from the group of high molecular weight polymers or low molecular compounds. In a further embodiment of the invention the stabilizer is selected from polyethylene glycol (e.g. PEG 3350), polyvinyl alcohol (PVA), polyvinylpyrrolidone, carboxy-/hydroxycellulose or derivates thereof (e.g. HPC, HPC-SL, HPC-L and HPMC), cyclodextrins, sulphur-containing substances as monothioglycerol, thioglycolic acid and 2-methylthioethanol, and different salts (e.g. sodium chloride). Each one of these specific stabilizers constitutes an alternative embodiment of the invention.

The pharmaceutical compositions may also comprise additional stabilizing agents, which further enhance stability of a therapeutically active polypeptide therein. Stabilizing agents of particular interest to the present invention include, but are not limited to, methionine and EDTA, which protect the polypeptide against methionine oxidation, and a nonionic surfactant, which protects the polypeptide against aggregation associated with freeze-thawing or mechanical shearing.

In a further embodiment of the invention the formulation further comprises a surfactant. In another embodiment of the invention the pharmaceutical composition comprises two different surfactants. The term "Surfactant" as used herein refers to any molecules or ions that are comprised of a water-soluble (hydrophilic) part, the head, and a fat-soluble (lipophilic) segment. Surfactants accumulate preferably at interfaces, which the hydrophilic part is orientated towards the water (hydrophilic phase) and the lipophilic part towards the oil- or hydrophobic phase (*i.e.* glass, air, oil etc.). The concentration at which surfactants begin to form micelles is known as the critical micelle concentration or CMC. Furthermore, surfactants lower the surface tension of a liquid. Surfactants are also known as amphipathic compounds. The term "Detergent" is a synonym used for surfactants in general.

Anionic surfactants may be selected from the group of: Chenodeoxycholic acid, Chenodeoxycholic acid sodium salt, Cholic acid, Dehydrocholic acid, Deoxycholic acid, Deoxycholic acid methyl ester, Digitonin, Digitoxigenin, N,N-Dimethyldodecylamine N-oxide, Docusate sodium, Glycochenodeoxycholic acid sodium, Glycocholic acid hydrate, Glycodeoxycholic acid monohydrate, Glycodeoxycholic acid sodium salt, Glycodeoxycholic acid sodium salt, Glycolithocholic acid 3-sulfate disodium salt, Glycolithocholic acid ethyl ester, N-Lauroylsarcosine sodium salt, N-Lauroylsarcosine sodium salt, N-Lauroylsarcosine, N-Lauroylsarcosine, Lithium dodecyl sulfate, Lugol, 1-Octanesulfonic acid sodium salt, 1-Octanesulfonic acid sodium salt, Sodium 1-butanesulfonate, Sodium 1-decanesulfonate, Sodium 1-dodecanesulfonate, Sodium 1-heptanesulfonate, Sodium 1-heptanesulfonate, Sodium 1-nonanesulfonate, Sodium 1-propanesulfonate monohydrate, Sodium 2-bromoethanesulfonate, Sodium cholate hydrate, ox or sheep bile, Sodium cholate hydrate, Sodium choleate, Sodium deoxycholate, Sodium dodecyl sulfate, Sodium dodecyl sulfate, Sodium hexanesulfonate, Sodium octyl sulfate, Sodium pentanesulfonate, Sodium taurocholate, Taurochenodeoxycholic acid sodium salt, Taurodeoxycholic acid sodium salt monohydrate, Taurolithocholic acid 3-sulfate disodium salt, Tauroursodeoxycholic acid sodium salt, Trizma® dodecyl sulfate, DSS (docusate sodium, CAS registry no [577-11-7]), docusate calcium, CAS registry no [128-49-4]), docusate potassium, CAS registry no [7491-09-0]), SDS (sodium dodecyl sulfate or sodium lauryl sulfate), Dodecylphosphocholine (FOS-Choline-12), Decylphosphocholine (FOS-Choline-10), Nonylphosphocholine (FOS-Choline-9), dipalmitoyl phosphatidic acid, sodium caprylate, and/or Ursodeoxycholic acid.

Cationic surfactants may be selected from the group of: Alkyltrimethylammonium bromide

Benzalkonium chloride, Benzalkonium chloride, Benzyldimethylhexadecylammonium chloride, Benzyldimethyltetradecylammonium chloride,

Benzyltrimethylammonium tetrachloroiodate, Dimethyldioctadecylammonium bromide, Dodecylethyldimethylammonium bromide, Dodecyltrimethylammonium bromide, Dodecyltrimethylammonium bromide, Ethylhexadecyldimethylammonium bromide, Hexadecyltrimethylammonium bromide, Hexadecyltrimethylammonium bromide, Polyoxyethylene(10)-N-tallow-1,3-diaminopropane, Thonzonium bromide, and/or Trimethyl(tetradecyl)ammonium bromide.

Nonionic surfactants may be selected from the group of: BigCHAP, Bis(polyethylene glycol bis[imidazoyl carbonyl]), block copolymers as polyethyleneoxide/polypropyleneoxide block copolymers such as poloxamers, poloxamer 188 and poloxamer 407, Brij® 35, Brij® 56, Brij® 72, Brij® 76, Brij® 92V, Brij® 97, Brij® 58P, Cremophor® EL, Decaethylene glycol monododecyl ether, N-Decanoyl-N-methylglucamine, n-Dodecanoyl-N-methylglucamide, alkyl-polyglucosides, ethoxylated castor oil, Heptaethylene glycol monodecyl ether, Heptaethylene glycol monododecyl ether, Heptaethylene glycol monotetradecyl ether, Hexaethylene glycol monododecyl ether, Hexaethylene glycol monohexadecyl ether, Hexaethylene glycol monooctadecyl ether, Hexaethylene glycol monotetradecyl ether, Igepal CA-630, Igepal CA-630, Methyl-6-O-(N-heptylcarbamoyl)-beta-D-glucopyranoside, Nonaethylene glycol monododecyl ether, N-Nonanoyl-N-methylglucamine, N-Nonanoyl-N-methylglucamine, Octaethylene glycol monodecyl ether, Octaethylene glycol monododecyl ether, Octaethylene glycol monohexadecyl ether, Octaethylene glycol monooctadecyl ether, Octaethylene glycol monotetradecyl ether, Octyl-β-D-glucopyranoside, Pentaethylene glycol monodecyl ether, Pentaethylene glycol monododecyl ether, Pentaethylene glycol monohexadecyl ether, Pentaethylene glycol monohexyl ether, Pentaethylene glycol monooctadecyl ether, Pentaethylene glycol monooctyl ether, Polyethylene glycol diglycidyl ether, Polyethylene glycol ether W-1, Polyoxyethylene 10 tridecyl ether, Polyoxyethylene 100 stearate, Polyoxyethylene 20 isohexadecyl ether, Polyoxyethylene 20 oleyl ether, Polyoxyethylene 40 stearate, Polyoxyethylene 50 stearate, Polyoxyethylene 8 stearate, Polyoxyethylene bis(imidazolyl carbonyl), Polyoxyethylene 25 propylene glycol stearate, Saponin from Quillaja bark, Span® 20, Span® 40, Span® 60, Span® 65, Span® 80, Span® 85, Tergitol, Type 15-S-12, Tergitol, Type 15-S-30, Tergitol, Type 15-S-5, Tergitol, Type 15-S-7, Tergitol, Type 15-S-9, Tergitol, Type NP-10, Tergitol, Type NP-4, Tergitol, Type NP-40, Tergitol, Type NP-7, Tergitol, Type NP-9, Tetradecyl-β-D-maltoside, Tetraethylene glycol monodecyl ether, Tetraethylene glycol monododecyl ether, Tetraethylene glycol monotetradecyl ether, Triethylene glycol monodecyl ether, Triethylene glycol monododecyl ether, Triethylene glycol monohexadecyl ether, Triethylene glycol monooctyl ether, Triethylene glycol monotetradecyl ether, Triton CF-21, Triton CF-32, Triton DF-12, Triton DF-16, Triton GR-5M, Triton QS-15,

Triton QS-44, Triton X-100, Triton X-102, Triton X-15, Triton X-151, Triton X-200, Triton X-207, Triton® X-100, Triton® X-114, Triton® X-165 solution, Triton® X-305 solution, Triton® X-405, Triton® X-45, Triton® X-705-70, TWEEN® 20, TWEEN® 40, TWEEN® 60, TWEEN® 6, TWEEN® 65, TWEEN® 80, TWEEN® 81, TWEEN® 85, Tyloxapol, sphingophospholipids (sphingomyelin), and sphingoglycolipids (ceramides, gangliosides), phospholipids, and/or n-Undecyl β-D-glucopyranoside. ´

Zwitterionic surfactants may be selected from the group of: CHAPS, CHAPSO, 3-(Decyldimethylammonio)propanesulfonate inner salt, 3-(Dodecyldimethylammonio)-propanesulfonate inner salt, 3-(Dodecyldimethylammonio)propanesulfonate inner salt, 3-(N,N-Dimethylmyristylammonio)propanesulfonate, 3-(N,N-Dimethyloctadecylammonio)-propanesulfonate, 3-(N,N-Dimethyloctylammonio)propanesulfonate inner salt, 3-(N,N-Dimethylpalmitylammonio)propanesulfonate, N-alkyl-N,N-dimethylammonio-1-propanesulfonates, 3-cholamido-1-propyldimethylammonio-1-propanesulfonate, Dodecylphosphocholine, myristoyl lysophosphatidylcholine, Zwittergent 3-12 (*N*-dodecyl-*N*,*N*-dimethyl-3-ammonio-1-propanesulfonate), Zwittergent 3-10 (3-(Decyldimethylammonio)-propanesulfonate inner salt), Zwittergent 3-08 (3-(Octyldimethylammonio)pro-panesulfonate), glycerophospholipids (lecithins, kephalins, phosphatidyl serine), glyceroglycolipids (galactopyranoside), alkyl, alkoxyl (alkyl ester), alkoxy (alkyl ether)- derivatives of lysophosphatidyl and phosphatidylcholines, e.g. lauroyl and myristoyl derivatives of lysophosphatidylcholine, dipalmitoylphosphatidylcholine, and modifications of the polar head group, that is cholines, ethanolamines, phosphatidic acid, serines, threonines, glycerol, inositol, lysophosphatidylserine and lysophosphatidylthreonine, acylcarnitines and derivatives, N^{beta}-acylated derivatives of lysine, arginine or histidine, or side-chain acylated derivatives of lysine or arginine, N^{beta}-acylated derivatives of dipeptides comprising any combination of lysine, arginine or histidine and a neutral or acidic amino acid, N^{beta}-acylated derivative of a tripeptide comprising any combination of a neutral amino acid and two charged amino acids, or the surfactant may be selected from the group of imidazoline derivatives, long-chain fatty acids and salts thereof C_6-C_{12} (eg. oleic acid and caprylic acid), N-Hexadecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate, anionic (alkyl-aryl-sulphonates) monovalent surfactants, palmitoyl lysophosphatidyl-L-serine, lysophospholipids (e.g. 1-acyl-sn-glycero-3-phosphate esters of ethanolamine, choline, serine or threonine), or mixtures thereof.

The term “alkyl-polyglucosides” as used herein in relates to an straight or branched C_{5-20}-alkyl, -alkenyl or -alkynyl chain which is substituted by one or more glucoside moieties such as maltoside, saccharide etc. Embodiments of these alkyl-polyglucosides include C_{6-18}-alkyl-polyglucosides. Specific embodiments of these alkyl-polyglucosides includes the even

numbered carbon-chains such as C_6, C_8, C_{10}, C_{12}, C_{14}, C_{16}, C_{18} and C_{20} alkyl chain. Specific embodiments of the glucoside moieties include pyranoside, glucopyranoside, maltoside, maltotrioside and sucrose. In embodiments of the invention less than 6 glucosid moieties are attached to the alkyl group. In embodiments of the invention less than 5 glucosid moieties are attached to the alkyl group. In embodiments of the invention less than 4 glucosid moieties are attached to the alkyl group. In embodiments of the invention less than 3 glucosid moieties are attached to the alkyl group. In embodiments of the invention less than 2 glucosid moieties are attached to the alkyl group. Specific embodiments of alkyl-polyglucosides are alkyl glucosides such n-decyl β-D-glucopyranoside, decyl β-D-maltopyranoside, dodecyl β-D-glucopyranoside, n-dodecyl β-D-maltoside, n-dodecyl β-D-maltoside, n-dodecyl β-D-maltoside, tetradecyl β-D-glucopyranoside, decyl β-D-maltoside, hexadecyl β-D-maltoside, decyl β-D-maltotrioside, dodecyl β-D-maltotrioside, tetradecyl β-D-maltotrioside, hexadecyl β-D-maltotrioside, n-dodecyl-sucrose, n-decyl-sucrose, sucrose monocaprate, sucrose monolaurate, sucrose monomyristate, and sucrose monopalmitate.

The use of a surfactant in pharmaceutical compositions is well-known to the skilled person. For convenience reference is made to Remington: *The Science and Practice of Pharmacy*, 19th edition, 1995.

In a further embodiment of the invention the formulation further comprises protease inhibitors such as EDTA (ethylenediamine tetraacetic acid) and benzamidineHCl, but other commercially available protease inhibitors may also be used. The use of a protease inhibitor is particular useful in pharmaceutical compositions comprising zymogens of proteases in order to inhibit autocatalysis.

It is possible that other ingredients may be present in the peptide pharmaceutical formulation of the present invention. Such additional ingredients may include wetting agents, emulsifiers, antioxidants, bulking agents, tonicity modifiers, chelating agents, metal ions, oleaginous vehicles, proteins (e.g., human serum albumin, gelatine or proteins) and a zwitterion (e.g., an amino acid such as betaine, taurine, arginine, glycine, lysine and histidine). Such additional ingredients, of course, should not adversely affect the overall stability of the pharmaceutical formulation of the present invention.

Pharmaceutical compositions containing a compound according to the present invention may be administered to a patient in need of such treatment at several sites, for example, at topical sites, for example, skin and mucosal sites, at sites which bypass absorption, for example, administration in an artery, in a vein, in the heart, and at sites which involve absorption, for example, administration in the skin, under the skin, in a muscle or in the abdomen.

Administration of pharmaceutical compositions according to the invention may be through several routes of administration, for example, lingual, sublingual, buccal, in the mouth, oral, in the stomach and intestine, nasal, pulmonary, for example, through the bronchioles and alveoli or a combination thereof, epidermal, dermal, transdermal, vaginal, rectal, ocular, for examples through the conjunctiva, uretal, and parenteral to patients in need of such a treatment.

Compositions of the current invention may be administered in several dosage forms, for example, as solutions, suspensions, emulsions, microemulsions, multiple emulsion, foams, salves, pastes, plasters, ointments, tablets, coated tablets, rinses, capsules, for example, hard gelatine capsules and soft gelatine capsules, suppositories, rectal capsules, drops, gels, sprays, powder, aerosols, inhalants, eye drops, ophthalmic ointments, ophthalmic rinses, vaginal pessaries, vaginal rings, vaginal ointments, injection solution, in situ transforming solutions, for example in situ gelling, in situ setting, in situ precipitating, in situ crystallization, infusion solution, and implants.

Compositions of the invention may further be compounded in, or attached to, for example through covalent, hydrophobic and electrostatic interactions, a drug carrier, drug delivery system and advanced drug delivery system in order to further enhance stability of the compound of the present invention, increase bioavailability, increase solubility, decrease adverse effects, achieve chronotherapy well known to those skilled in the art, and increase patient compliance or any combination thereof. Examples of carriers, drug delivery systems and advanced drug delivery systems include, but are not limited to, polymers, for example cellulose and derivatives, polysaccharides, for example dextran and derivatives, starch and derivatives, poly(vinyl alcohol), acrylate and methacrylate polymers, polylactic and polyglycolic acid and block co-polymers thereof, polyethylene glycols, carrier proteins, for example albumin, gels, for example, thermogelling systems, for example block co-polymeric systems well known to those skilled in the art, micelles, liposomes, microspheres, nanoparticulates, liquid crystals and dispersions thereof, L2 phase and dispersions there of, well known to those skilled in the art of phase behaviour in lipid-water systems, polymeric micelles, multiple emulsions, self-emulsifying, self-microemulsifying, cyclodextrins and derivatives thereof, and dendrimers.

Compositions of the current invention are useful in the formulation of solids, semisolids, powder and solutions for pulmonary administration of compounds of the present invention, using, for example a metered dose inhaler, dry powder inhaler and a nebulizer, all being devices well known to those skilled in the art.

Compositions of the current invention are specifically useful in the formulation of controlled, sustained, protracting, retarded, and slow release drug delivery systems. More specifically,

but not limited to, compositions are useful in formulation of parenteral controlled release and sustained release systems (both systems leading to a many-fold reduction in number of administrations), well known to those skilled in the art. Even more preferably, are controlled release and sustained release systems administered subcutaneous. Without limiting the scope of the invention, examples of useful controlled release system and compositions are hydrogels, oleaginous gels, liquid crystals, polymeric micelles, microspheres, nanoparticles, Methods to produce controlled release systems useful for compositions of the current invention include, but are not limited to, crystallization, condensation, co-crystallization, precipitation, co-precipitation, emulsification, dispersion, high pressure homogenisation, encapsulation, spray drying, microencapsulating, coacervation, phase separation, solvent evaporation to produce microspheres, extrusion and supercritical fluid processes. General reference is made to Handbook of Pharmaceutical Controlled Release (Wise, D.L., ed. Marcel Dekker, New York, 2000) and Drug and the Pharmaceutical Sciences vol. 99: Protein Formulation and Delivery (MacNally, E.J., ed. Marcel Dekker, New York, 2000).

Parenteral administration may be performed by subcutaneous, intramuscular, intraperitoneal or intravenous injection by means of a syringe, optionally a pen-like syringe. Alternatively, parenteral administration can be performed by means of an infusion pump. A further option is a composition which may be a solution or suspension or a powder for the administration of the compound of the present invention in the form of a nasal or pulmonal liquid or powder spray. As a still further option, the pharmaceutical compositions containing the compound of the invention can also be adapted to transdermal administration, *e.g.* by needle-free injection or from a patch, optionally an iontophoretic patch, or transmucosal, *e.g.* buccal, administration.

The compounds of the present invention can be administered via the pulmonary route in a vehicle, as a solution, suspension or dry powder using any of known types of devices suitable for pulmonary drug delivery. Examples of these comprise, but are not limited to, the three general types of aerosol-generating for pulmonary drug delivery, and may include jet or ultrasonic nebulizers, metered-dose inhalers, or dry powder inhalers (Cf. Yu J, Chien YW. Pulmonary drug delivery: Physiologic and mechanistic aspects. Crit Rev Ther Drug Carr Sys 14(4) (1997) 395-453).

Based on standardised testing methodology, the aerodynamic diameter (d_a) of a particle is defined as the geometric equivalent diameter of a reference standard spherical particle of unit density (1 g/cm^3). In the simplest case, for spherical particles, d_a is related to a reference diameter (d) as a function of the square root of the density ratio as described by:

Modifications to this relationship occur for non-spherical particles (cf. Edwards DA, Ben-Jebria A, Langer R. Recent advances in pulmonary drug delivery using large, porous inhaled particles. J Appl Physiol 84(2) (1998) 379-385). The terms "MMAD" and "MMEAD" are well-described and known to the art (cf. Edwards DA, Ben-Jebria A, Langer R and represents a measure of the median value of an aerodynamic particle size distribution. Recent advances in pulmonary drug delivery using large, porous inhaled particles. J Appl Physiol 84(2) (1998) 379-385). Mass median aerodynamic diameter (MMAD) and mass median effective aerodynamic diameter (MMEAD) are used inter-changeably, are statistical parameters, and empirically describe the size of aerosol particles in relation to their potential to deposit in the lungs, independent of actual shape, size, or density (cf. Edwards DA, Ben-Jebria A, Langer R. Recent advances in pulmonary drug delivery using large, porous inhaled particles. J Appl Physiol 84(2) (1998) 379-385). MMAD is normally calculated from the measurement made with impactors, an instrument that measures the particle inertial behaviour in air.
In a further embodiment, the formulation could be aerosolized by any known aerosolisation technology, such as nebulisation, to achieve a MMAD of aerosol particles less than 10 µm, more preferably between 1-5 µm, and most preferably between 1-3 µm. The preferred particle size is based on the most effective size for delivery of drug to the deep lung, where protein is optimally absorbed (cf. Edwards DA, Ben-Jebria A, Langer A, Recent advances in pulmonary drug delivery using large, porous inhaled particles. J Appl Physiol 84(2) (1998) 379-385).
Deep lung deposition of the pulmonal formulations comprising the compound of the present invention may optional be further optimized by using modifications of the inhalation techniques, for example, but not limited to: slow inhalation flow (eg. 30 L/min), breath holding and timing of actuation.

The term "stabilized formulation" refers to a formulation with increased physical stability, increased chemical stability or increased physical and chemical stability.

The term "physical stability" of the protein formulation as used herein refers to the tendency of the protein to form biologically inactive and/or insoluble aggregates of the protein as a result of exposure of the protein to thermo-mechanical stresses and/or interaction with interfaces and surfaces that are destabilizing, such as hydrophobic surfaces and interfaces. Physical stability of the aqueous protein formulations is evaluated by means of visual inspection and/or turbidity measurements after exposing the formulation filled in suitable containers (e.g. cartridges or vials) to mechanical/physical stress (e.g. agitation) at different temperatures for various time periods. Visual inspection of the formulations is performed in a

sharp focused light with a dark background. The turbidity of the formulation is characterized by a visual score ranking the degree of turbidity for instance on a scale from 0 to 3 (a formulation showing no turbidity corresponds to a visual score 0, and a formulation showing visual turbidity in daylight corresponds to visual score 3). A formulation is classified physical unstable with respect to protein aggregation, when it shows visual turbidity in daylight. Alternatively, the turbidity of the formulation can be evaluated by simple turbidity measurements well-known to the skilled person. Physical stability of the aqueous protein formulations can also be evaluated by using a spectroscopic agent or probe of the conformational status of the protein. The probe is preferably a small molecule that preferentially binds to a non-native conformer of the protein. One example of a small molecular spectroscopic probe of protein structure is Thioflavin T. Thioflavin T is a fluorescent dye that has been widely used for the detection of amyloid fibrils. In the presence of fibrils, and perhaps other protein configurations as well, Thioflavin T gives rise to a new excitation maximum at about 450 nm and enhanced emission at about 482 nm when bound to a fibril protein form. Unbound Thioflavin T is essentially non-fluorescent at the wavelengths.
Other small molecules can be used as probes of the changes in protein structure from native to non-native states. For instance the “hydrophobic patch” probes that bind preferentially to exposed hydrophobic patches of a protein. The hydrophobic patches are generally buried within the tertiary structure of a protein in its native state, but become exposed as a protein begins to unfold or denature. Examples of these small molecular, spectroscopic probes are aromatic, hydrophobic dyes, such as antrhacene, acridine, phenanthroline or the like. Other spectroscopic probes are metal-amino acid complexes, such as cobalt metal complexes of hydrophobic amino acids, such as phenylalanine, leucine, isoleucine, methionine, and valine, or the like.

The term “chemical stability” of the protein formulation as used herein refers to chemical covalent changes in the protein structure leading to formation of chemical degradation products with potential less biological potency and/or potential increased immunogenic properties compared to the native protein structure. Various chemical degradation products can be formed depending on the type and nature of the native protein and the environment to which the protein is exposed. Elimination of chemical degradation can most probably not be completely avoided and increasing amounts of chemical degradation products is often seen during storage and use of the protein formulation as well-known by the person skilled in the art. Most proteins are prone to deamidation, a process in which the side chain amide group in glutaminyl or asparaginyl residues is hydrolysed to form a free carboxylic acid. Other

degradations pathways involves formation of high molecular weight transformation products where two or more protein molecules are covalently bound to each other through transamidation and/or disulfide interactions leading to formation of covalently bound dimer, oligomer and polymer degradation products (*Stability of Protein Pharmaceuticals, Ahern. T.J. & Manning M.C., Plenum Press, New York 1992*). Oxidation (of for instance methionine residues) can be mentioned as another variant of chemical degradation. The chemical stability of the protein formulation can be evaluated by measuring the amount of the chemical degradation products at various time-points after exposure to different environmental conditions (the formation of degradation products can often be accelerated by for instance increasing temperature). The amount of each individual degradation product is often determined by separation of the degradation products depending on molecule size and/or charge using various chromatography techniques (e.g. SEC-HPLC and/or RP-HPLC).

Hence, as outlined above, a "stabilized formulation" refers to a formulation with increased physical stability, increased chemical stability or increased physical and chemical stability. In general, a formulation must be stable during use and storage (in compliance with recommended use and storage conditions) until the expiration date is reached.

In one embodiment of the invention the pharmaceutical formulation comprising the compound of the present invention is stable for more than 6 weeks of usage and for more than 3 years of storage.
In another embodiment of the invention the pharmaceutical formulation comprising the compound of the present invention is stable for more than 4 weeks of usage and for more than 3 years of storage.
In a further embodiment of the invention the pharmaceutical formulation comprising the compound of the present invention is stable for more than 4 weeks of usage and for more than two years of storage.
In an even further embodiment of the invention the pharmaceutical formulation comprising the compound of the present invention is stable for more than 2 weeks of usage and for more than two years of storage.

In another aspect the present invention relates to the use of a compound according to the invention for the preparation of a medicament.

In one embodiment a compound according to the invention is used for the preparation of a medicament for the treatment or prevention of hyperglycemia, type 2 diabetes, impaired glucose tolerance, type 1 diabetes, obesity, hypertension, syndrome X, dyslipidemia, cognitive

disorders, atheroschlerosis, myocardial infarction, stroke, coronary heart disease and other cardiovascular disorders, inflammatory bowel syndrome, dyspepsia and gastric ulcers.

In another embodiment a compound according to the invention is used for the preparation of a medicament for delaying or preventing disease progression in type 2 diabetes.

In another embodiment a compound according to the invention is used for the preparation of a medicament for decreasing food intake, decreasing β-cell apoptosis, increasing β-cell function and β-cell mass, and/or for restoring glucose sensitivity to β-cells.

The treatment with a compound according to the present invention may also be combined with a second or more pharmacologically active substances, e.g. selected from antidiabetic agents, antiobesity agents, appetite regulating agents, antihypertensive agents, agents for the treatment and/or prevention of complications resulting from or associated with diabetes and agents for the treatment and/or prevention of complications and disorders resulting from or associated with obesity. Examples of these pharmacologically active substances are : Insulin, sulphonylureas, biguanides, meglitinides, glucosidase inhibitors, glucagon antagonists, DPP-IV (dipeptidyl peptidase-IV) inhibitors, inhibitors of hepatic enzymes involved in stimulation of gluconeogenesis and/or glycogenolysis, glucose uptake modulators, compounds modifying the lipid metabolism such as antihyperlipidemic agents as HMG CoA inhibitors (statins), Gastric Inhibitory Polypeptides (GIP analogs), compounds lowering food intake, RXR agonists and agents acting on the ATP-dependent potassium channel of the β-cells; Cholestyramine, colestipol, clofibrate, gemfibrozil, lovastatin, pravastatin, simvastatin, probucol, dextrothyroxine, neteglinide, repaglinide; β-blockers such as alprenolol, atenolol, timolol, pindolol, propranolol and metoprolol, ACE (angiotensin converting enzyme) inhibitors such as benazepril, captopril, enalapril, fosinopril, lisinopril, alatriopril, quinapril and ramipril, calcium channel blockers such as nifedipine, felodipine, nicardipine, isradipine, nimodipine, diltiazem and verapamil, and α-blockers such as doxazosin, urapidil, prazosin and terazosin; CART (cocaine amphetamine regulated transcript) agonists, NPY (neuropeptide Y) antagonists, PYY agonist, PYY2 agonists, PYY4 agonits, mixed PPY2/PYY4 agonists, MC4 (melanocortin 4) agonists, orexin antagonists, TNF (tumor necrosis factor) agonists, CRF (corticotropin releasing factor) agonists, CRF BP (corticotropin releasing factor binding protein) antagonists, urocortin agonists, β3 agonists, MSH (melanocyte-stimulating hormone) agonists, MCH (melanocyte-concentrating hormone) antagonists, CCK (cholecystokinin) agonists, serotonin re-uptake inhibitors, serotonin and noradrenaline re-uptake inhibitors, mixed serotonin and noradrenergic compounds, 5HT (serotonin) agonists, bombesin agonists, galanin antagonists, growth hormone, growth hormone releasing compounds, TRH (thyreotropin releasing hormone) agonists, UCP 2 or 3

(uncoupling protein 2 or 3) modulators, leptin agonists, DA agonists (bromocriptin, doprexin), lipase/amylase inhibitors, RXR (retinoid X receptor) modulators, TR β agonists; histamine H3 antagonists, Gastric Inhibitory Polypeptide agonists or antagonists (GIP analogs), gastrin and gastrin analogs.

The treatment with a compound according to this invention may also be combined with surgery- a surgery that influence the glucose levels and/or lipid homeostasis such as gastric banding or gastric bypass.

It should be understood that any suitable combination of the compounds according to the invention with one or more of the above-mentioned compounds and optionally one or more further pharmacologically active substances are considered to be within the scope of the present invention.

The present invention is further illustrated by the following examples which, however, are not to be construed as limiting the scope of protection. The features disclosed in the foregoing description and in the following examples may, both separately and in any combination thereof, be material for realising the invention in diverse forms thereof.

EXAMPLES

Abbreviations used:

r.t: Room temperature
DIPEA: diisopropylethylamine
H_2O: water
CH_3CN: acetonitrile
DMF: NN dimethylformamide
HBTU: 2-(1H-Benzotriazol-1-yl-)-1,1,3,3 tetramethyluronium hexafluorophosphate
Fmoc: 9 H-fluoren-9-ylmethoxycarbonyl
Boc: tert butyloxycarbonyl
OtBu: tert butyl ester
tBu: tert butyl
Trt: triphenylmethyl
Pmc: 2,2,5,7,8-Pentamethyl-chroman-6-sulfonyl
Dde: 1-(4,4-Dimethyl-2,6-dioxocyclohexylidene)ethyl
ivDde: 1-(4,4-Dimethyl-2,6-dioxocyclohexylidene)-3-methylbutyl
Mtt: 4-methyltrityl
Mmt: 4-methoxytrityl
DCM: dichloromethane

TIS: triisopropylsilane)
TFA: trifluoroacetic acid
Et_2O: diethylether
NMP: 1-Methyl-pyrrolidin-2-one
DIPEA: Diisopropylethylamine
HOAt: 1-Hydroxy-7-azabenzotriazole
HOBt: 1-Hydroxybenzotriazole
DIC: Diisopropylcarbodiimide

A: Synthesis of resin bound peptide.

The protected peptidyl resin was synthesized according to the Fmoc strategy on an Applied Biosystems 433 peptide synthesizer in 0.25 mmol or 1.0 mmol scale using the manufacturer supplied FastMoc UV protocols which employ HBTU (2-(1H-Benzotriazol-1-yl-)-1,1,3,3 tetramethyluronium hexafluorophosphate) or HATU (O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate) mediated couplings in NMP (N-methyl pyrrolidone), and UV monitoring of the deprotection of the Fmoc protection group. The starting resin used for the synthesis of the GLP-1 peptide amides was Rink-Amide resin and either Wang or chlorotrityl resin was used for GLP-1 peptides with a carboxy C-terminal. The protected amino acid derivatives used were standard Fmoc-amino acids (supplied from e.g. Anaspec, or Novabiochem) supplied in preweighed cartridges suitable for the ABI433A synthesizer with the exception of unnatural aminoacids such as Fmoc-Aib-OH (Fmoc-aminoisobutyric acid). The N terminal amino acid was Boc protected at the alpha amino group (e.g. Boc-His(Boc)OH was used for peptides with His at the N-terminal). The epsilon amino group of lysine in position 26 was either protected with Mtt, Mmt, Dde, ivDde, or Boc, depending on the route for attachment of the albumin binding moiety and spacer. The synthesis of the peptides may in some cases be improved by the use of dipeptides protected on the dipeptide amide bond with a group that can be cleaved under acidic conditions such but not limited to 2-Fmoc-oxy-4-methoxybenzyl or 2,4,6-trimethoxybenzyl. In cases where a serine or a threonine is present in the peptide, the use of pseudoproline dipeptides may be used (see e.g. catalogue from Novobiochem 2002/2003 or newer version, or W.R. Sampson (1999), J. Pep. Sci. 5, 403.

<u>Procedure for removal of ivDde or Dde-protection</u>.

The resin (0.25 mmol) was placed in a manual shaker/filtration apparatus and treated with 2% hydrazine in N-methyl pyrrolidone (20 ml, 2x12 min) to remove the Dde or

ivDde group and wash with N-methyl pyrrolidone (4x20 ml).

Procedure for removal of Mtt or Mmt-protection.

The resin (0.25 mmol) was placed in a manual shaker/filtration apparatus and treated with 2% TFA and 2-3% TIS in DCM (20 ml, 5-10 min repeated 6-12 times) to remove the Mtt or Mmt group and wash with DCM (2x20 ml), 10%MeOH and 5% DIPEA in DCM (2x20ml) and N-methyl pyrrolidone (4x20 ml).

Procedure for attachment of sidechains to Lysine residue.

The albumin binding residue (B-U- sidechain of formula I) can be attached to the GLP-1 peptide either by acylation to resin bound peptide or acylation in solution to the unprotected peptide using standard acylating reagent such as but not limited to DIC, HOBt/DIC, HOAt/DIC, or HBTU.

Attachment to resin bound peptide:

Route I

Activated (active ester or symmetric anhydride) albumin binding residue (B-U- sidechain of formula I) such as octadecanedioic acid mono-(2,5-dioxo-pyrrolidin-1-yl) ester (Ebashi *et al.* EP511600, 4 molar equivalents relative to resin bound peptide) was dissolved in NMP (25 mL), added to the resin and shaken overnight at room temperature. The reaction mixture was filtered and the resin was washed extensively with NMP, dichloromethane, 2-propanol, methanol and diethyl ether.

Route II

The albumin binding residue (B-U- sidechain of formula I) was dissolved in N-methyl pyrrolidone/methylene chloride (1:1, 10 ml). The activating reagent such as hydroxybenzotriazole (HOBt) (4 molar equivalents relative to resin) and diisopropylcarbodiimide (4 molar equivalents relative to resin) was added and the solution was stirred for 15 min. The solution was added to the resin and diisopropyethylamine (4 molar equivalents relative to resin) was added. The resin was shaken 2 to 24 hours at room temperature. The resin was washed with N-methyl pyrrolidone (2x20 ml), N-methyl pyrrolidone/Methylene chloride (1:1) (2x20ml) and methylene chloride (2x20 ml).

Route III

Activated (active ester or symmetric anhydride) albumin binding residue (B-U- sidechain of formula I) such as octadecanedioic acid mono-(2,5-dioxo-pyrrolidin-1-yl) ester (Ebashi *et al.*

EP511600, 1-1.5 molar equivalents relative to the GLP-1 peptide was dissolved in an organic solvent such as acetonitrile, THF, DMF, DMSO or in a mixture of water/organic solvent (1-2 ml) and added to a solution of the peptide in water (10-20ml) together with 10 molar equivalents of DIPEA. In case of protecting groups on the albumin binding residue such as tert.-butyl, the reaction mixture was lyophilized O/N and the isolated crude peptide deprotected afterwards – in case of a *tert*-butyl group the peptide was dissolved in a mixture of trifluoroacetic acid, water and triisopropylsilane (90:5:5). After for 30min the mixture was, evaporated in vacuo and the finale petide purified by preparative HPLC.

Procedure for removal of Fmoc-protection: The resin (0.25 mmol) was placed in a filter flask in a manual shaking apparatus and treated with N-methyl pyrrolidone/methylene chloride (1:1) (2x20 ml) and with N-methyl pyrrolidone (1x20 ml), a solution of 20% piperidine in N-methyl pyrrolidone (3x20 ml, 10 min each). The resin was washed with N-methyl pyrrolidone (2x20 ml), N-methyl pyrrolidone/Methylene chloride (1:1) (2x20ml) and methylene chloride (2x20 ml).

Procedure for cleaving the peptide off the resin:
The peptide was cleaved from the resin by stirring for 180 min at room temperature with a mixture of trifluoroacetic acid, water and triisopropylsilane (95:2.5:2.5 to 92:4:4). The cleavage mixture was filtered and the filtrate was concentrated to an oil by a stream of nitrogen. The crude peptide was precipitated from this oil with 45 ml diethyl ether and washed 1 to 3 times with 45 ml diethyl ether.

Purification: The crude peptide was purified by semipreparative HPLC on a 20 mm x 250 mm column packed with either 5μ or 7μ C-18 silica. Depending on the peptide one or two purification systems were used.
TFA: After drying the crude peptide was dissolved in 5 ml 50% acetic acid H_2O and diluted to 20 ml with H_2O and injected on the column which then was eluted with a gradient of 40-60 % CH_3CN in 0.1% TFA 10 ml/min during 50 min at 40 °C. The peptide containing fractions were collected. The purified peptide was lyophilized after dilution of the eluate with water.
Ammonium sulphate: The column was equilibrated with 40% CH_3CN in 0.05M $(NH_4)_2SO_4$, which was adjusted to pH 2.5 with concentrated H_2SO_4. After drying the crude peptide was dissolved in 5 ml 50% acetic acid H_2O and diluted to 20 ml with H_2O and injected on the column which then was eluted with a gradient of 40% - 60% CH_3CN in 0.05M $(NH_4)_2SO_4$, pH 2.5 at 10 ml/min during 50 min at 40 °C. The peptide containing fractions were collected and diluted with 3 volumes of H_2O and passed through a Sep-Pak® C18 cartridge (Waters part.

#:51910) which has been equilibrated with 0.1% TFA. It was then eluted with 70% CH_3CN containing 0.1% TFA and the purified peptide was isolated by lyophilisation after dilution of the eluate with water.

The final product obtained was characterised by analytical RP-HPLC (retention time) and by LCMS

The RP-HPLC analysis was performed using UV detection at 214 nm and a Vydac 218TP54 4.6mm x 250mm 5µ C-18 silica column (The Separations Group, Hesperia, USA) which was eluted at 1 ml/min at 42 °C. Two different elution conditions were used:

A1: Equilibration of the column with in a buffer consisting of 0.1M $(NH_4)_2SO_4$, which was adjusted to pH 2.5 with concentrated H_2SO_4 and elution by a gradient of 0% to 60% CH_3CN in the same buffer during 50 min.

B1: Equilibration of the column with 0.1% TFA / H_2O and elution by a gradient of 0% CH_3CN / 0.1% TFA / H_2O to 60% CH_3CN / 0.1% TFA / H_2O during 50 min.

B6: Equilibration of the column with 0.1% TFA / H_2O and elution by a gradient of 0% CH_3CN / 0.1% TFA / H_2O to 90% CH_3CN / 0.1% TFA / H_2O during 50 min.

Alternative the RP-HPLC analysis was performed using UV detection at 214 nm and a Symmetry300, 3.6mm x 150mm, 3.5µ C-18 silica column (Waters) which was eluted at 1 ml/min at 42 °C.

B4: Equilibration of the column with 0.05% TFA / H_2O and elution by a gradient of 5% CH_3CN / 0.05% TFA / H_2O to 95% CH_3CN / 0.05% TFA / H_2O during 15 min.

The following instrumentation was used:

LCMS was performed on a setup consisting of Sciex API 100 Single quadropole mass spectrometer, Perkin Elmer Series 200 Quard pump ,Perkin Elmer Series 200 autosampler, Applied Biosystems 785A UV detector, Sedex 75 evaporative light scattering detector

The instrument control and data acquisition were done by the Sciex Sample control software running on a Windows 2000 computer.

The HPLC pump is connected to two eluent reservoirs containing:

A: 0.05% Trifluoro acetic acid in water

B: 0.05% Trifluoro acetic acid in acetonitrile

The analysis is performed at room temperature by injecting an appropriate volume of the sample (preferably 20 µl) onto the column which is eluted with a gradient of acetonitrile.

The HPLC conditions, detector settings and mass spectrometer settings used are giving in the following table.

Column : Waters Xterra MS C-18 X 3 mm id 5 μm
Gradient : 5% - 90 % acetonitrile linear during 7.5 min at 1.5ml/min
Detection : 210 nm (analogue output from DAD)
ELS (analogue output from ELS), 40 °C
MS ionisation mode API-ES

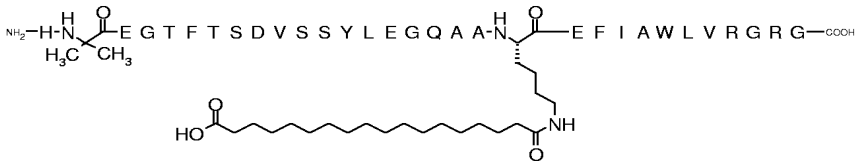
Alternatively LCMS was performed on a setup consisting of Hewlett Packard series 1100 G1312A Bin Pump, Hewlett Packard series 1100 Column compartment, Hewlett Packard series 1100 G1315A DAD diode array detector, Hewlett Packard series 1100 MSD and Sedere 75 Evaporative Light Scattering detectorcontrolled by HP Chemstation software. The HPLC pump is connected to two eluent reservoirs containing:
A: 10mM NH_4OH in water
B: 10mM NH_4OH in 90% acetonitrile
The analysis was performed at 23° C by injecting an appropriate volume of the sample (preferably 20 μl) onto the column which is eluted with a gradient of A and B.
The HPLC conditions, detector settings and mass spectrometer settings used are giving in the following table.
Column Waters Xterra MS C-18 X 3 mm id 5 m
Gradient 5% - 100% acetonitrile linear during 6.5 min at 1.5ml/min
Detection 210 nm (analogue output from DAD)
ELS (analogue output from ELS)
MS ionisation mode API-ES. Scan 100-1000 amu step 0.1 amu

Radioligand binding to plasma membranes expressing the human GLP-1 receptor

The binding assay was performed with purified plasma membranes containing the human GLP-1 receptor. The plasma membranes containing the receptors were purified from stably expressing BHK tk-ts 13 cells. The membranes were diluted in Assay Buffer (50 mM HEPES, 5 mM EGTA, 5 mM $MgCl_2$, 0.005% Tween 20, pH=7.4) to a final concentration of 0.2 mg/ml of protein and destributed to 96-well microtiter plates precoated with 0.3 % PEI. Membranes in the presence of 0.05 nM [^{125}I]GLP-1, unlabelled ligands in increasing

concentrations and different HSA concentrations (0.005%, 0.05%, and 2%) were incubated 2 hr at 30°C. After incubation, unbound ligands were separated from bound ligands by filtration through a vacuum-manifold followed by 2X100 µl washing with ice cold assaybuffer. The filters were dried overnight at RT, punched out and quantified in a γ-counter.

Example 1



N-ε^{26} (17-carboxyheptadecanoyl)-[Aib8,Arg34]GLP-1-(7-37)-peptide

A resin (Fmoc-Gly-NovaSyn TGT, 0.22 mmol/g Novabiochem 0.25 mmole) was used to produce the primary sequence on an ABI433A machine according to manufacturers guidelines. All protecting groups were acid labile with the exception of the residue used in position 26 (FmocLys(ivDde)-OH, Novabiochem) allowing specific deprotection of this lysine rather than any other lysine.

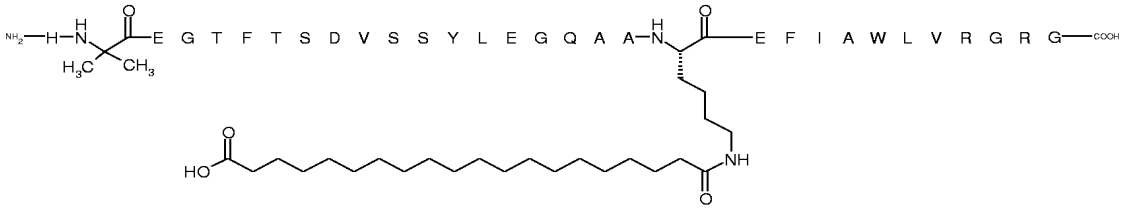
<u>Procedure</u>

The resin (0.09 mmole) was placed in a manual shaker/filtration apparatus and treated with 4% hydrazine in N-methyl pyrrolidone in (4x10 min. 4x4 ml) to remove the ivDde group. The resin was washed with N-methyl pyrrolidone (3x4 ml).Octadecanedioic acid mono-(2,5-dioxo-pyrrolidone-1-yl)ester) (4 molar equivalents relative to resin) was dissolved in DMF (4ml). The solution was added to the resin and diisopropylethylamine (8 molar equivalents relative to resin) was added. The resin was shaken 24 hours at room temperature. The resin was washed with N-methyl pyrrolidone (4x4 ml) and DCM (4x4ml). The peptide was cleaved from the resin by stirring for 180 min at room temperature with a mixture of trifluoroacetic acid, water and triisopropylsilane (92.5:5.0:2.5 4 ml).The cleavage mixture was filtered and the crude peptide was precipitated from 40 ml diethyl ether and washed 3 times with 45 ml diethyl ether. The crude peptide was purified by preparative HPLC on a 20 mm x 250 mm column packed with 7µ C-18 silica. The crude peptide was dissolved in 5 ml 50% acetic acid in water and diluted to 20 ml with H_2O and injected on the column which then was eluted with a gradient of 25-65 % (CH_3CN in water with 0.1% TFA) 20 ml/min during 40 min at RT. The peptide containing fractions were collected. The purified peptide was lyophilized after dilution of the eluate with water.

HPLC (method B4): RT= 9.94 min (91%)

LCMS: m/z = 1232 (MH_3^{3+}) Calculated for (MH_3^{3+}) = 1232

Example 2



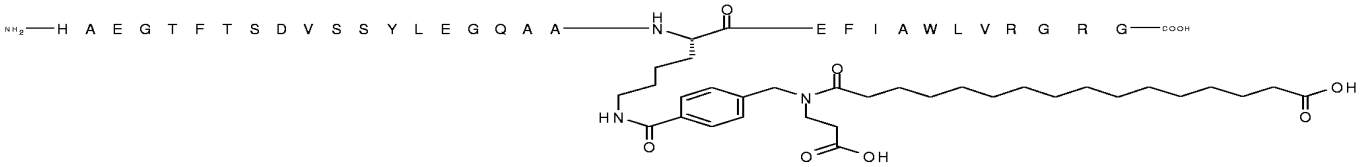
N-ε^{26}-(19-carboxynonadecanoyl)-[Aib8,Arg34]GLP-1-(7-37)-peptide

Prepared as in Example 1 and in accordance with "synthetic methods".

HPLC (method B4): RT= 10.42 min (91%)

LCMS: m/z = 1242 (MH_3^{3+}), Calculated for (MH_3^{3+}) = 1242

Example 3



N-ε^{26}-(4-{[N-(2-carboxyethyl)-N-(15-carboxypentadecanoyl)amino]methyl}benzoyl[Arg34]GLP-1-(7-37)-peptide

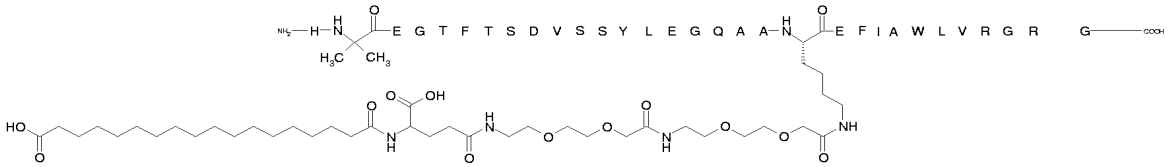
To a solution of 4-(N-(2-(*tert*-butoxycarbonyl)ethyl)-N-(15-(*tert*-butoxycarbonyl)pentadecanoyl)aminomethyl)benzoic acid (36 mg, 60 µmol) in THF (1 ml) were added DIPEA (7 µl) and O-(1-succinimidyl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (TSTU, 17 mg, 56 µl). After stirring for 1 h at room temperature, the mixture was diluted with THF (1 ml), and 1 ml of the resulting solution was added to a solution of [Arg34]GLP-1-(7-37) peptide (approx 100 mg) and DIPEA (103 µl) in water (5 ml). After 0.5 h more of the THF-solution of acylating agent (0.4 ml) was added. After stirring at room temperature for a total of 1.5 h the reaction mixture was filtered and applied to a preparative HPLC (gradient elution with 35-55% MeCN/55-35% water/10% water with 1% TFA). Fractions containing the desired product were combined and lyophylized. The product was then treated with 25 ml of a mixture of TFA and water (95/5 vol) for 15 min at room temperature, concentrated, and purified once more by HPLC. 15.4 mg of the title compound was obtained.

HPLC (method B4): RT = 9.41 min (99%)

LCMS: m/z = 1287 (MH_3^{3+}). Calculated for (MH_3^{3+}): 1287

Example 4

N-ε^{26}-[2-(2-[2-(2-[2-(2-[4-(17-Carboxyheptadecanoylamino)-4(S)-carboxybutyrylamino]ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][Aib8,Arg34]GLP-1-(7-37)peptide.

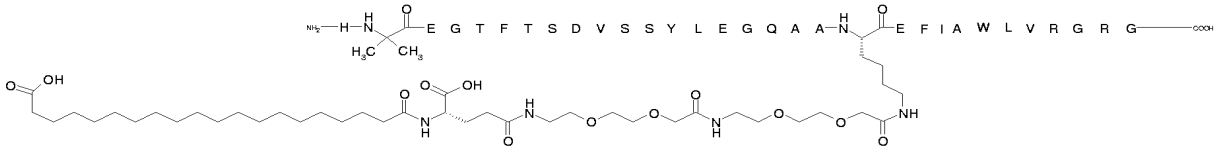


[Aib8,Arg34]GLP-1-(7-37)-peptide was prepared by standard Fmoc-solid phase peptide synthesis and purified by preparative HPLC. [Aib8,Arg34]GLP-1-(7-37)-peptide was dissolved in water (15ml) and DIPEA (50ul) was added. 17-((S)-1-tert-Butoxycarbonyl-3-{2-[2-({2-[2-(2,5-dioxopyrrolidin-1-yloxycarbonylmethoxy) ethoxy]ethylcarbamoyl}methoxy)ethoxy]ethylcarbamoyl}propylcarbamoyl)heptadecanoic acid tert-butyl ester (21 mg) was dissolved in acetonitrile/water 2:1 (1.5 ml) and added in small portions. The reaction was monitored by HPLC. When no more [Aib8,Arg34]GLP-1-(7-37)-peptide was found the reaction mixture was lyophilized O/N. To the isolated compound was added 10 ml of 90% TFA / 5% TIS/ 5% water and the reaction mixture was standing for 2 hours, evaporated *in vacuo*, and co-evaporated with heptane. The residual oil was dissolved in 15ml of water contaning 1%of NH3-aq and purified by preparative HPLC to give the title compound.

HPLC (method B4): RT = 9.60 min (100%)

LCMS: m/z = 1372 (MH_3^{3+}). Calculated for (MH_3^{3+}): 1372

Example 5



N-ε^{26}-[2-(2-[2-(2-[2-(2-[4-(19-Carboxynonadecanoylamino)-4(S)-carboxybutyrylamino]ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][Aib8,Arg34]GLP-1-(7-37)peptide.

The peptide was prepared according to: A. Synthesis of resin bound peptide in 0.25 mMol scale on a Fmoc-Gly-Wang resin (0.66 mmol/g Novabiochem) was used to produce the primary sequence on an ABI433A machine according to manufacturers guidelines. All

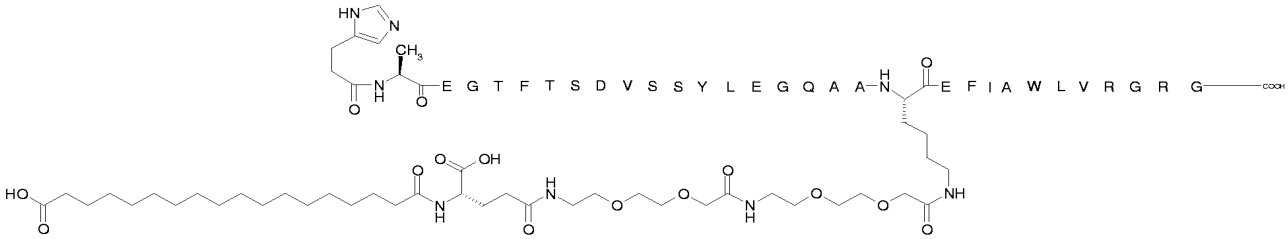
protecting groups were acid labile with the exception of the residue used in position 26 (FmocLys(Mtt)-OH, Novabiochem) which is super acid labile, allowing specific deprotection of this lysine rather than any other lysine.

Procedure for removal of Mtt-protection. The resin (0.25 mmol) was placed in a manual shaker/filtration apparatus and treated with 2% TFA, 3% TIS in DCM (20 ml, 5-10 min repeated 6-12 times) to remove the Mtt group and wash with DMF. Synthesis was continued with Procedure for attachment of sidechains to Lysine residue, following Route II, with the appropriate Procedure for removal of Fmoc-protection. Final deprotection, HPLC-purification and analysis by HPLC and LC-MS according to the procedures.

HPLC (method B6): RT = 34.56 min (100%)

LCMS: m/z = 1381.8 (MH_3^{3+}). Calculated for ($M+H^+$): 4142.7

Example 6



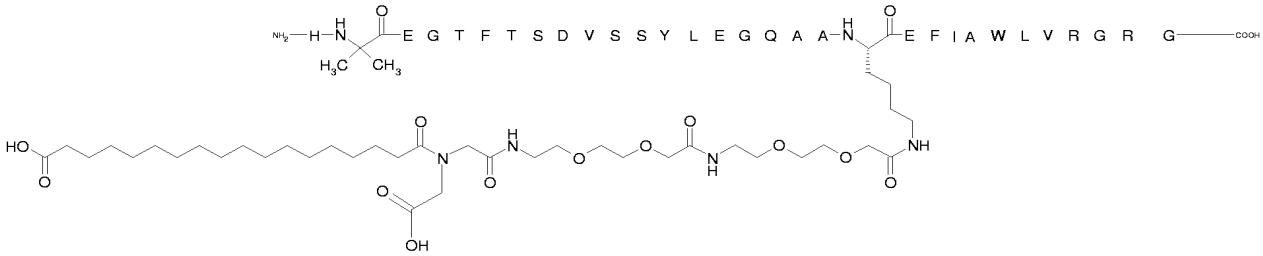
N-ε^{26}-[2-(2-[2-(2-[2-(2-[4-(17-Carboxyheptadecanoylamino)-4(S)-carboxybutyrylamino]ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][3-(4-Imidazolyl)Propionyl7,Arg34]GLP-1-(7-37)peptide

Prepared as in Example 5 and in accordance with "synthetic methods".

HPLC (method B6): RT = 32.89 min (100%)

LCMS: m/z = 1362.3 (MH_3^{3+}). Calculated for ($M+H^+$): 4085.6

Example 7



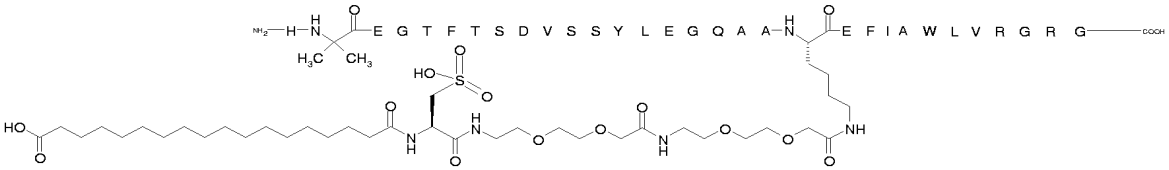
N-ε^{26}-[2-(2-[2-(2-[2-(2-[4-(17-Carboxyheptadecanoylamino)-(Carboxymethyl-amino)acetylamino]ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][Aib8,Arg34]GLP-1-(7-37)peptide

Prepared as in Example 5 and in accordance with "synthetic methods".

HPLC (method B6): RT = 32.67 min (100%)

LCMS: m/z = 1367.3 (MH_3^{3+}). Calculated for ($M+H^+$): 4100.6

Example 8



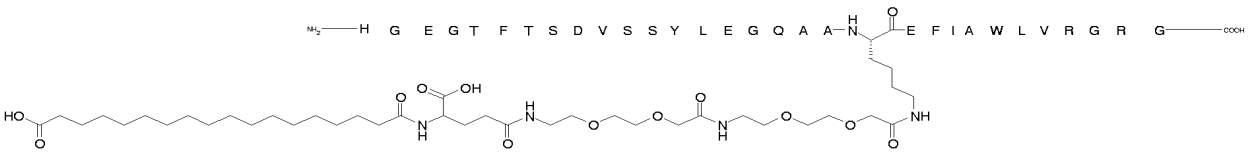
N-ε^{26}-[2-(2-[2-(2-[2-(2-[4-(17-Carboxyheptadecanoylamino)-3(S)-Sulfopropionylamino]ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][Aib8,Arg34]GLP-1-(7-37)peptide

Prepared as in Example 5 and in accordance with "synthetic methods".

HPLC (method B6): RT = 32.04 min (100%)

LCMS: m/z = 1379.8 (MH_3^{3+}). Calculated for ($M+H^+$): 4136.7

Example 9



N-ε^{26}-[2-(2-[2-(2-[2-(2-[4-(17-Carboxyheptadecanoylamino)-4(S)-carboxybutyrylamino]ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][Gly8,Arg34]GLP-1-(7-37)peptide.

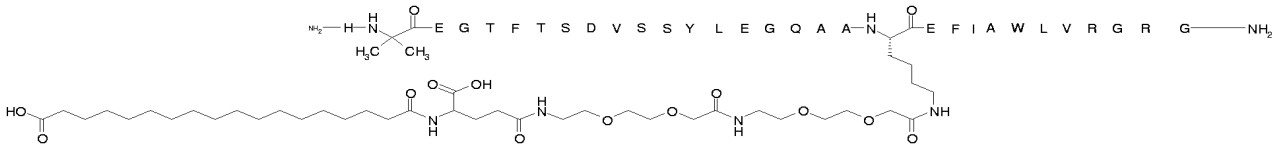
[Gly8,Arg34]GLP-1(7-37) peptide starting from 150 mg 2-chlorotrityl chloride resin (1.4 mmol/g) was prepared by Fmoc-solid phase peptide synthesis using Apex396 from Advanced Chemtech. The Lys residue at position 26 was protected as Lys(ivDde) while the functional side-chains for the other amino acids were protected with standard acid labile protecting groups. The Lys residue was deprotected with 3% hydrazine/3% piperidine in NMP for 1 hr. Then, the two units of 8-amino-3,6-dioxaoctanoic acid, γ–glutamic acid and octadecanedioic acid were coupled to the resin attached peptide using DIC/HOAt. The

peptide was finally deprotected and cleaved from the resin with TFA/TIS/H_2O/thioanisol (90/5/3/2). The peptide was isolated by LC-MS.

HPLC: Elutes at 46% acetonitrile

MALDI: 4087 (MH+)

Example 10



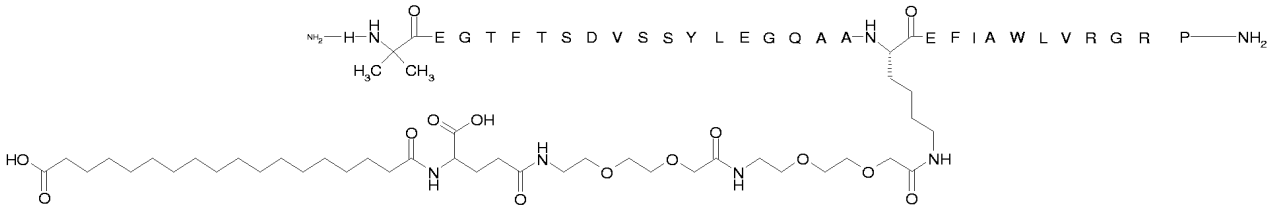
N-ε^{26}-[2-(2-[2-(2-[2-(2-[4-(17-Carboxyheptadecanoylamino)-4(S)-carboxybutyrylamino]ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][Aib8,Arg34]GLP-1-(7-37)-amide

[Aib8,34]GLP-1(7-37) amide starting from 200 mg Tentagel RAM S resin (0.26 mmol/g) was prepared by Fmoc-solid phase peptide synthesis using Apex396 from Advanced Chemtech. The Lys residue at position 26 was protected as Lys(ivDde) while the functional side-chains for the other amino acids were protected with standard acid labile protecting groups. The Lys residue was deprotected with 3% hydrazine/3% piperidine in NMP for 1 hr. Then, the two units of,8-amino-3,6-dioxaoctanoic acid, γ-glutamic acid octadecanedioic acid were coupled to the resin attached peptide using DIC/HOAt. The peptide was finally deprotected and cleaved from the resin with TFA/TIS/H_2O/thioanisol (90/5/3/2). The peptide was isolated by LC-MS.

HPLC: Elutes at 49% acetonitrile

MALDI: 4114 (MH+)

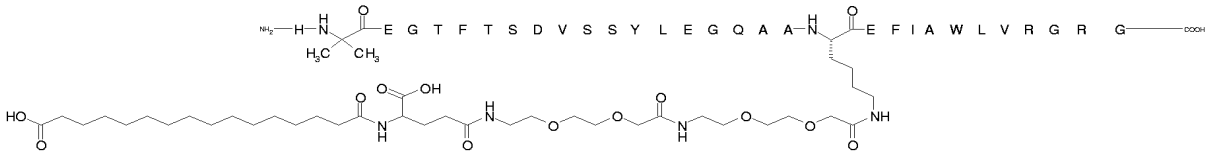
Example 11



N-ε^{26}-[2-(2-[2-(2-[2-(2-[4-(17-Carboxyheptadecanoylamino)-4(S)-carboxybutyrylamino]ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][Aib8,Arg34,Pro37]GLP-1-(7-37)amide

The peptide was prepared on a Rink amide resin (0.70 mmol/g Novabiochem) and else as in Example 5 and in accordance with "synthetic methods".
HPLC (method B6): RT = 32.13 min (100%). (method A1): RT = 44.33 min (98.4%)
LCMS: m/z = 1385.3 (MH_3^{3+}). Calculated for ($M+H^+$): 4153.8

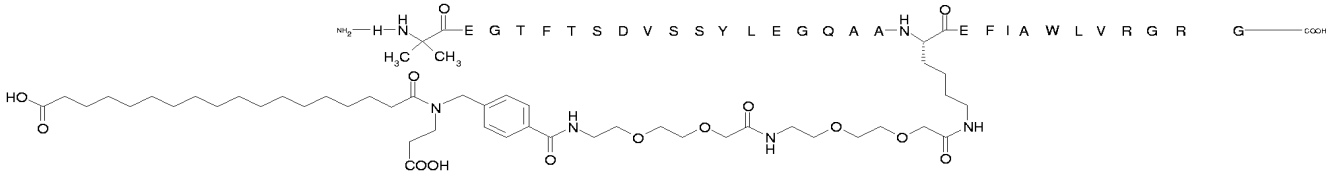
Example 12



Aib[8],Lys[26](N-ε[26]-{2-(2-(2-(2-[2-(2-(4-(pentadecanoylamino)-4-carboxybutyrylamino)ethoxy)ethoxy]acetyl)ethoxy)ethoxy)acetyl)}),Arg[34])GLP-1 H(7-37)-OH

HPLC (method B6): RT= 30.41 min
LCMS: m/z = 1362.9 (MH_3^{3+}) Calculated for (M^+) = 4085.61

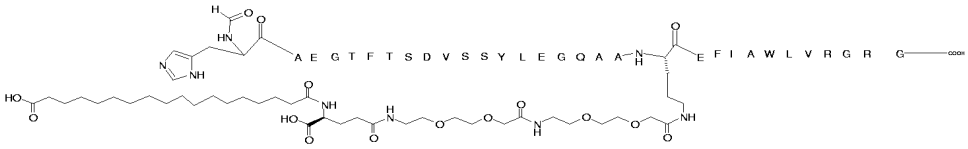
Example 13



N-ε[26]-[2-(2-[2-(2-[2-(2-[4-{[N-(2-carboxyethyl)-N-(17-carboxyheptadecanoyl)amino]methyl}benzoyl)amino]ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][Aib8,Arg34]GLP-1(7-37)
[Aib8,Arg34]GLP-1(7-37) peptide starting from 150 mg 2-chlorotrityl chloride resin (1.4 mmol/g) was prepared by Fmoc-solid phase peptide synthesis using Apex396 from Advanced Chemtech. The Lys residue at position 26 was protected as Lys(ivDde) while the functional side-chains for the other amino acids were protected with standard acid labile protecting groups. The Lys residue was deprotected with 3% hydrazine/3% piperidine in NMP for 1 hr. The two units of 8-amino-3,6-dioxaoctanoic acid and 4{[(2-tert-butoxycarbonyl-ethyl)-(17-tert-butoxycarbonyl-heptadecanoyl)-amino]-methyl}-benzoic acid were coupled to the resin attached peptide using DIC/HOAt. The peptide was finally deprotected and cleaved from the resin with TFA/TIS/H_2O/thioanisol (90/5/3/2). The peptide was isolated by preparative LC-MS.
HPLC: Elutes at 52% acetonitrile

MALDI: 4191 (MH+)

Example 14

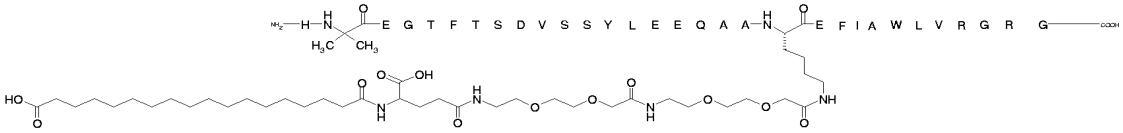


N-α^7-formyl, N-ε^{26}-[2-(2-[2-(2-[2-(2-[4-(17-Carboxyheptadecanoylamino)-4(S)-carboxybutyrylamino]ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][Arg^{34}]GLP-1-(7-37)-peptide

HPLC (method B6): RT= 32,6 min

LCMS: m/z = 1377.3 (MH_3^{3+}) Calculated for (M^+) = 4128.0

Example 15



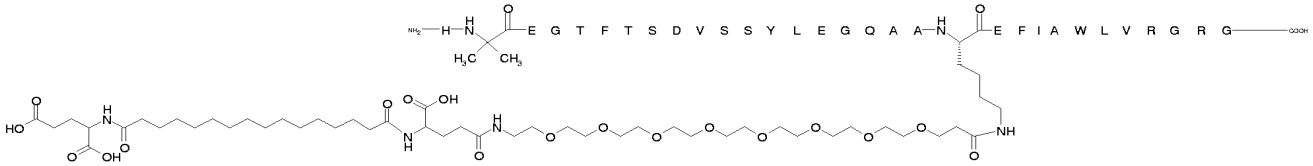
N-ε^{26}26-[2-(2-[2-(2-[2-(2-[4-(17-Carboxyheptadecanoylamino)-4(S)-carboxybutyrylamino]ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][Aib8,Glu22,Arg34]GLP-1-(7-37)peptide.

[Aib8,Glu22,Arg34]GLP-1(7-37) peptide starting from 150 mg Fmoc-Gly-Wang resin (0.66mmol/g) was prepared by Fmoc-solid phase peptide synthesis using Apex396 from Advanced Chemtech. The Lys residue at position 26 was protected as Lys(Mtt) while the functional side-chains for the other amino acids were protected with standard acid labile protecting groups. The Lys residue was deprotected with 2% TFA/2% TIS in DCM for 4 x 5 min. The two units of 8-amino-3,6-dioxaoctanoic acid, γ-glutamic and octadecanoic acid tert-butyl ester were coupled to the resin attached peptide using DIC/HOAt. The peptide was finally deprotected and cleaved from the resin with TFA/TIS/H_2O/thioanisol (90/5/3/2). The peptide was isolated by LC-MS.

HPLC: Elutes at 50% acetonitrile

MALDI: 4187 (MH+)

Example 16



N-ε^{26}\{3-[2-(2-\{2-[2-(2-\{2-[2-(2-[4-(15-(N-((S)-1,3-dicarboxypropyl)carbamoyl)pentadecanoylamino)-(S)-4-carboxybutyrylamino]ethoxy)ethoxy]ethoxy\}ethoxy)ethoxy]ethoxy\}ethoxy)ethoxy]propionyl\}[Aib8,Arg34]GLP-1-(7-37)-peptide

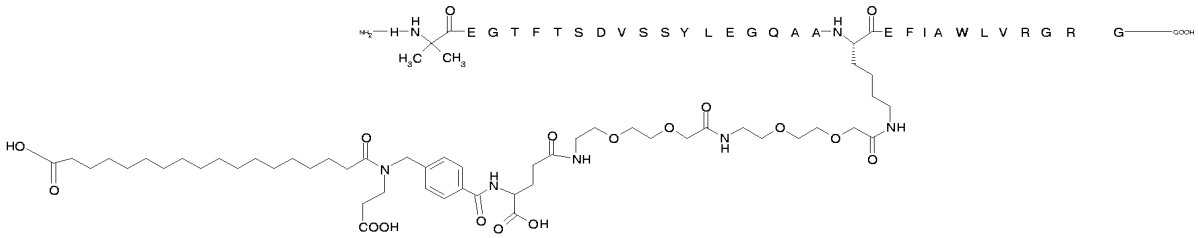
Method and analysis

Prepared as in Example 3 and in accordance with "synthetic methods".

HPLC (method B4): RT = 10.29 min (92%)

LCMS: m/z = 1450 (MH_3^{3+}). Calculated for (MH_3^{3+}): 1450

Example 17



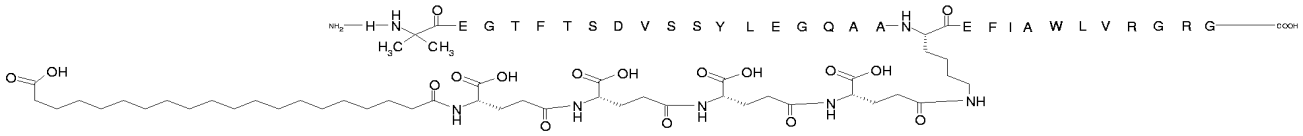
N-ε^{26}-[2-(2-[2-(2-[2-(2-[4-\{[N-(2-carboxyethyl)-N-(17-carboxyheptadecanoyl)amino]methyl\}benzoyl)amino](4(S)-carboxybutyrylamino)ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][Aib8,Arg34]GLP-1(7-37)

[Aib8,Arg34]GLP-1(7-37) peptide starting from 150 mg Fmoc-Gly Wang resin (0.66mmol/g) was prepared by Fmoc-solid phase peptide synthesis using Apex396 from Advanced Chemtech. The Lys residue at position 26 was protected as Lys(Mtt) while the functional side-chains for the other amino acids were protected with standard acid labile protecting groups. The Lys residue was deprotected with 2% TFA/2% TIS in DCM for 4 x 5 min. The two units of 8-amino-3,6-dioxaoctanoic acid, γ-glutamic acid and 4\{[(2-tert-butoxycarbonyl-ethyl)-(17-tert-butoxycarbonyl-heptadecanoyl)-amino]-methyl\}-benzoic acid were coupled to the resin attached peptide using DIC/HOAt. The peptide was finally deprotected and cleaved from the resin with TFA/TIS/H_2O/thioanisol (90/5/3/2). The peptide was isolated by preparative HPLC.

HPLC: Elutes at 51% acetonitrile

MALDI: 4320 (MH+)

Example 18



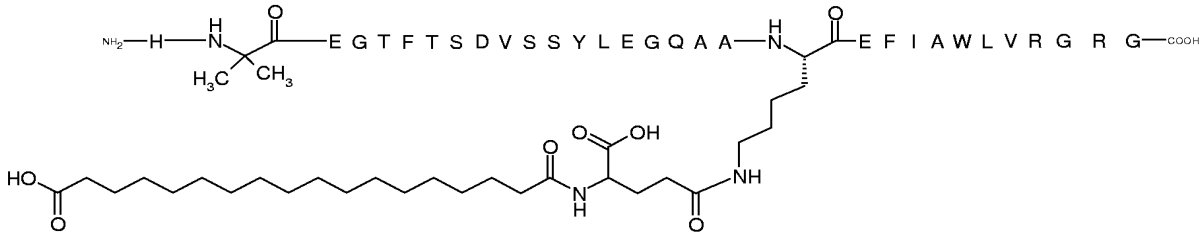
N-ε^{26}-{(S)-4-carboxy-4-((S)-4-carboxy-4-((S)-4-carboxy-4-((S)-4-carboxy-4-(19-carboxynonadecanoylamino)butyrylamino)butyrylamino)butyrylamino)butyrylamino}[Aib8,Arg 34]GLP-1-(7-37)

The peptide was synthesized using Fmoc chemistry on a Liberty Microwave Peptide Synthesizer (CEM Corporation). The synthesis was performed on a Gly-Wang resin (Novabiochem) with a loading of 0.66 mmol/g using 4 fold excess of amino acids and DIC/HOAt for coupling. The N-terminal histidine was Boc-protected and the lysine to be modified was Mtt-protected. After synthesis of the peptide backbone, the Mtt group was removed with 3% TFA in DCM and the side chain was built on the Liberty using standard peptide synthesis protocols. In the last step the fatty diacid was added as a mono-t-butyl-ester.

After cleavage with TFA/TIS/water (95:2.5:2.5), the peptide was dissolved in 50% acetonitrile by addition of DIPEA and purified on a Waters LC-MS system using a 7.8 x 300 mm X-Terra Prep MS C18 10 µm column running at room temperature. After 5 minutes at 30% CH_3CN, 0.08% TFA, 4 ml/min, the column was eluted with a linear gradient of 30 to 70% CH_3CN over 40 minutes. The fractions containing the desired compound were collected and the concentration of the peptide in the eluate was determined by measurement of the UV absorption at 280 nm assuming molar extinction coefficients of 1280 and 3690 for tyrosine and tryptophan respectively. The identity and purity was confirmed by MALDI. After the concentration determination the eluate was aliquotted into vials containing the desired amount and dried by vacuum centrifugation.

HPLC: Elutes at 52% acetonitrile
MALDI: 4239 (MH+)

Example 19



N-ε^{26}-4-(17-Carboxyheptadecanoylamino)-4(S)-carboxybutyryl-[Aib8,Arg34]GLP-1-(7-37)-peptide

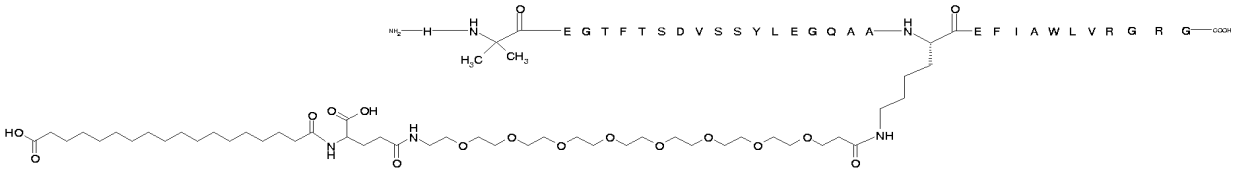
Method and analysis

Prepared as in Example 4 and in accordance with "synthetic methods".

HPLC (method B4): Rt = 9.64 min (97 %)

LCMS: m/z: = 1276 (MH_3^{3+}), Calculated for (MH_3^{3+}) 1276

Example 20

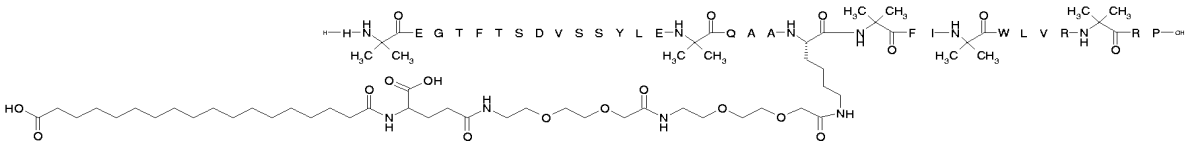


N-ε^{26}-{3-[2-(2-{2-[2-(2-{2-[2-(2-[4-(17-carboxyheptadecanoylamino)-4(S)-carboxybutyrylamino]
ethoxy)ethoxy]ethoxy}ethoxy)ethoxy]ethoxy}ethoxy)ethoxy]propionyl}[Aib8,Arg34]GLP-1-(7-37)-peptide.

LCMS*: m/z: = 1417 (MH_3^{3+}), Calculated for (MH_3^{3+}) 1417

*HPLC (Eluted at 0.5 mL/min at 42°C by a linear gradient from 5 ---->80% acetonitrile, 85----->10% water and 10% of a solution of 1.0% trifluoroacetic acid over 50min. UV detection at 214 on a Symmetry300, 5um, 3.9 mm x 150 mm C-18 silica column.)method B4): Rt = 32.09 min (95 %)

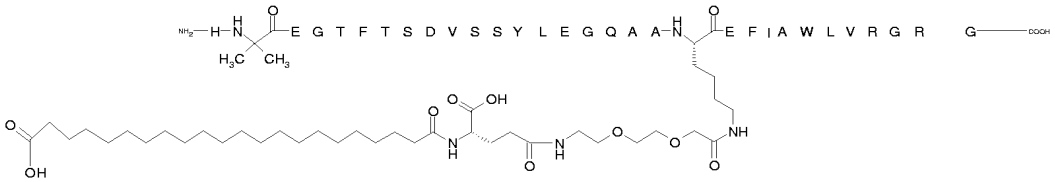
Example 21



N-ε^{26}-{2-(2-(2-(2-[2-(2-(4-(17-carboxyheptadecanoylamino)-4-carboxybutyrylamino)ethoxy)ethoxy]acetyl)ethoxy)ethoxy)acetyl)}-[Aib^{8,22,27,30,35},Arg^{34},Pro^{37}, Lys^{26}] GLP-1 (7-37)amide

HPLC (method B6): RT= 35.0 min
LCMS: m/z = 1394.0 (MH_3^{3+}) Calculated for (M^+) = 4180.0

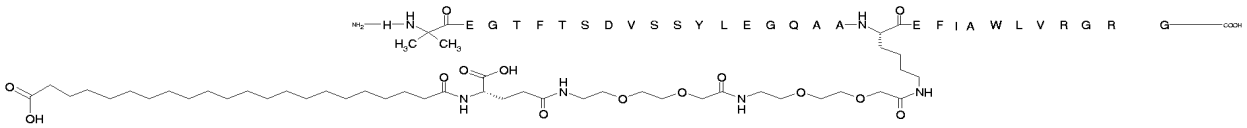
Example 22



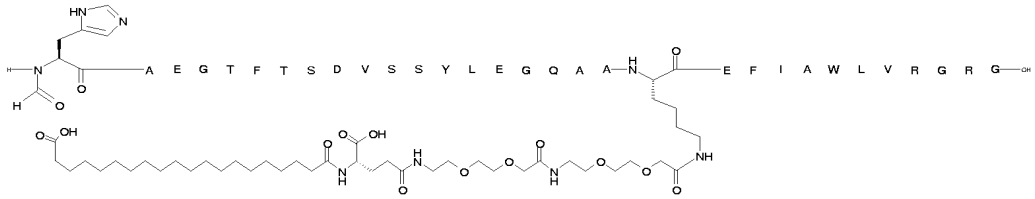
N-ε^{26}-[2-(2-[2-[4-(21-Carboxyuneicosanoylamino)-4(S)-carboxybutyrylamino]ethoxy]ethoxy)acetyl][Aib8,Arg34]GLP-1-(7-37)
Prepared using the same method as in Example 19.

HPLC: Elutes at 53.4% acetonitrile
MALDI: 4025 (MH+)

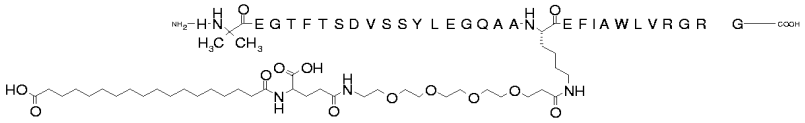
Other compounds of this invention include:

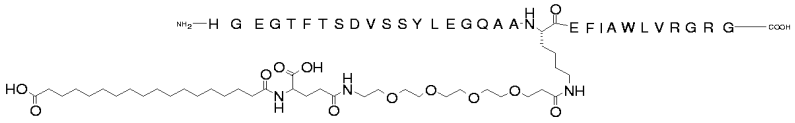


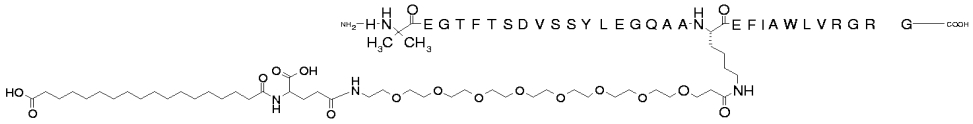
N-ε^{26}-[2-(2-[2-(2-[2-(2-[4-(21-Carboxyuneicosanoylamino)-4(S)-carboxybutyrylamino]ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][Aib8,Arg34]GLP-1-(7-37)peptide.

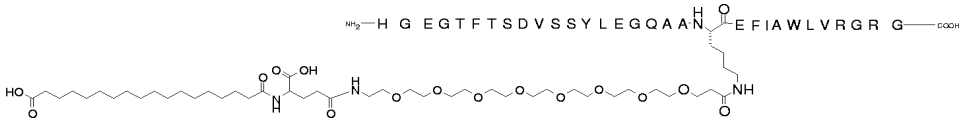


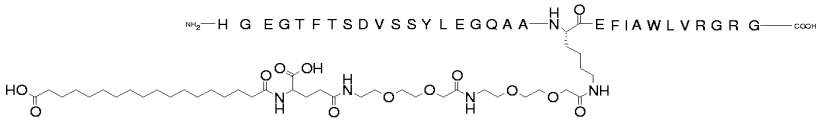
N-α1-formyl-N-ε^{26}-[2-(2-[2-(2-[2-(2-[4-(19-Carboxynonadecanoylamino)-4(S)-carboxybutyrylamino]ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][Arg34]GLP-1-(7-37)peptide.

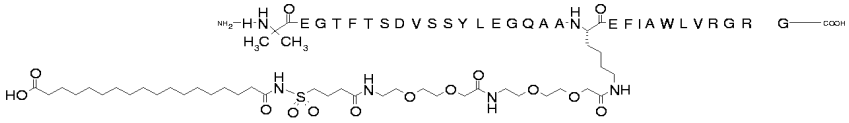


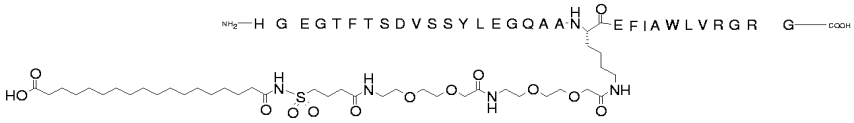


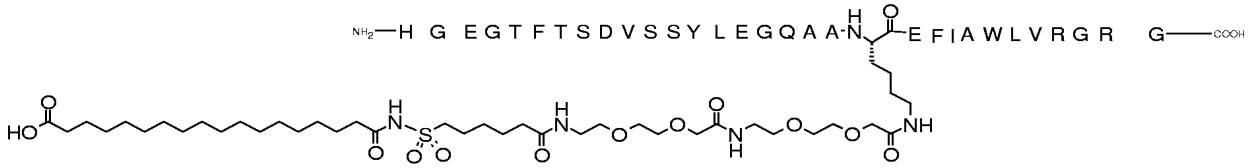


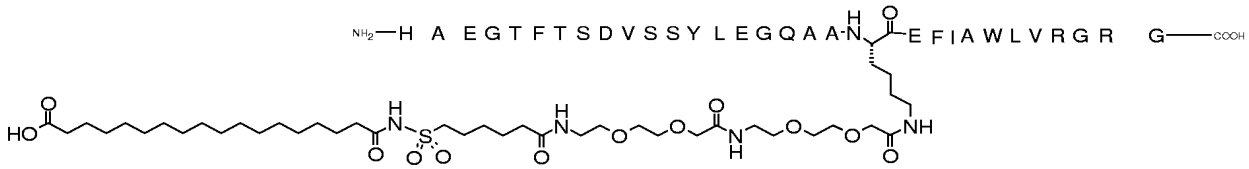


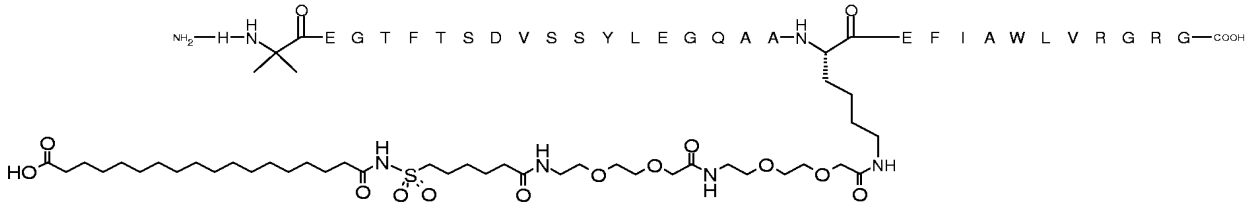


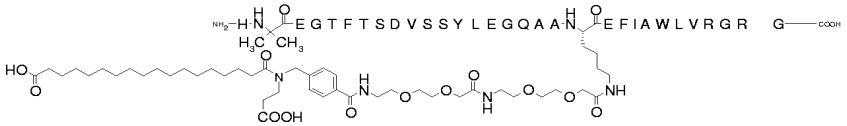


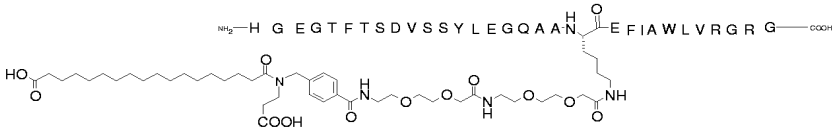


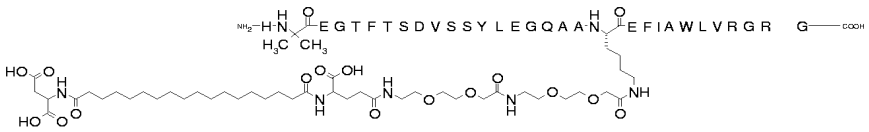


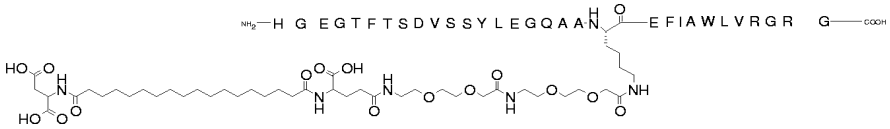


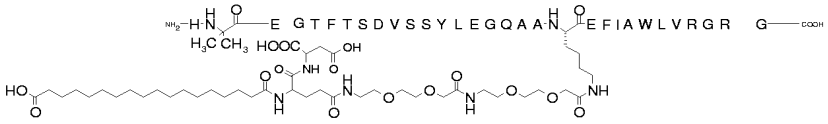


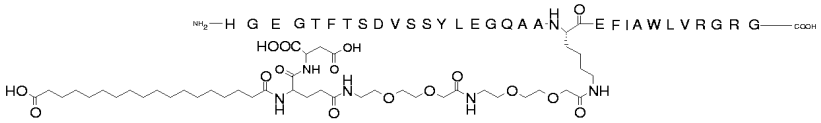












Pharmacodynamic study using db/db mice

In one aspect of this invention the GLP-1 agonists have a duration of action of at least 24hrs after dosing of 30nmol/kg to db/db mice

The efficacy and duration of action are measured in db/db mice.

Male db/db mice are shipped from Taconic, Denmark at the age of 8-10 weeks. From the time of arrival, the mice are housed under standard conditions but at 24 °C. The mice are kept 10 per cage until experimentation with free access to standard chow (Altromin,

Brogaarden APS., Denmark) and tap water at a normal day: light cycle (light on at 6 am). The mice are used for 1 experiment per week for 3 weeks. After this, the mice are euthanized.

After an acclimatisation period of 1 week, the blood glucose is measured by sampling from the tail tip capillary. In brief, 5 µl blood is sampled in heparinised glass capillary tubes and immediately suspended in 250 µl EBIO buffer solution (Eppendorf, Germany) in an 1.5 ml Eppendorf tube. The blood glucose concentration is measured by the glucose oxidase method at the EBIO Plus Auto analyser (Eppendorf, Germany).

The cut of value for blood glucose is 10 mM. When evaluating the mice, it is essential, that all 42 mice entering the experiment have blood glucose values above 10 mM, but also that the inter-mice variance is small. Therefore, if many mice are not severely diabetic, whereas some are, the start up of experiments should be postponed one week and new basal blood glucose measurements be made.

Based on the basal blood glucose values, the mice are allocated to 7 groups of n=6 with matching group mean blood glucose values.

On the day of testing the basal blood glucose morning values are assessed as described above and the basal body weight of each mouse is assessed. A time 0, the compound is dosed subcutaneously in the scruff of the neck (dosing volume app. 300 µl/50 g mouse).

The blood glucose values are followed up to 48 hours (time 1, 3, 6, 24 and 48 h) and the terminal body weight is assessed.

All data are entered into Graphpad Prism where mean blood glucose and mean delta body weights are calculated.

One aspect of this invention is to prepare GLP-1 analogues/derivatives with extended plasma half-lives that are suitable for once weekly administration. The pharmaco kinetic properties can be evaluated in mini pigs or domestic pigs as described below

Pharmacokinetic screening of once weekly GLP-1 analogues

Pharmacokinetic screening of GLP-1 analogues for identification of suitable once weekly candidates were performed on candidates that according to the project screenings plan were shown to be sufficiently potent with respect to glucose lowering potential in a diabetic mouse model (db/db mice) and subsequently had a time of duration of 48 hours or more in the db/db mouse model.

Primary screening

The first part of the pharmacokinetic screening consisted of a single dose subcutaneous administration of 2 nmol/kg to three minipigs weighing 8-12 kg. Blood samples were drawn from each animal at predose, 0.5, 1, 2, 4, 6, 8, 12, 24, 48, 72, 96 and 120 hours post-injection. All blood samples were stabilised with a special stabilisation buffer consisting of: EDTA (di-sodium) 0.18 M, Aprotenin 15000 KIE/ml, Val-Pyr 0.30 mM, pH adjusted to 7.4 in order to prevent enzymatic degradation of the GLP-1 analogues. Plasma was collected from each stabilised blood samples by centrifugation (4ºC, 10 min., 1270 G (4000 rpm), and analysed for the content of GLP-1 analogue by ELISA assays. Three different ELISA assays were used for the plasma analysis: "The "Total assay" using the antibody combination F1/Ra2135 detecting both the N-terminally intact 7-37GLP-1 molecule and the N-terminal enzymatically degraded 9-37GLP-1 molecule with a limit of detection (LOD) of 35 pM and a dynamic analytical range of 35-30000 pM. The "Intact assay" using the antibody combination F1/Mab26.1. This assay was detecting the N-terminally intact 7-37GLP-1 molecule only. The LOD was 35 pM and a dynamic analytical range of 35-30000 pM. The "Aib-intact assay" using the antibody combination F1/GLP162-3F15. This assay was detecting the Aib stabilised N-terminal of the GLP-1 molecule enabling detection of stabilised GLP-1 analogues. The LOD was 45 pM and the dynamic analytical range 45-30000 pM.

All plasma concentration-time profiles were analysed pharmacokinetically by a non-compartmental analysis. The following pharmacokinetic parameters were calculated if data permitted: t_{max}, C_{max}, AUC, AUC/Dose, $AUC_{\%Extrapol}$, λ_z, $t_{½}$, CL/F, V_z/F and MRT.

Secondary screening

A second part of the pharmacokinetic screening was conducted on those compounds with an initial terminal half-life of 60-70 hours or more. This screening consisted of a single dose intravenous and subcutaneous administration of 2 nmol/kg to six minipigs for each route of administration. The blood sampling schedule was extended from 0-120 hours to 0-432 and 0-504 hours after intravenous and subcutaneous administration respectively. This was done in order to increase the precision and accuracy of the pharmacokinetic parameter estimates, especially the terminal half-life, AUC and the derived parameters clearance and volume of distribution, and to estimate the bioavailability after subcutaneous administration.

GLP-1 (AIB8- INTACT) ASSAY

The assay was a two-site assay with simultaneous incubation of the analyte with catcher and detector antibody. A ready to use chemiluminescent substrate was used to maximize signal. The assay neither recognizes endogen GLP-1 (7-37) nor the DPPIV cleaved GLP-1 (9-37).

Reference plasma for GLP-1 assays

0-plasma was prepared from pooled EDTA plasma without Valine Pyrrolidide and Aprotinin from fasting animals. The pooled EDTA plasma was incubated at 37°C for 4 hours to remove traces of GLP-1 and after incubation Valine Pyrrolidide and Aprotinin were added.

Buffers

Coating buffer

PBS was used as coating buffer: 10mM sodium phosphate and 145mM sodium chloride adjusted to pH 7.4.

Washing buffer

PBS with 0.05% (v/v) Tween 20

Assay buffer

PBS with 0.05% (v/v) Tween 20, 10g/L BSA and 10mg/L anti-TNP.

Streptavidin buffer

Washing buffer with an additional 0.5M NaCl.

Substrate

Ready to use substrate SuperSignal ELISA Femto (Pierce, cat.no. 37075).

Standards

Standards were prepared from a 25 µM stock solution of 0113-0000-0217. The peptide was serially diluted into reference plasma to make standards with final concentrations of 30000-10000-3333-1111-370-123-41 and 0 pM. Standards were stored in Micronic tubes in 100µL aliquots at -20°C.

Assay procedure

Crystal 2000 Microplates (black) were coated with monoclonal antibody GLPb1-7F1, 100µL of 5 µg/mL in PBS overnight at 4°C.

Plates were washed 5 times with washing buffer in an automated plate washer (SkanWasher, Skatron) and allowed to stand for at least 30min. with washing buffer to block remaining sites.

20µL of sample or standard was added to each well in duplicate immediately followed by 100µL GLP162-3F15 biotinylated, 1µg/mL in assay buffer. Plates were incubated for 2 hours at room temperature on a plate shaker followed by 5 wash cycles as previously described.

100µL of streptavidin-peroxidase solution (KPL, code 14-30-00, 1:20000 in streptavidin buffer) was added to each well and incubated for 1 hour at room temperature on a plate shaker. Plates were washed as previously described and after emptying 100µL of SuperSignal femto was added. Plates were put on a shaker for 1 minute and measured in Orion Luminometer (Berthold). Data were transferred to MultiCalc and standard curves

calculated using the weighted 4PL method. Sample concentrations were calculated from the standard curve.

GLP-1 (TOTAL) ASSAY

The assay was a two-site assay with simultaneous incubation of the analyte with catcher and detector antibody. The assay recognizes N-terminally cleaved GLP-1 up to GLP-1(12-37).

Buffers

Coating buffer

PBS was used as coating buffer: 10mM sodium phosphate and 145mM sodium chloride adjusted to pH 7.4.

Washing buffer

PBS with 0.05% (v/v) Tween 20

Assay buffer

PBS with 0.05% (v/v) Tween 20, 10g/L BSA and 10mg/L anti-TNP.

Streptavidin buffer

Washing buffer with an additional 0.5M NaCl.

Substrate

Ready-to-use substrate TMB (KemEnTec code 4380A)

Stop buffer

4 M H_3PO_4

Standards

Standards were prepared from a 25 µM stock solution of 0113-0000-0217. The peptide was serially diluted into reference plasma to make standards with final concentrations of 30000-10000-3333-1111-370-123-41 and 0 pM. Standards were stored in Micronic tubes in 100µL aliquots at -20°C.

Assay procedure

Maxisorp microtiter plates (NUNC) were coated with monoclonal antibody GLPb1-7F1, 100µL of 5 µg/mL in PBS overnight at 4°C.

Plates were washed 5 times with washing buffer in an automated plate washer (SkanWasher, Skatron) and allowed to stand for at least 30min. with washing buffer to block remaining sites.

20µL of sample or standard was added to each well immediately followed by 100µL Ra2135-biotinylated, 1µg/mL in assay buffer. Plates were incubated for 2 hours at room temperature on a plate shaker followed by 5 wash cycles as previously described.

100µL of streptavidin-peroxidase solution (Amersham Bioscinces code RPN4401V, 1:8000 in assay buffer) was added to each well and incubated for 1 hour at room temperature on a

plate shaker. Plates were washed as previously described and after emptying 100µL of TMB was added and after 5 minutes stopped with 100 µL H_3PO_4 .
Plates were measured in Victor Multilabel Reader (Wallac). Data were transferred to MultiCalc and standard curves calculated using the weighted 4PL method. Sample concentrations were calculated from the standard curve.

The in-life experimental procedures, plasma analysis and pharmacokinetic analysis were identical to that described under the primary screening.

Pharmaceutical formulation:

A compound of the invention may be formulated as:

Compound of example 4	6,25 mg/ml
Propyleneglycol	14,0 mg/ml
Phenol	5.5 mg/ml
Phosphate Buffer pH 8.15	

Optionally the compound is treated with heat and/or base before formulation as described in PCT/ EP2005/055946.

Claims

1. A GLP-1 analog having a modification of at least one non-proteogenic amino acid residue in positions 7 and/or 8 relative to the sequence GLP-1(7-37) (SEQ ID No 1), which is acylated with a moiety to the lysine residue in position 26, and where said moiety comprises at least two acidic groups, wherein one acidic group is attached terminally.

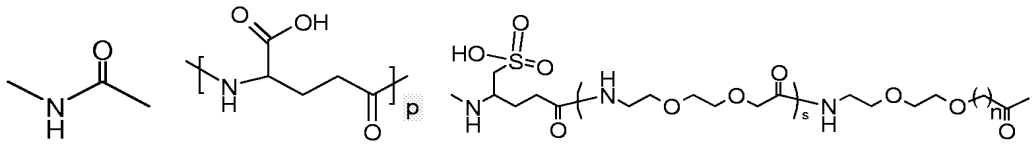
2. A GLP-1 analog according to claim 1, wherein the moiety attached in position 26 comprises a hydrophilic linker.

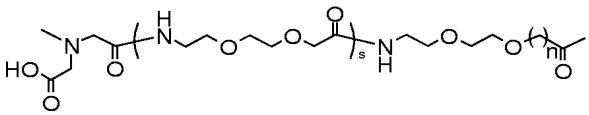
3. A GLP-1 analog according to claim 2, wherein the hydrophilic linker comprises at least 5 non-hydrogen atoms where 30-50% of these are either N or O.

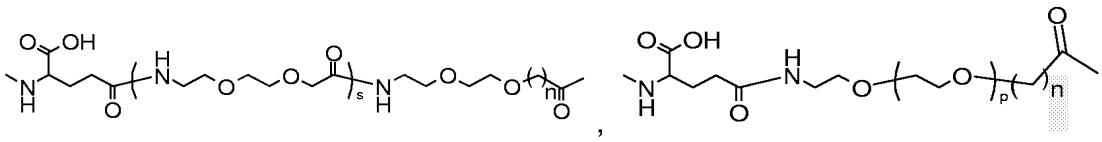
4. A GLP-1 analog according to any of the above claims, wherein the moiety attached in position 26 comprises an albumin binding moiety seperated from the peptide by the hydrophilic linker.

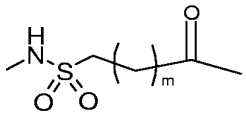
5. A GLP-1 analog according to claim 4 wherein the albumin binding moiety is a linear or branched lipophilic moiety containing 4-40 carbon atoms having a distal acidic group.

6. A GLP-1 analog according to any of the above claims, wherein the acylated moiety is B-U', where U' is selected from





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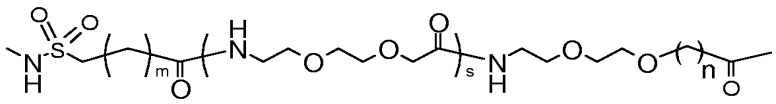
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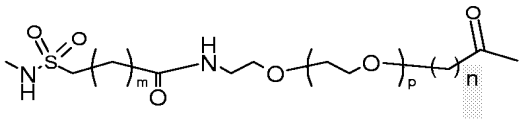

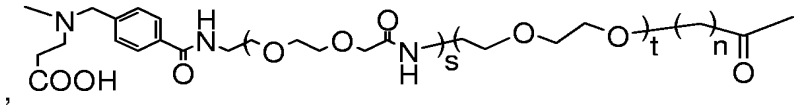
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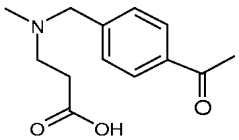
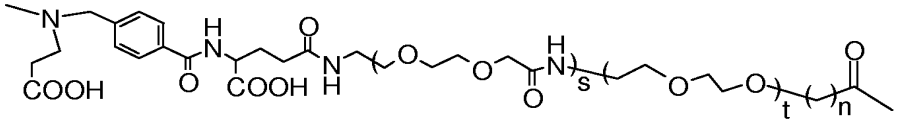


,

m is 0, 1, 2, 3, 4, 5, or 6,

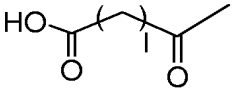
n is 1, 2 or 3

s is 0, 1, 2, or 3,

t is 0, 1, 2, 3, or 4

p is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17,18, 19, 20, 21, 22, or 23;

and where B is an acidic group selected from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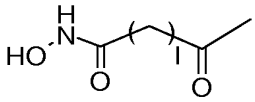
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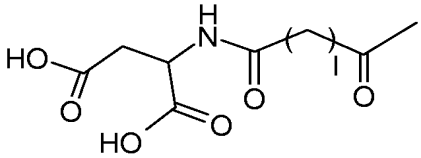
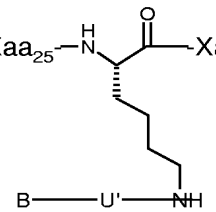


where l is 12, 13, 14, 15, 16, 17, 18, 19 or 20

7. A GLP-1 analog which is a compound of formula I (SEQ ID No. 2) :

Xaa_7-Xaa_8-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Xaa_{16}-Ser-Xaa_{18}-Xaa_{19}-Xaa_{20}-Glu-Xaa_{22}-

Xaa_{23}-Ala-Xaa_{25}-  -Xaa_{27}-Phe-Ile-Xaa_{30}-Trp-Leu-Xaa_{33}-Xaa_{34}-Xaa_{35}-Xaa_{36}-Xaa_{37}

Formula I

wherein

Xaa_7 is L-histidine, imidazopropionyl, α-hydroxy-histidine, D-histidine, desamino-histidine, 2-amino-histidine, β-hydroxy-histidine, homohistidine, N^{α}-acetyl-histidine, N^{α}-formyl-histidine, α-

fluoromethyl-histidine, α-methyl-histidine, 3-pyridylalanine, 2-pyridylalanine or 4-pyridylalanine

Xaa_8 is Ala, Gly, Val, Leu, Ile, Thr, Ser, Lys, Aib, (1-aminocyclopropyl) carboxylic acid, (1-aminocyclobutyl) carboxylic acid, (1-aminocyclopentyl) carboxylic acid, (1-aminocyclohexyl) carboxylic acid, (1-aminocycloheptyl) carboxylic acid, or (1-aminocyclooctyl) carboxylic acid;

Xaa_{16} is Val or Leu;

Xaa_{18} is Ser, Lys or Arg;

Xaa_{19} is Tyr or Gln;

Xaa_{20} is Leu or Met;

Xaa_{22} is Gly, Glu or Aib;

Xaa_{23} is Gln, Glu, Lys or Arg;

Xaa_{25} is Ala or Val;

Xaa_{27} is Glu or Leu;

Xaa_{30} is Ala, Glu or Arg;

Xaa_{33} is Val or Lys;

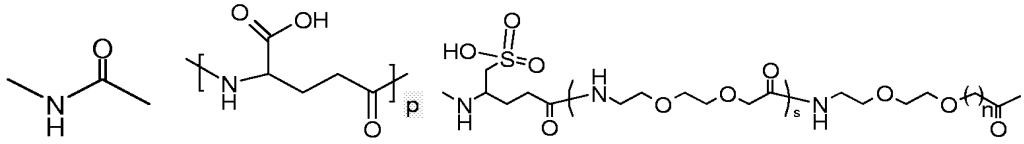
Xaa_{34} is Lys, Glu, Asn or Arg;

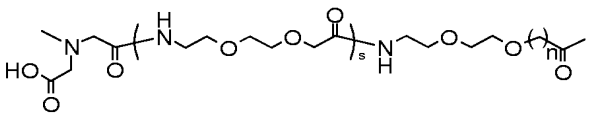
Xaa_{35} is Gly or Aib;

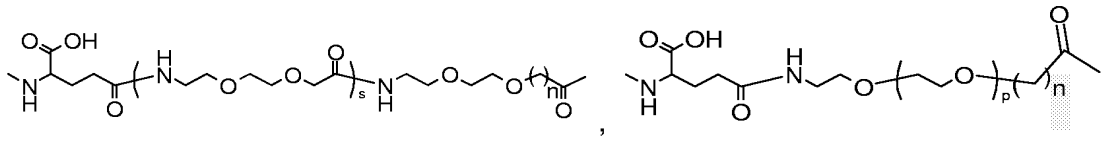
Xaa_{36} is Arg, Gly or Lys, or is absent;

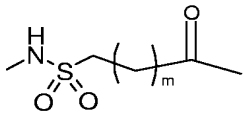
Xaa_{37} is Gly, Ala, Glu, Pro, Lys, or is absent;

and B and U' together is the acylated moiety, where U' is selected from





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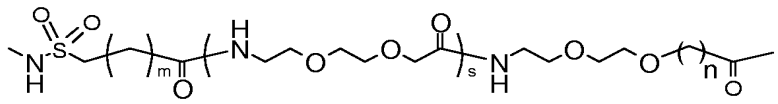
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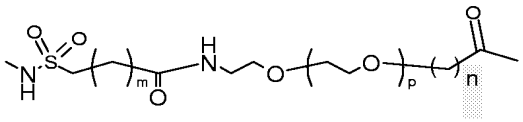

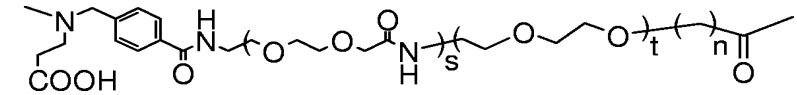
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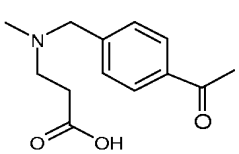
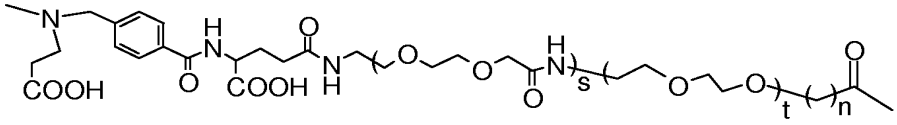
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m is 0, 1, 2, 3, 4, 5, or 6,

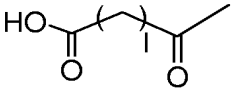
n is 1, 2 or 3

s is 0, 1, 2, or 3,

t is 0, 1, 2, 3, or 4

p is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17,18, 19, 20, 21, 22, or 23;

and where B is an acidic group selected from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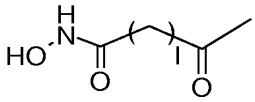
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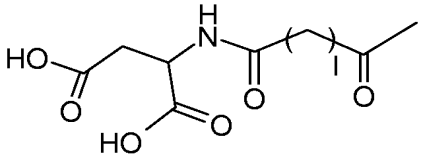
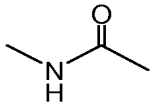


where l is 12, 13, 14, 15, 16, 17, 18, 19 or 20;

8. A GLP-1 analog according to claims 6-7, wherein U' is selected from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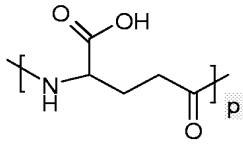
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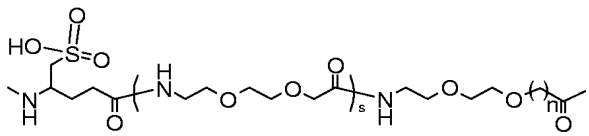

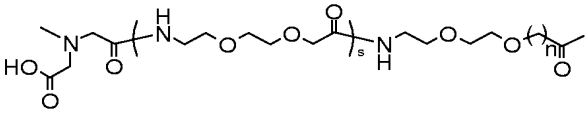

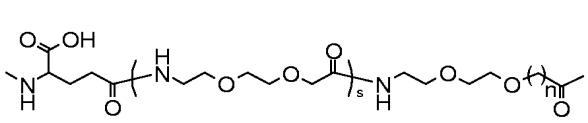
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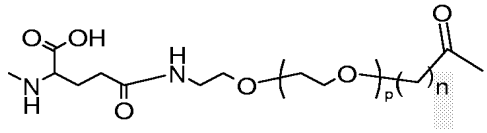

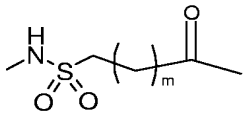
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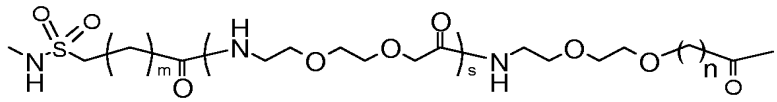
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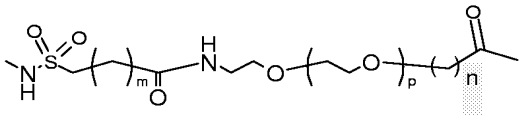

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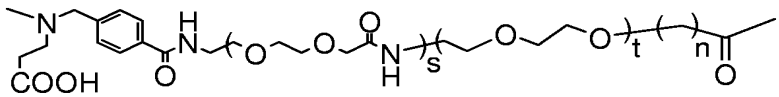
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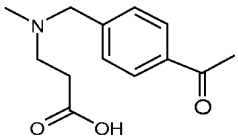
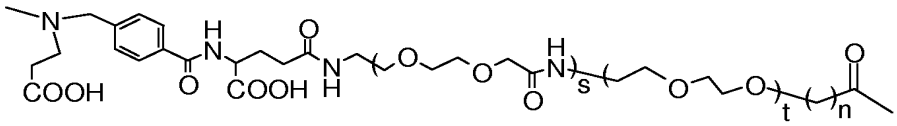
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m is 2, 3, 4 or 5,

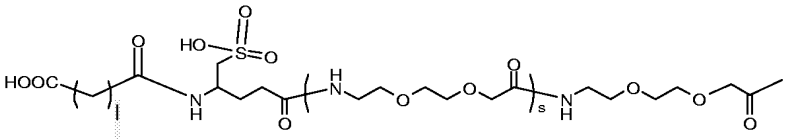
n is 1 or 2

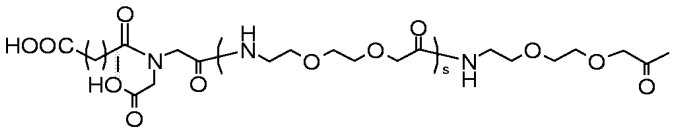
s is 0, 1, or 2,

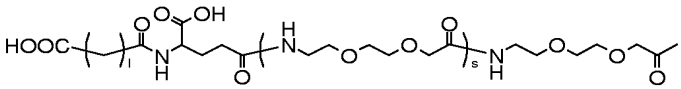
t is 0, 1, 2, or 3

p is 1, 2, 3, 4, 7, 11 or 23

9. A GLP-1 analog according to claims 6-8 wherein B-U'- is



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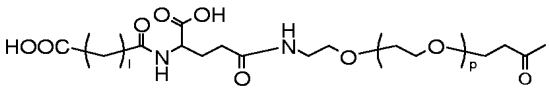

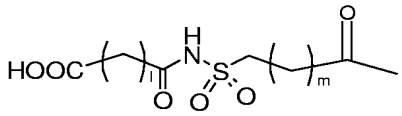
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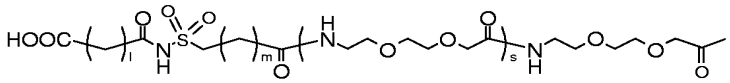
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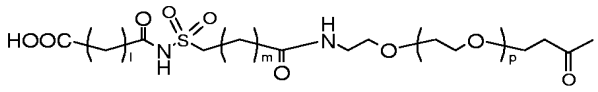
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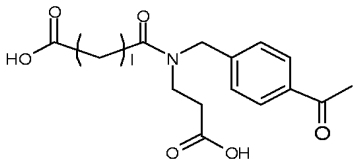
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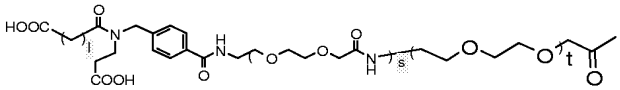
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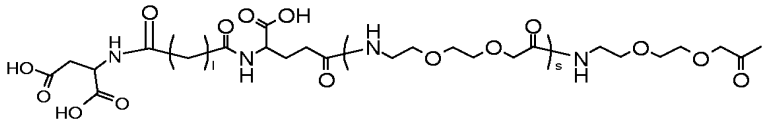
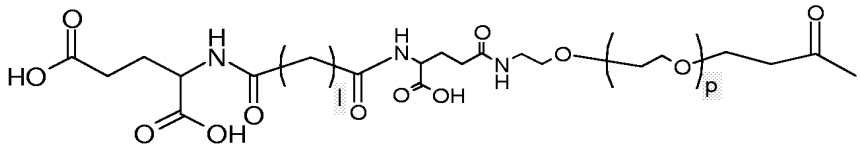



where l is 14, 15, 16, 17, 18, 19 or 20;
p is 1, 2, 3, 4, 7, 8 , 9, 10, 11 or 12.
s is 0, 1 or 2
t is 0 or 1;
m is 2, 3 or 4;

10. A GLP-1 analog according to claim 9, wherein
where l is 14, 15, 16, 17 or 18
p is 1, 2, 3, 4 or 11;
s is 0, 1 or 2;
t is 0 or 1;

11. A GLP-1 analog according to any of the claims 6-10, wherein s is 1.

12. A GLP-1 analog according any of the claims 6-10 wherein l is 16

13. A GLP-1 analog according to any of the claims 6-10 wherein p is 3 or 4.

14. A GLP-1 analog according to any of the claims 6-10 wherein n is 1.

15. A GLP-1 analog according to any of the claims 7-14, wherein
Xaa_7 is His or desamino-histidine;
Xaa_8 is Ala, Gly, Val, Leu, Ile, Lys or Aib;
Xaa_{16} is Val;
Xaa_{18} is Ser;
Xaa_{19} is Tyr;
Xaa_{20} is Leu;
Xaa_{22} is Gly, Glu or Aib;
Xaa_{23} is Gln or Glu;
Xaa_{25} is Ala;
Xaa_{27} is Glu;
Xaa_{30} is Ala or Glu;
Xaa_{33} is Val;
Xaa_{34} is Lys or Arg;
Xaa_{35} is Gly or Aib;
Xaa_{36} is Arg or Lys
Xaa_{37} is Gly, amide or is absent;

16. A GLP-1 analog according to claim 15, wherein
Xaa_7 is His
Xaa_8 is Gly, or Aib;
Xaa_{16} is Val;
Xaa_{18} is Ser;
Xaa_{19} is Tyr;
Xaa_{20} is Leu;
Xaa_{22} is Glu or Aib;
Xaa_{23} is Gln;
Xaa_{25} is Ala;
Xaa_{27} is Glu;
Xaa_{30} is Ala;
Xaa_{33} is Val;
Xaa_{34} is Lys or Arg;
Xaa_{35} is Gly or Aib;
Xaa_{36} is Arg

Xaa_{37} is Gly

17. A GLP-1 analog according to any one of the claims 1-15, wherein said GLP-1 analog comprises a modification of the N-terminal L-histidine in position 7 of the GLP-1(7-37) sequence.

18. A GLP-1 analog according to claim 17, wherein said GLP-1 analog comprises imidazopropionyl^7, α-hydroxy-histidine^7 or N-methyl-histidine^7, D-histidine^7, desamino-histidine^7, 2-amino-histidine^7, β-hydroxy-histidine^7, homohistidine^7, N^{α}-acetyl-histidine^7, α-fluoromethyl-histidine^7, α-methyl-histidine^7, 3-pyridylalanine^7, 2-pyridylalanine^7 or 4-pyridylalanine^7.

19. A GLP-1 analog according to any one of the claims 1-18, wherein said GLP-1 analog comprises a substitution of the L-alanine in position 8 of the GLP-1(7-37) sequence for another amino acid residue.

20. A GLP-1 analog according to claim 19, wherein said GLP-1 analog comprises Aib^8, Gly^8, Val^8, Ile^8, Leu^8, Ser^8, Thr^8, (1-aminocyclopropyl) carboxylic acid, (1-aminocyclobutyl) carboxylic acid, (1-aminocyclopentyl) carboxylic acid, (1-aminocyclohexyl) carboxylic acid, (1-aminocycloheptyl) carboxylic acid, or (1-aminocyclooctyl) carboxylic acid.

21. A GLP-1 analog according to claim 20, wherein said GLP-1 analog comprises Aib^8;

22. A GLP-1 analog according to any one of the preceding claims, wherein said GLP-1 analog comprises no more than fifteen amino acid residues which have been exchanged, added or deleted as compared to GLP-1(7-37) (SEQ ID No. 1),

23. A GLP-1 analog according to claim 22, wherein no more than ten amino acid residues which have been exchanged, added or deleted as compared to GLP-1(7-37) (SEQ ID No. 1).

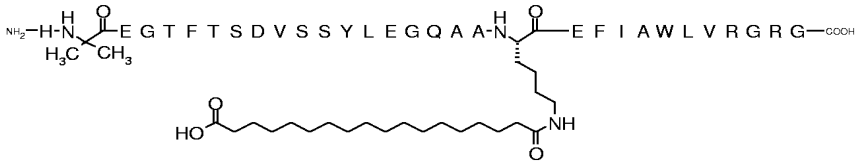
24. A GLP-1 analog according to claim 23, wherein said GLP-1 analog comprises no more than six amino acid residues which have been exchanged, added or deleted as compared to GLP-1(7-37) (SEQ ID No. 1).

25. A GLP-1 analog according to any of the above claims, wherein said GLP-1 analog comprises no more than 3 amino acid residues which are not encoded by the genetic code.

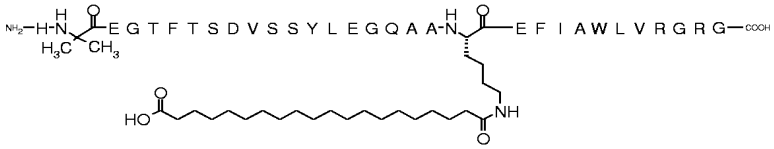
26. A GLP-1 analog according to any one of the preceding claims, wherein said GLP-1 analog comprises only one lysine residue.

27. A GLP-1 analog according to any of the above claims, which is
Aib^{8},Arg^{34}-GLP-1(7-37)
Aib^{8,22},Arg^{34}-GLP-1(7-37).
Arg^{34}-GLP-1(7-37).
[3-(4-Imidazolyl)Propionyl7,Arg34]GLP-1-(7-37)peptide
Gly^{8},Arg^{34}-GLP-1(7-37)
Aib^{8},Arg^{34}, Pro^{37}-GLP-1(7-37)
Aib^{8,22,27,30,35},Arg^{34},Pro^{37}- GLP-1 (7-37)amide,
all of which are substituted by B-U' in position 26.

28. A compound according to any one of the preceding claims, which is selected from



N-ε^{26}--(17-carboxyheptadecanoyl)-[Aib8,Arg34]GLP-1-(7-37)-peptide,



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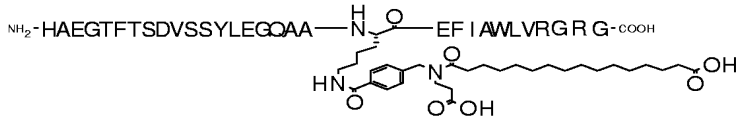

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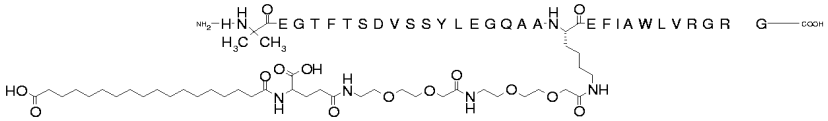
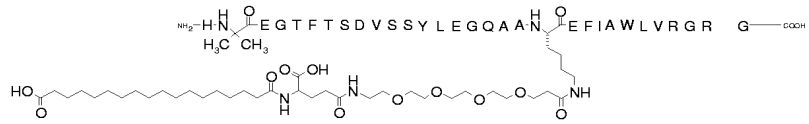


N-ε^{26}-[2-(2-[2-(2-[2-(2-[4-(17-Carboxyheptadecanoylamino)-4(S)-carboxybutyrylamino]ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][Aib8,Arg34]GLP-1-(7-37)peptide,



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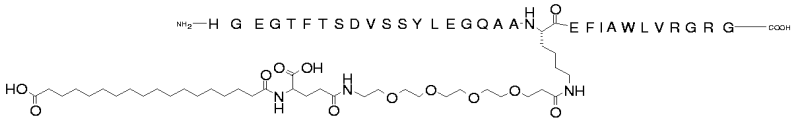

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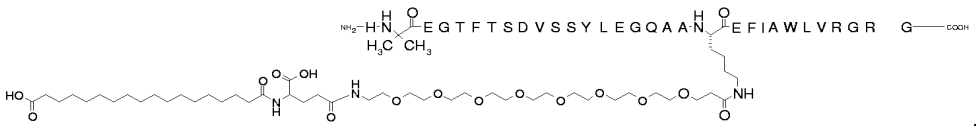

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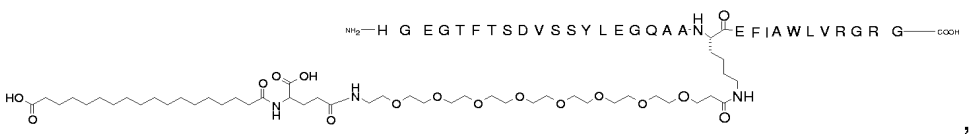

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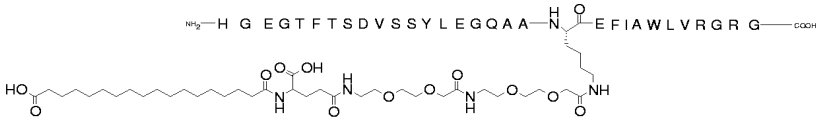

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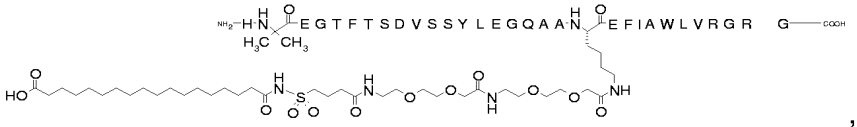

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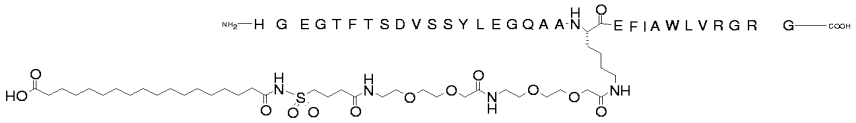

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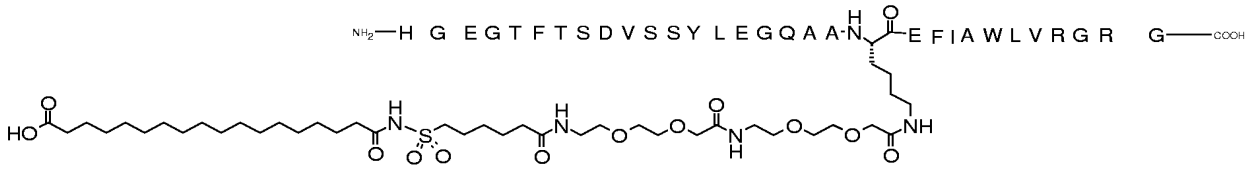

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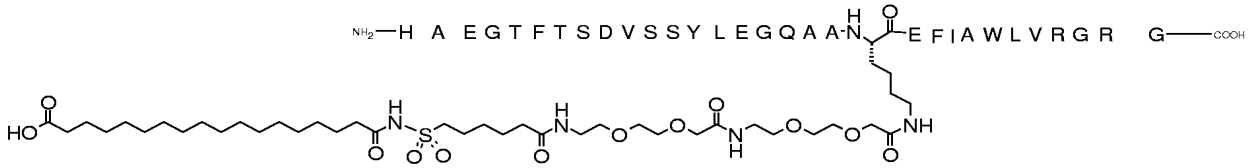

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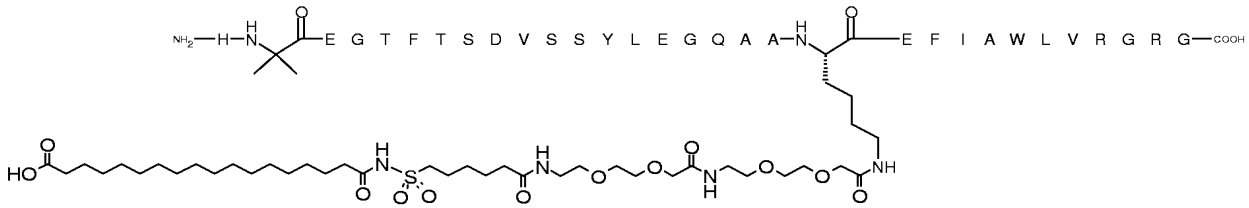

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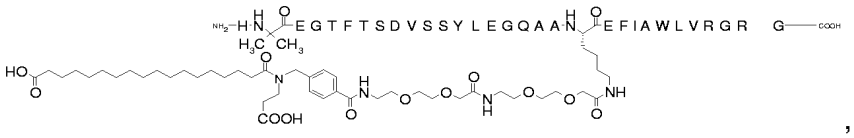

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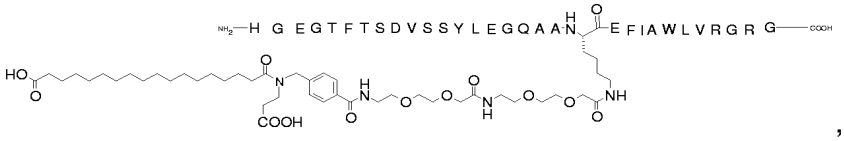

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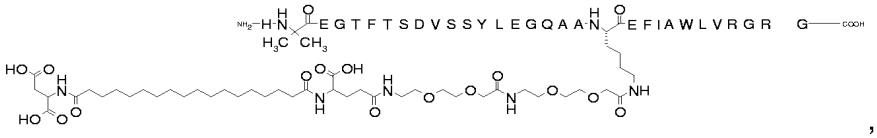

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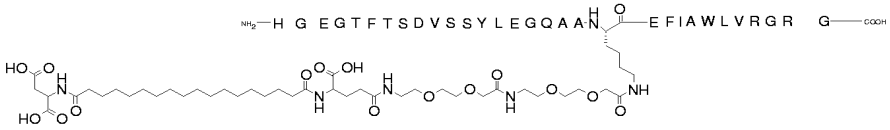

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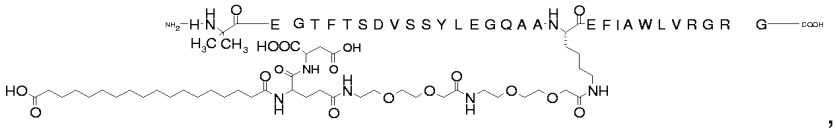

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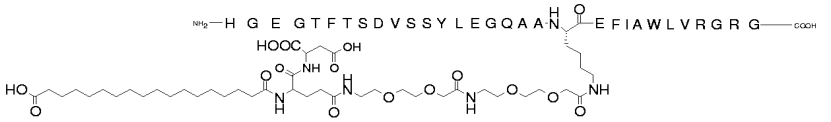

29. A method for increasing the time of action in a patient of a GLP-1 analog, characterised in acylating said GLP-1 analog with a moiety B-U' as disclosed in any of the preceding claims, on the lysine residue in position 26 of said GLP-1 analog.

30. A method for increasing the time of action in a patient of a GLP-1 analog to more than about 40 hours, characterised in modifying at least one of the amino acid residues in positions 7 and 8 of a GLP-1(7-37) peptide or an analog thereof, and acylating said GLP-1 analog with a moiety B-U'- as disclosed in any of the preceding claims on the lysine residue in position 26 of said GLP-1 analog.

31. A pharmaceutical composition comprising a compound according to any one of claims 1-28, and a pharmaceutically acceptable excipient.

32. The pharmaceutical composition according to claim 31, which is suited for parenteral administration.

33. Use of a compound according to any one of the claims 1-28 for the preparation of a medicament.

34. Use of a compound according to any one of the claims 1-28 for the preparation of a medicament for the treatment or prevention of hyperglycemia, type 2 diabetes, impaired glucose tolerance, type 1 diabetes, obesity, hypertension, syndrome X, dyslipidemia, cognitive disorders, atheroschlerosis, myocardial infarction, coronary heart disease and other cardiovascular disorders, stroke, inflammatory bowel syndrome, dyspepsia and gastric ulcers.

35. Use of a compound according to any one of the claims 1-28 for the preparation of a medicament for delaying or preventing disease progression in type 2 diabetes.

36. Use of a compound according to any one of the claims 1-28 for the preparation of a medicament for decreasing food intake, decreasing β-cell apoptosis, increasing β-cell function and β-cell mass, and/or for restoring glucose sensitivity to β-cells.

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WO 2006/097537 A2

(54) Title: ACYLATED GLP-1 COMPOUNDS

(57) Abstract: Protracted GLP-1 compounds and therapeutic uses thereof.

ACYLATED GLP-1 COMPOUNDS

FIELD OF THE INVENTION

This invention relates to the field of therapeutic peptides, i.e. to new protracted GLP-1 compounds.

BACKGROUND OF THE INVENTION

A range of different approaches have been used for modifying the structure of glucagon-like peptide 1 (GLP-1) compounds in order to provide a longer duration of action in vivo.
WO 96/29342 discloses peptide hormone derivatives wherein the parent peptide hormone has been modified by introducing a lipophilic substituent in the C-terminal amino acid residue or in the N-terminal amino acid residue.
WO 98/08871 discloses GLP-1 derivatives wherein at least one amino acid residue of the parent peptide has a lipophilic substituent attached.
WO 99/43708 discloses GLP-1(7-35) and GLP-1(7-36) derivatives which have a lipophilic substituent attached to the C-terminal amino acid residue.
WO 00/34331 discloses acylated GLP-1 analogs.
WO 00/69911 discloses activated insulinotropic peptides to be injected into patients where they are supposed to react with blood components to form conjugates and thereby alledgedly providing longer duration of action in vivo.
WO 02/46227 discloses GLP-1 and exendin-4 analogs fused to human serum albumin in order to extend in vivo half-life.

Many diabetes patients particularly in the type 2 diabetes segment are subject to so-called “needle-phobia”, i.e. a substantial fear of injecting themselves. In the type 2 diabetes segment most patients are treated with oral hypoglycaemic agents, and since GLP-1 compounds are expected to be the first injectable product these patients will be administered, the fear of injections may become a serious obstacle for the widespread use of the clinically very promising GLP-1 compounds. Thus, there is a need to develop new GLP-1 compounds which can be administered less than once daily, e.g. once every second or third day preferably once weekly, while retaining an acceptable clinical profile.

SUMMARY OF THE INVENTION

The invention provides a GLP-1 analog having a modification of at least one non-proteogenic amino acid residue in positions 7 and/or 8 relative to the sequence GLP-1(7-37) (SEQ ID No 1), which is acylated with a moiety to the lysine residue in position 26, and where said moiety comprises at least two acidic groups, wherein one acidic group is attached terminally.

The present invention also provides pharmaceutical compositions comprising a compound according to the present invention and the use of compounds according to the present invention for preparing medicaments for treating disease.

The invention provides a method for increasing the time of action in a patient of a GLP-1 analog, characterised in acylating said GLP-1 analog with a moiety B-U' as disclosed in any of the preceding claims, on the lysine residue in position 26 of said GLP-1 analog.

DESCRIPTION OF THE INVENTION

In the present specification, the following terms have the indicated meaning :

The term "polypeptide" and "peptide" as used herein means a compound composed of at least five constituent amino acids connected by peptide bonds. The constituent amino acids may be from the group of the amino acids encoded by the genetic code and they may be natural amino acids which are not encoded by the genetic code, as well as synthetic amino acids. Natural amino acids which are not encoded by the genetic code are e.g., γ-carboxyglutamate, ornithine, phosphoserine, D-alanine and D-glutamine. Synthetic amino acids comprise amino acids manufactured by chemical synthesis, i.e. D-isomers of the amino acids encoded by the genetic code such as D-alanine and D-leucine, Aib (α-aminoisobutyric acid), Abu (α-aminobutyric acid), Tle (tert-butylglycine), β-alanine, 3-aminomethyl benzoic acid, anthranilic acid.

The 22 proteogenic amino acids are:

Alanine, Arginine, Asparagine, Aspartic acid, Cysteine, Cystine, Glutamine, Glutamic acid, Glycine, Histidine, Hydroxyproline, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Proline, Serine, Threonine, Tryptophan, Tyrosine, Valine.

Thus a non-proteogenic amino acid is a moiety which can be incorporated into a peptide via peptide bonds but is not a proteogenic amino acid. Examples are γ-carboxyglutamate, ornithine, phosphoserine, the D-amino acids such as D-alanine and D-glutamine, Synthetic non-proteogenic amino acids comprise amino acids manufactured by chemical synthesis, i.e. D-isomers of the amino acids encoded by the genetic code such as D-alanine and D-leucine,

Aib (α-aminoisobutyric acid), Abu (α-aminobutyric acid), Tle (tert-butylglycine), 3-aminomethyl benzoic acid, anthranilic acid, des-amino-Histidine, the beta analogs of amino acids such as β-alanine etc. D-histidine, desamino-histidine, 2-amino-histidine, β-hydroxy-histidine, homohistidine, N^{α}-acetyl-histidine, α-fluoromethyl-histidine, α-methyl-histidine, 3-pyridylalanine, 2-pyridylalanine or 4-pyridylalanine, (1-aminocyclopropyl) carboxylic acid, (1-aminocyclobutyl) carboxylic acid, (1-aminocyclopentyl) carboxylic acid, (1-aminocyclohexyl) carboxylic acid, (1-aminocycloheptyl) carboxylic acid, or (1-aminocyclooctyl) carboxylic acid;

The term "analogue" as used herein referring to a polypeptide means a modified peptide wherein one or more amino acid residues of the peptide have been substituted by other amino acid residues and/or wherein one or more amino acid residues have been deleted from the peptide and/or wherein one or more amino acid residues have been deleted from the peptide and or wherein one or more amino acid residues have been added to the peptide. Such addition or deletion of amino acid residues can take place at the N-terminal of the peptide and/or at the C-terminal of the peptide. A simple system is often used to describe analogues : For example [Arg^{34}]GLP-1(7-37)Lys designates a GLP-1(7-37) analogue wherein the naturally occurring lysine at position 34 has been substituted with arginine and wherein a lysine has been added to the terminal amino acid residue, i.e. to the Gly^{37}. All amino acids for which the optical isomer is not stated is to be understood to mean the L-isomer. In embodiments of the invention a maximum of 17 amino acids have been modified. In embodiments of the invention a maximum of 15 amino acids have been modified. In embodiments of the invention a maximum of 10 amino acids have been modified. In embodiments of the invention a maximum of 8 amino acids have been modified. In embodiments of the invention a maximum of 7 amino acids have been modified. In embodiments of the invention a maximum of 6 amino acids have been modified. In embodiments of the invention a maximum of 5 amino acids have been modified. In embodiments of the invention a maximum of 4 amino acids have been modified. In embodiments of the invention a maximum of 3 amino acids have been modified. In embodiments of the invention a maximum of 2 amino acids have been modified. In embodiments of the invention 1 amino acid has been modified.

The term "derivative" as used herein in relation to a peptide means a chemically modified peptide or an analogue thereof, wherein at least one substituent is not present in the unmodified peptide or an analogue thereof, i.e. a peptide which has been covalently modified. Typical modifications are amides, carbohydrates, alkyl groups, acyl groups, esters and the like. An example of a derivative of GLP-1(7-37) is $N^{\varepsilon 26}$-((4S)-4-(hexadecanoylamino)-carboxy-butanoyl)[Arg^{34}, Lys^{26}]GLP-1-(7-37).

The term "GLP-1 peptide" as used herein means GLP-1(7-37) (SEQ ID No 1), a GLP-1(7-37) analogue, a GLP-1(7-37) derivative or a derivative of a GLP-1(7-37) analogue. In one embodiment the GLP-1 peptide is an insulinotropic agent.

The term "insulinotropic agent" as used herein means a compound which is an agonist of the human GLP-1 receptor, i.e. a compound which stimulates the formation of cAMP in a suitable medium containing the human GLP-1 receptor (one such medium disclosed below). The potency of an insulinotropic agent is determined by calculating the EC_{50} value from the dose-response curve as described below.

Baby hamster kidney (BHK) cells expressing the cloned human GLP-1 receptor (BHK- 467-12A) were grown in DMEM media with the addition of 100 IU/mL penicillin, 100 µg/mL streptomycin, 5% fetal calf serum and 0.5 mg/mL Geneticin G-418 (Life Technologies). The cells were washed twice in phosphate buffered saline and harvested with Versene. Plasma membranes were prepared from the cells by homogenisation with an Ultraturrax in buffer 1 (20 mM HEPES-Na, 10 mM EDTA, pH 7.4). The homogenate was centrifuged at 48,000 x g for 15 min at 4°C. The pellet was suspended by homogenization in buffer 2 (20 mM HEPES-Na, 0.1 mM EDTA, pH 7.4), then centrifuged at 48,000 x g for 15 min at 4°C. The washing procedure was repeated one more time. The final pellet was suspended in buffer 2 and used immediately for assays or stored at -80°C.

The functional receptor assay was carried out by measuring cyclic AMP (cAMP) as a response to stimulation by the insulinotropic agent. cAMP formed was quantified by the AlphaScreen™ cAMP Kit (Perkin Elmer Life Sciences). Incubations were carried out in half-area 96-well microtiter plates in a total volume of 50 µL buffer 3 (50 mM Tris-HCl, 5 mM HEPES, 10 mM $MgCl_2$, pH 7.4) and with the following addiditions: 1 mM ATP, 1 µM GTP, 0.5 mM 3-isobutyl-1-methylxanthine (IBMX), 0.01 % Tween-20, 0.1% BSA, 6 µg membrane preparation, 15 µg/mL acceptor beads, 20µg/mL donor beads preincubated with 6 nM biotinyl-cAMP. Compounds to be tested for agonist activity were dissolved and diluted in buffer 3. GTP was freshly prepared for each experiment. The plate was incubated in the dark with slow agitation for three hours at room temperature followed by counting in the Fusion™ instrument (Perkin Elmer Life Sciences). Concentration-response curves were plotted for the individual compounds and EC_{50} values estimated using a four-parameter logistic model with Prism v. 4.0 (GraphPad, Carlsbad, CA).

The term "DPP-IV protected" as used herein referring to a polypeptide means a polypeptide which has been chemically modified in order to render said compound resistant to the plasma peptidase dipeptidyl aminopeptidase-4 (DPP-IV). The DPP-IV enzyme in plasma is known to be involved in the degradation of several peptide hormones, e.g. GLP-1, GLP-2, Exendin-4 etc. Thus, a considerable effort is being made to develop analogues and derivatives

of the polypeptides susceptible to DPP-IV mediated hydrolysis in order to reduce the rate of degradation by DPP-IV. In one embodiment a DPP-IV protected peptide is more resistant to DPP-IV than GLP-1(7-37) or Exendin-4(1-39).

Resistance of a peptide to degradation by dipeptidyl aminopeptidase IV is determined by the following degradation assay :

Aliquots of the peptide (5 nmol) are incubated at 37 ºC with 1 µL of purified dipeptidyl aminopeptidase IV corresponding to an enzymatic activity of 5 mU for 10-180 minutes in 100 µL of 0.1 M triethylamine-HCl buffer, pH 7.4. Enzymatic reactions are terminated by the addition of 5 µL of 10% trifluoroacetic acid, and the peptide degradation products are separated and quantified using HPLC analysis. One method for performing this analysis is : The mixtures are applied onto a Vydac C18 widepore (30 nm pores, 5 µm particles) 250 x 4.6 mm column and eluted at a flow rate of 1 ml/min with linear stepwise gradients of acetonitrile in 0.1% trifluoroacetic acid (0% acetonitrile for 3 min, 0-24% acetonitrile for 17 min, 24-48% acetonitrile for 1 min) according to Siegel et al., Regul. Pept. 1999;79:93-102 and Mentlein et al. Eur. J. Biochem. 1993;214:829-35. Peptides and their degradation products may be monitored by their absorbance at 220 nm (peptide bonds) or 280 nm (aromatic amino acids), and are quantified by integration of their peak areas related to those of standards. The rate of hydrolysis of a peptide by dipeptidyl aminopeptidase IV is estimated at incubation times which result in less than 10% of the peptide being hydrolysed.

The term "C_{1-6}-alkyl" as used herein means a saturated, branched, straight or cyclic hydrocarbon group having from 1 to 6 carbon atoms. Representative examples include, but are not limited to, methyl, ethyl, n-propyl, isopropyl, butyl, isobutyl, *sec*-butyl, *tert*-butyl, n-pentyl, isopentyl, neopentyl, *tert*-pentyl, n-hexyl, isohexyl, cyclohexane and the like.

The term "pharmaceutically acceptable" as used herein means suited for normal pharmaceutical applications, i.e. giving rise to no adverse events in patients etc.

The term "excipient" as used herein means the chemical compounds which are normally added to pharmaceutical compositions, e.g. buffers, tonicity agents, preservatives and the like.

The term "effective amount" as used herein means a dosage which is sufficient to be effective for the treatment of the patient compared with no treatment.

The term "pharmaceutical composition" as used herein means a product comprising an active compound or a salt thereof together with pharmaceutical excipients such as buffer, preservative, and optionally a tonicity modifier and/or a stabilizer. Thus a pharmaceutical composition is also known in the art as a pharmaceutical formulation.

The term "treatment of a disease" as used herein means the management and care of a patient having developed the disease, condition or disorder. The purpose of treatment is to combat the disease, condition or disorder. Treatment includes the administration of the active compounds to eliminate or control the disease, condition or disorder as well as to alleviate the symptoms or complications associated with the disease, condition or disorder.

In another aspect the present invention relates to an acylated GLP-1 analogue that can bind to albumin and the GLP-1 receptor simultaneously.

In another aspect the present invention relates to an acylated GLP-1 analogue that bind to the GLP-1 receptor with an affinity below 100nM, preferable below 30 nM in the presence of 2% albumin.

In another aspect the present invention relates to an acylated GLP-1 analogue which affinity to the GLP-1 receptor is only partly decreased when comparing the affinity in the presence of very low concentration (e.g. 0.005% to 0.2%) of human albumin to the affinity in the presence of 2% human albumin. The shift in binding affinity under these conditions is less than 50 fold, preferable below 30 fold and more preferable below 10 fold.

The term "albumin binding moiety" as used herein means a residue which binds non-covalently to human serum albumin. The albumin binding residue attached to the therapeutic polypeptide typically has an affinity below 10 µM to human serum albumin and preferably below 1 µM. A range of albumin binding residues are known among linear and branched lipohophillic moieties containing 4-40 carbon atoms having a distal acidic group.

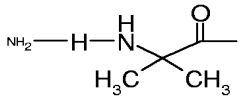
The term "hydrophilic linker" as used herein means a spacer that separates a peptide and an albumin binding residue with a chemical moiety which comprises at least 5 non-hydrogen atoms where 30-50% of these are either N or O.

The term "acidic groups" as used herein means organic chemical groups which are fully or partly negatively charged at physiological pH. The pKa value of such groups is below 7, preferable below 5. This includes but is not limited to carboxylic acids, sulphonic acids, phosphoric acids or heterocyclic ring systems which are fully or partly negatively charged at physiological pH.

In the below structural formula II the moiety U is a di-radical may be attached to the terminal groups B and the aminogroup of the lysine amino acid in the peptide in two different ways. In embodiments of the invention the U in formula II is attached with the group B attached at the end of the alkyl chain and the peptide at the other end.

In the formulas below the terminal bonds from the attached groups are to be regarded as attachment bonds and not ending in methylene groups unless stated.

In the formulas below


means the H_2N-His-Aib- N-terminal of the GLP-1 analogue.

In an embodiment the invention provides a GLP-1 analog acylated with a lipophillic albumin binding moiety containing at least two free acidic chemical groups attached via a non natural amino acid linker to the lysine residue in position 26.

In an embodiment, the term free acidic chemical groups is to be understood as having the same meaning as "acidic groups" as used herein.

In an embodiment the invention provides an acylated GLP-1 analog where said GLP-1 analog is stabilised against DPP-IV by modification of at least one amino acid residue in positions 7 and 8 relative to the sequence GLP-1(7-37) (SEQ ID No 1), and where said acylation is a diacid attached to the lysine residue in position 26 optionally via a non natural amino acid hydrophilic linker.

In an embodiment of the invention a GLP-1 analog having a modification of at least one non-proteogenic amino acid residue in positions 7 and/or 8 relative to the sequence GLP-1(7-37) (SEQ ID No 1), which is acylated with a moiety to the lysine residue in position 26, and where said moiety comprises at least two acidic groups, wherein one acidic group is attached terminally.

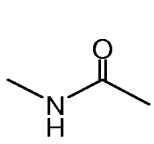
An embodiment provides a GLP-1 analog according to the above embodiment, wherein the moiety attached in position 26 comprises a hydrophilic linker.

An embodiment provides a GLP-1 analog according to the above embodiments, wherein the hydrophilic linker comprises at least 5 non-hydrogen atoms where 30-50% of these are either N or O.

An embodiment provides a GLP-1 analog according to any of the above embodiments, wherein the moiety attached in position 26 comprises an albumin binding moiety seperated from the peptide by the hydrophilic linker.

An embodiment provides a GLP-1 analog according to the above embodiment, wherein the albumin binding moiety is a linear or branched lipophilic moiety containing 4-40 carbon atoms having a distal acidic group.

An embodiment provides a GLP-1 analog according to any of the above embodiments, wherein the acylated moiety is B-U', where U' is selected from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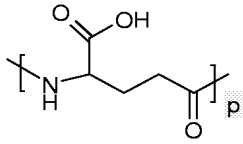
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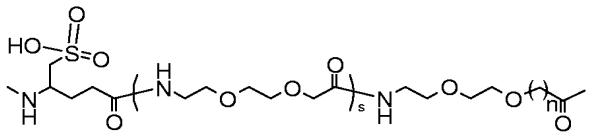

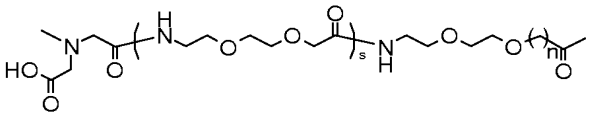

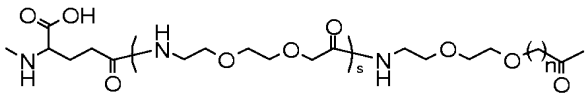

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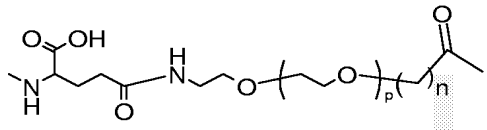

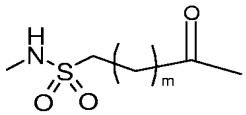

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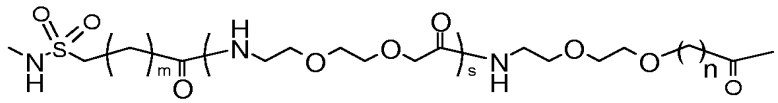

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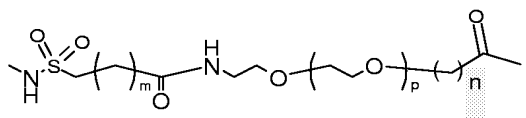

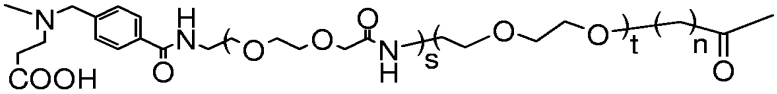

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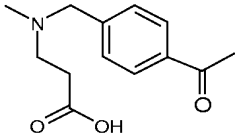

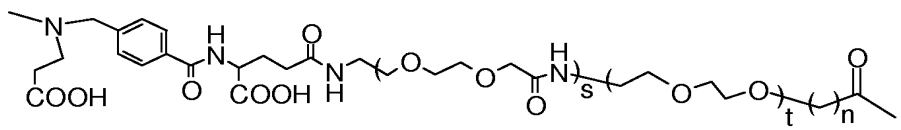

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m is 0, 1, 2, 3, 4, 5, or 6,

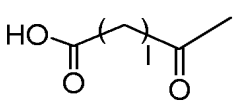
n is 1, 2 or 3

s is 0, 1, 2, or 3,

t is 0, 1, 2, 3, or 4

p is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17,18, 19, 20, 21, 22, or 23;

and where B is an acidic group selected from



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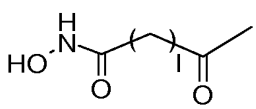

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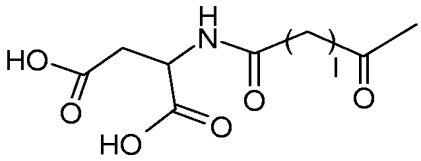
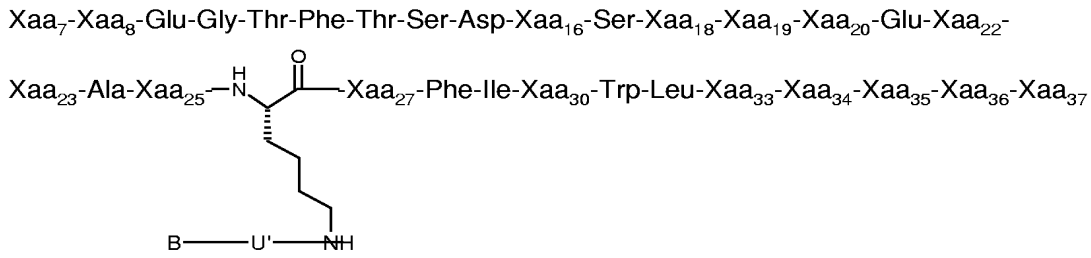


where l is 12, 13, 14, 15, 16, 17, 18, 19 or 20;

An embodiment provides a GLP-1 analog according to any of the above embodiments, which is a compound of formula I (SEQ ID No. 2) :



Formula I

wherein

Xaa_7 is L-histidine, imidazopropionyl, α-hydroxy-histidine, D-histidine, desamino-histidine, 2-amino-histidine, β-hydroxy-histidine, homohistidine, N^{α}-acetyl-histidine, N^{α}-formyl-histidine, α-fluoromethyl-histidine, α-methyl-histidine, 3-pyridylalanine, 2-pyridylalanine or 4-pyridylalanine

Xaa_8 is Ala, Gly, Val, Leu, Ile, Thr, Ser, Lys, Aib, (1-aminocyclopropyl) carboxylic acid, (1-aminocyclobutyl) carboxylic acid, (1-aminocyclopentyl) carboxylic acid, (1-aminocyclohexyl) carboxylic acid, (1-aminocycloheptyl) carboxylic acid, or (1-aminocyclooctyl) carboxylic acid;

Xaa_{16} is Val or Leu;

Xaa_{18} is Ser, Lys or Arg;

Xaa_{19} is Tyr or Gln;

Xaa_{20} is Leu or Met;

Xaa_{22} is Gly, Glu or Aib;

Xaa_{23} is Gln, Glu, Lys or Arg;

Xaa_{25} is Ala or Val;

Xaa_{27} is Glu or Leu;

Xaa_{30} is Ala, Glu or Arg;

Xaa_{33} is Val or Lys;

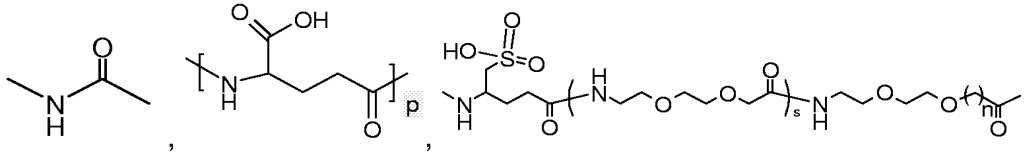
Xaa_{34} is Lys, Glu, Asn or Arg;

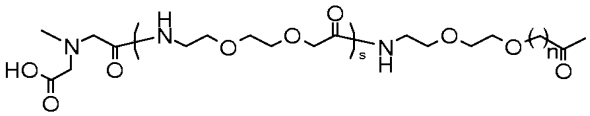
Xaa_{35} is Gly or Aib;

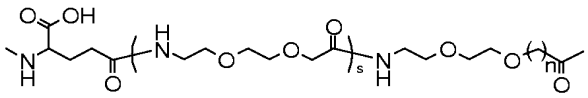
Xaa_{36} is Arg, Gly or Lys, or is absent;

Xaa_{37} is Gly, Ala, Glu, Pro, Lys, or is absent;

and B and U' together is the acylated moiety, where U' is selected from







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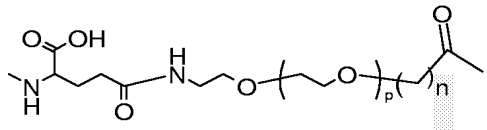

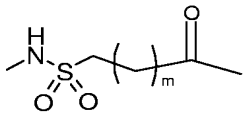

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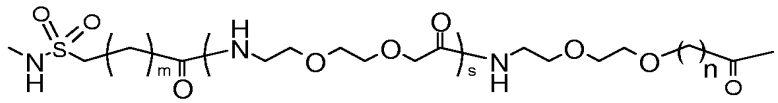

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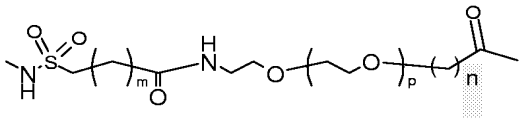

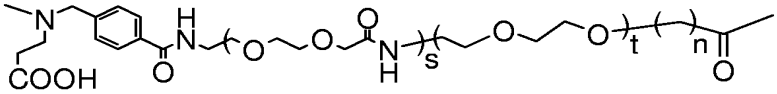

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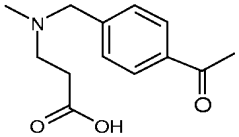

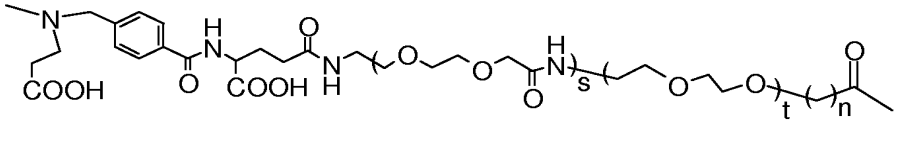

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m is 0, 1, 2, 3, 4, 5, or 6,

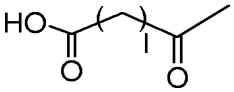
n is 1, 2 or 3

s is 0, 1, 2, or 3,

t is 0, 1, 2, 3, or 4

p is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17,18, 19, 20, 21, 22, or 23;

and where B is an acidic group selected from



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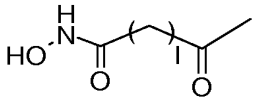

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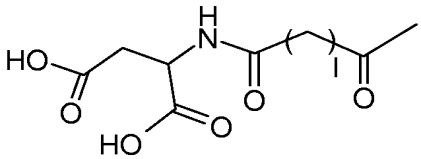
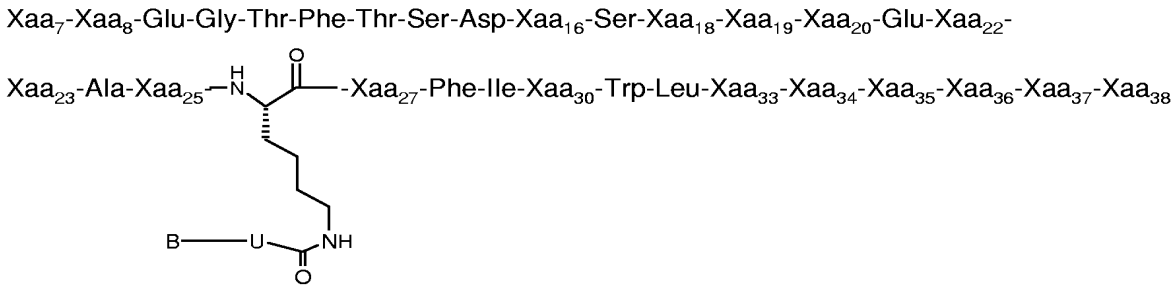


where l is 12, 13, 14, 15, 16, 17, 18, 19 or 20;

In an embodiment the invention provides a compound which is a compound of formula II (SEQ ID No. 3) :



Formula II

The formula II is identical to formula I as stated in an embodiment above, where the moiety B-U is replaced by B-U'. The difference being only the incorporation of the carboxy group in the U' relative to U, which is without the attaching carboxy group.

In formula II each of the Xaa's has the following meaning:

Xaa_7 is L-histidine, D-histidine, desamino-histidine, 2-amino-histidine, β-hydroxy-histidine, homohistidine, N^{α}-acetyl-histidine, α-fluoromethyl-histidine, α-methyl-histidine, 3-pyridylalanine, 2-pyridylalanine or 4-pyridylalanine;

Xaa_8 is Ala, Gly, Val, Leu, Ile, Lys, Aib, (1-aminocyclopropyl) carboxylic acid, (1-aminocyclobutyl) carboxylic acid, (1-aminocyclopentyl) carboxylic acid, (1-aminocyclohexyl) carboxylic acid, (1-aminocycloheptyl) carboxylic acid, or (1-aminocyclooctyl) carboxylic acid;

Xaa_{16} is Val or Leu;

Xaa_{18} is Ser, Lys or Arg;

Xaa_{19} is Tyr or Gln;

Xaa_{20} is Leu or Met;

Xaa_{22} is Gly, Glu or Aib;

Xaa_{23} is Gln, Glu, Lys or Arg;

Xaa_{25} is Ala or Val;

Xaa_{27} is Glu or Leu;

Xaa_{30} is Ala, Glu or Arg;

Xaa_{33} is Val or Lys;

Xaa_{34} is Lys, Glu, Asn or Arg;

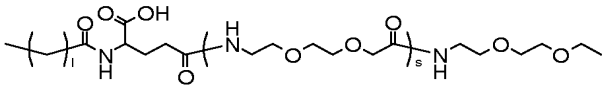
Xaa_{35} is Gly or Aib;

Xaa_{36} is Arg, Gly or Lys, or is absent;

Xaa_{37} is Gly, Ala, Glu, Pro, Lys, or is absent;

Xaa_{38} is Lys, Ser, amide or is absent;

and where U is a spacer selected from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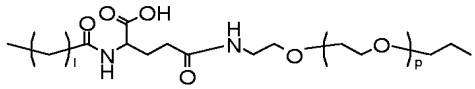

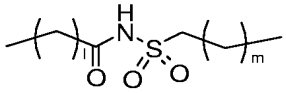
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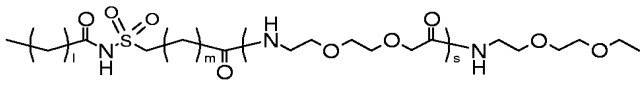
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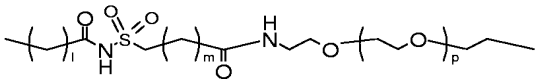
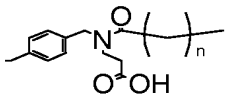



where n is 12, 13, 14, 15, 16, 17 or 18

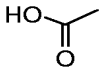
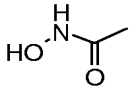
l is 12, 13, 14, 15, 16, 17 or 18,

m is 0, 1, 2, 3, 4, 5, or 6,

s is 0, 1, 2, or 3,

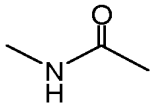
p is 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17,18, 19, 20, 21, 22, or 23;

and where B is an acidic group selected from

 and  .

In the embodiments below when referring to U' in formula I it is to be understood as also referring to formula II and U, with the only difference being the carboxy group.

An embodiment provides a GLP-1 analog according to the embodiments above, wherein U' is selected from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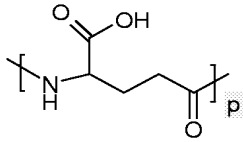
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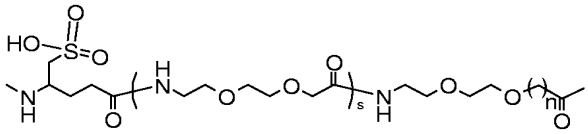

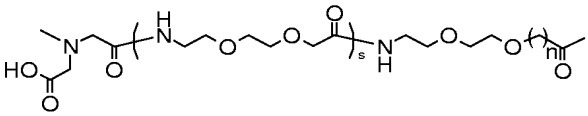

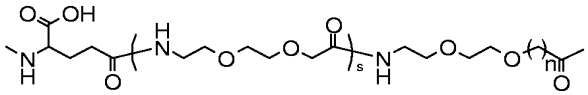
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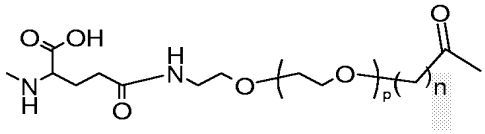

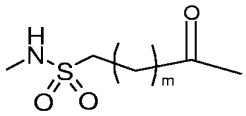
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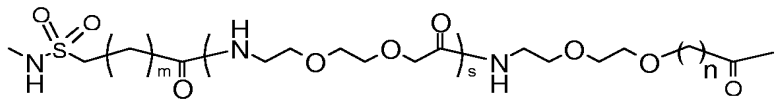
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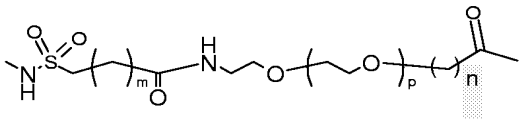

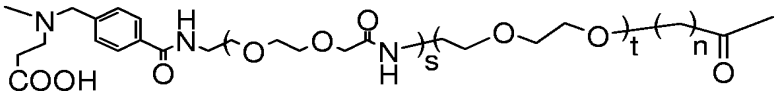
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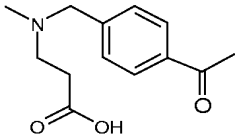
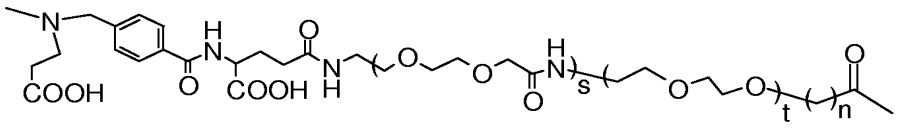


,

m is 2, 3, 4 or 5,

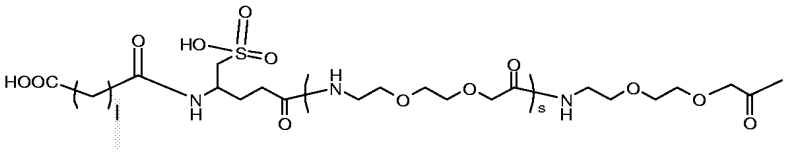
n is 1 or 2

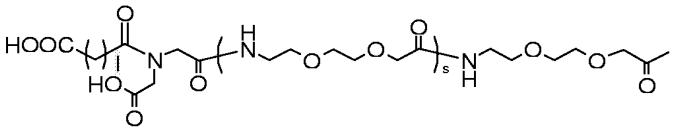
s is 0, 1, or 2,

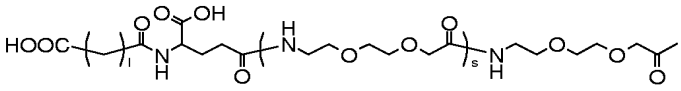
t is 0, 1, 2, or 3

p is 1, 2, 3, 4, 7, 11 or 23

An embodiment provides a GLP-1 analog according to the embodiments above, wherein B-U'- is



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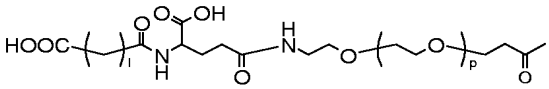

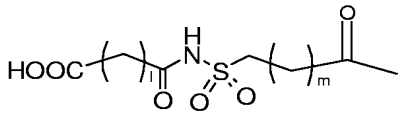
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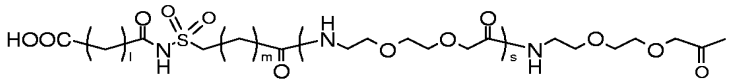
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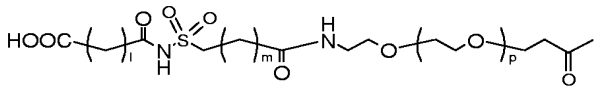
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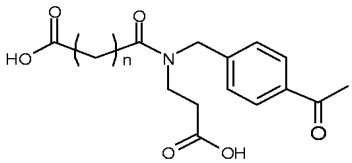
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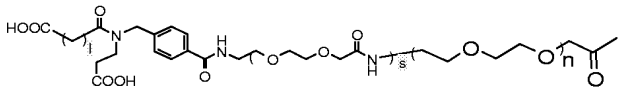
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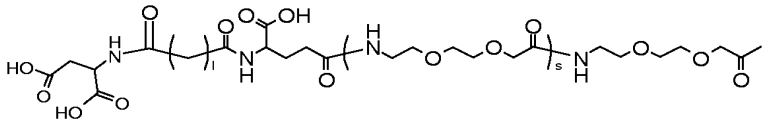
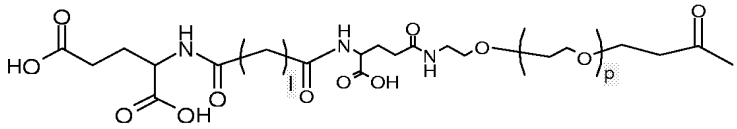



where l is 14, 15, 16, 17, 18, 19 or 20;

p is 1, 2, 3, 4, 7, 8 , 9, 10, 11 or 12.

s is 0, 1 or 2

t is 0 or 1;

An embodiment according to the above wherein

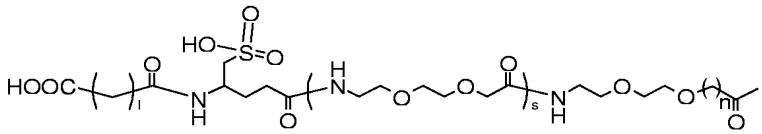
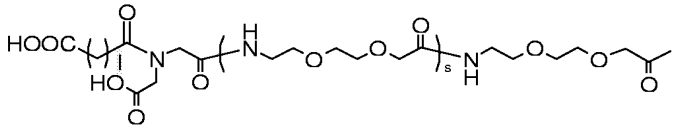
where l is 14, 15, 16, 17 or 18

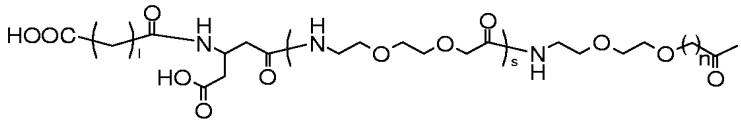
p is 1, 2, 3, 4 or 11;

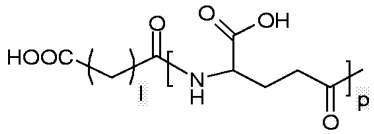
s is 0, 1 or 2;

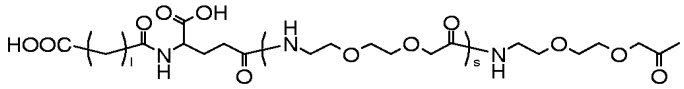
t is 0 or 1;

An embodiment provides a GLP-1 analog according to the embodiment above, wherein B-U' is







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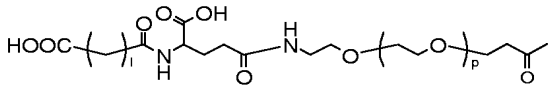

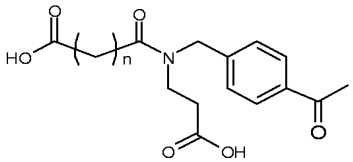

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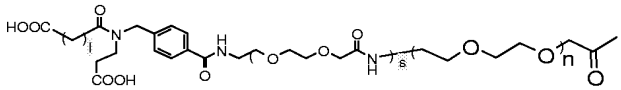


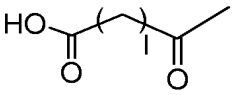
where l is 14, 15, 16, 17, 18, 19 or 20;

p is 1, 2, 3, or 4.

s is 0, 1 or 2

n is 0, 1 or 2

An embodiment according to any of the above embodiments is wherein B is



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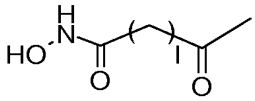

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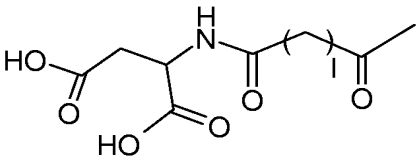
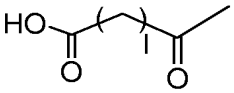


and l is 14,16, 18 or 20;

An embodiment provides a GLP-1 analog according to any of the embodiments above, wherein B is



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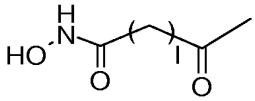

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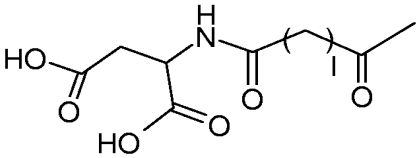


where l is 14, 15, or 16.

An embodiment provides a GLP-1 analog according to any of the embodiments above, wherein s is 1.

An embodiment provides a GLP-1 analog according to any of the embodiments above, wherein n is 1.

An embodiment provides a GLP-1 analog according any of the embodiments above, wherein l is 14, 15 or 16; In embodiments l is 17, 18, 19 or 20. In embodiments l is 15, 16 or 17. In embodiments l is 18, 19 or 20. In embodiments l is 14. In embodiments l is 16. In embodiments l is 18. In embodiments l is 20.

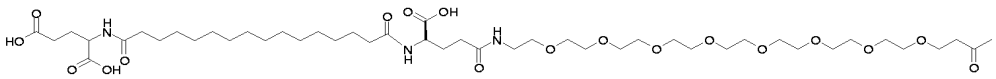
An embodiment provides a GLP-1 analog according to any of the embodiments above, wherein p is 1.

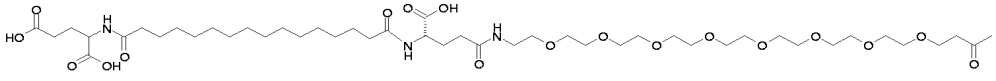
An embodiment provides a GLP-1 analog according to any of the embodiments above, wherein p is 2.

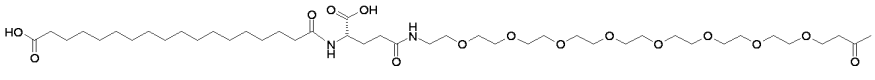
An embodiment provides a GLP-1 analog according to any of the embodiments above, wherein p is 3.

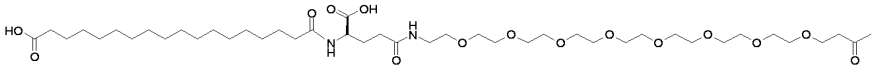
An embodiment provides a GLP-1 analog according to any of the embodiments above, wherein p is 4.

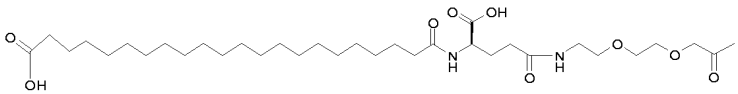
An embodiment provides a GLP-1 analog according to any of the embodiments above, wherein B-U' is

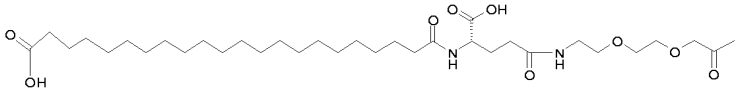


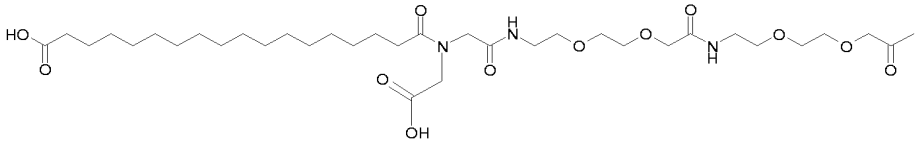




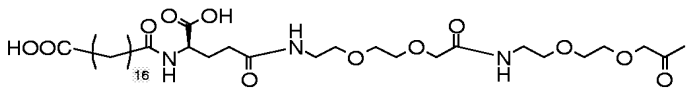




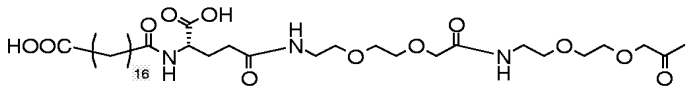




An embodiment provides a GLP-1 analog according to any of the embodiments above, wherein B-U' is



An embodiment provides a GLP-1 analog according to any of the embodiments above, wherein B-U' is



An embodiment provides a GLP-1 analog according to formula I above, wherein
Xaa_7 is His or desamino-histidine;
Xaa_8 is Ala, Gly, Val, Leu, Ile, Lys or Aib;
Xaa_{16} is Val;
Xaa_{18} is Ser;
Xaa_{19} is Tyr;
Xaa_{20} is Leu;
Xaa_{22} is Gly, Glu or Aib;
Xaa_{23} is Gln or Glu;
Xaa_{25} is Ala;
Xaa_{27} is Glu;
Xaa_{30} is Ala or Glu;
Xaa_{33} is Val;
Xaa_{34} is Lys or Arg;
Xaa_{35} is Gly or Aib;
Xaa_{36} is Arg or Lys
Xaa_{37} is Gly, amide or is absent;

An embodiment provides a GLP-1 analog according to formula I above, wherein
Xaa_7 is His
Xaa_8 is Gly, or Aib;
Xaa_{16} is Val;
Xaa_{18} is Ser;

Xaa_{19} is Tyr;
Xaa_{20} is Leu;
Xaa_{22} is Glu or Aib;
Xaa_{23} is Gln;
Xaa_{25} is Ala;
Xaa_{27} is Glu;
Xaa_{30} is Ala;
Xaa_{33} is Val;
Xaa_{34} is Lys or Arg;
Xaa_{35} is Gly or Aib;
Xaa_{36} is Arg
Xaa_{37} is Gly

An embodiment provides a GLP-1 analog according to any one of the above embodiments, wherein said GLP-1 analog comprises a modification of the N-terminal L-histidine in position 7 of the GLP-1(7-37) sequence.

An embodiment provides a GLP-1 analog according to the embodiment above, wherein said GLP-1 analog comprises imidazopropionyl^7, α-hydroxy-histidine^7 or N-methyl-histidine^7, D-histidine^7, desamino-histidine^7, 2-amino-histidine^7, β-hydroxy-histidine^7, homohistidine^7, N^{α}-acetyl-histidine^7, α-fluoromethyl-histidine^7, α-methyl-histidine^7, 3-pyridylalanine^7, 2-pyridylalanine^7 or 4-pyridylalanine^7.

An embodiment provides a GLP-1 analog according to any one of the embodiments above, wherein said GLP-1 analog comprises a substitution of the L-alanine in position 8 of the GLP-1(7-37) sequence for another amino acid residue.

An embodiment provides a GLP-1 analog according to the embodiment above, wherein said GLP-1 analog comprises Aib^8, Gly^8, Val^8, Ile^8, Leu^8, Ser^8, Thr^8, (1-aminocyclopropyl) carboxylic acid, (1-aminocyclobutyl) carboxylic acid, (1-aminocyclopentyl) carboxylic acid, (1-aminocyclohexyl) carboxylic acid, (1-aminocycloheptyl) carboxylic acid, or (1-aminocyclooctyl) carboxylic acid.

An embodiment provides a GLP-1 analog according to any of the the embodiment above, wherein said GLP-1 analog comprises Aib^8;

In one embodiment of the invention said GLP-1 analog is Aib^{8},Arg^{34}-GLP-1(7-37) or $Aib^{8,22}$,Arg^{34}-GLP-1(7-37).

An embodiment provides a GLP-1 analog according to any of the above embodiments, wherein said GLP-1 analog comprises no more than fifteen amino acid residues which have been exchanged, added or deleted as compared to GLP-1(7-37) (SEQ ID No. 1),

An embodiment provides a GLP-1 analog according to the embodiment above, wherein no more than ten amino acid residues which have been exchanged, added or deleted as compared to GLP-1(7-37) (SEQ ID No. 1).

An embodiment provides a GLP-1 analog according to the embodiment above, wherein said GLP-1 analog comprises no more than six amino acid residues which have been exchanged, added or deleted as compared to GLP-1(7-37) (SEQ ID No. 1).

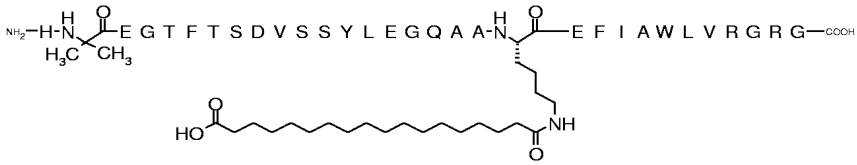
An embodiment provides a GLP-1 analog according to any of the above embodiments, wherein said GLP-1 analog comprises no more than 3 amino acid residues which are not encoded by the genetic code.

An embodiment provides a GLP-1 analog according to any of the above embodiments, wherein said GLP-1 analog comprises only one lysine residue.

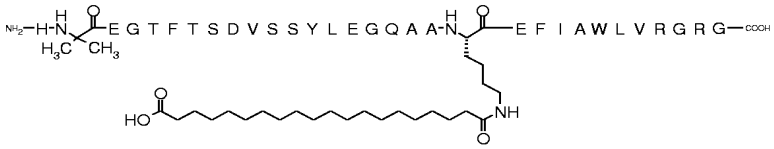
An embodiment provides a GLP-1 analog according to any of the above embodiments, which is

Aib^{8},Arg^{34}-GLP-1(7-37)
$Aib^{8,22}$,Arg^{34}-GLP-1(7-37).
Arg^{34}-GLP-1(7-37).
[3-(4-Imidazolyl)Propionyl7,Arg34]GLP-1-(7-37)peptide
Gly^{8},Arg^{34}-GLP-1(7-37)
Aib^{8},Arg^{34}, Pro^{37}-GLP-1(7-37)
$Aib^{8,22,27,30,35}$,Arg^{34},Pro^{37}- GLP-1 (7-37)amide,
all of which are substituted by B-U' in position 26.

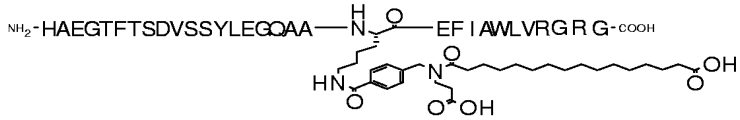
An embodiment provides a GLP-1 analog according to any one of the preceding embodiments, which is selected from



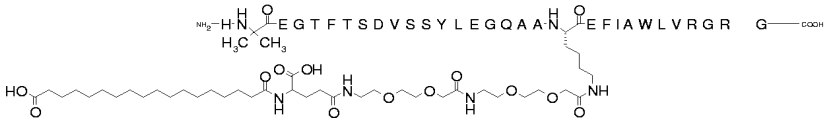
N-ε^{26}-(17-carboxyheptadecanoyl)-[Aib8,Arg34]GLP-1-(7-37)-peptide,



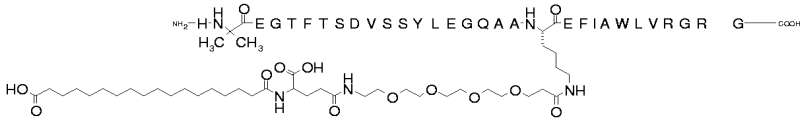
N-ε^{26}-(19-carboxynonadecanoyl)-[Aib8,Arg34]GLP-1-(7-37)-peptide,



N-ε^{26}-(4-{[N-(2-carboxyethyl)-N-(15-carboxypentadecanoyl)amino]methyl}benzoyl)[Arg34]GLP-1-(7-37),



N-ε^{26}-[2-(2-[2-(2-[2-(2-[4-(17-Carboxyheptadecanoylamino)-4(S)-carboxybutyrylamino]ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][Aib8,Arg34]GLP-1-(7-37)peptide,



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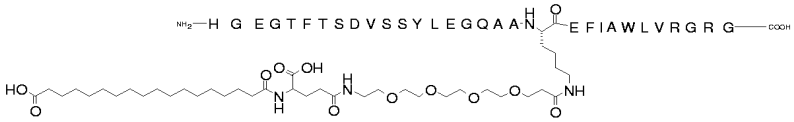

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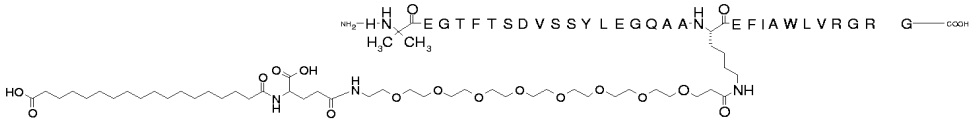

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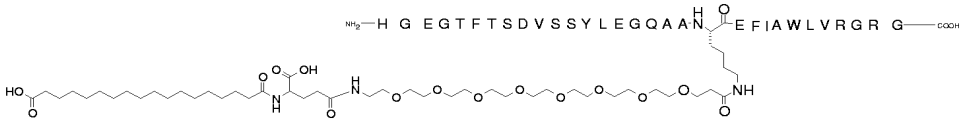

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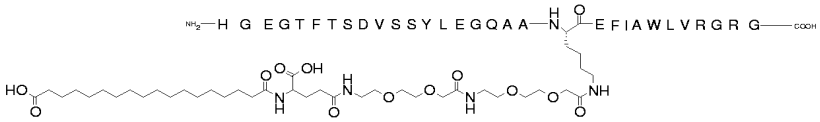

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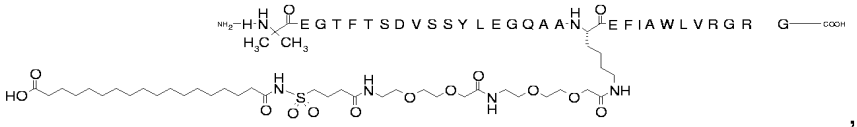

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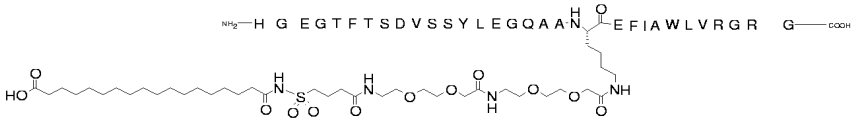

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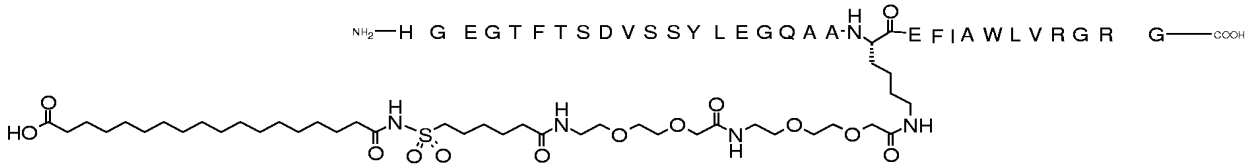

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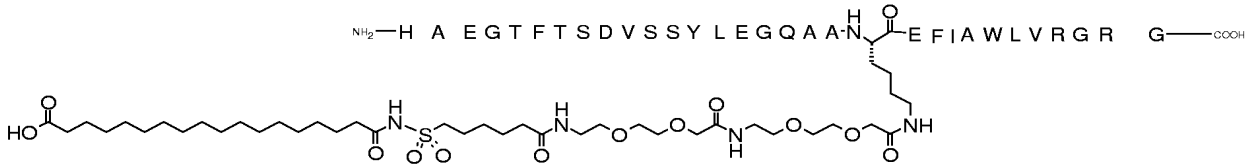

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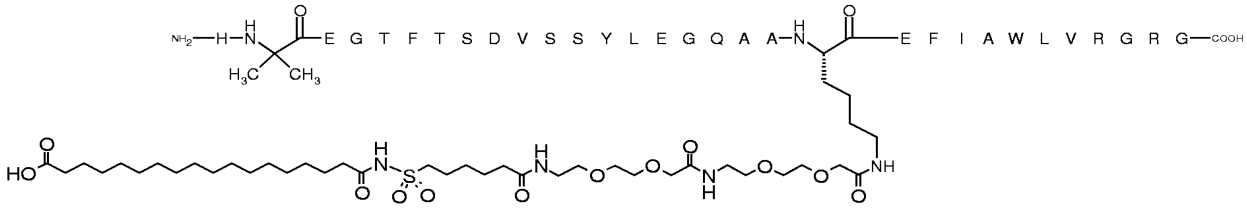

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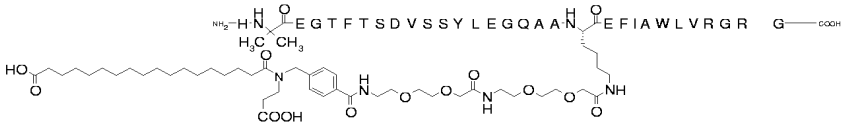

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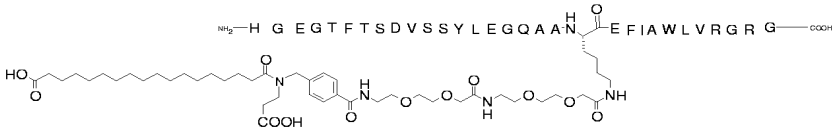

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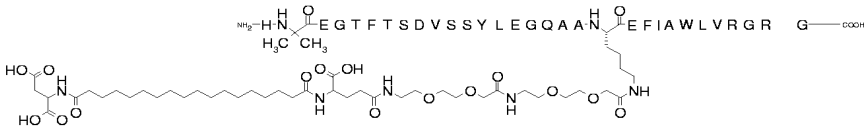

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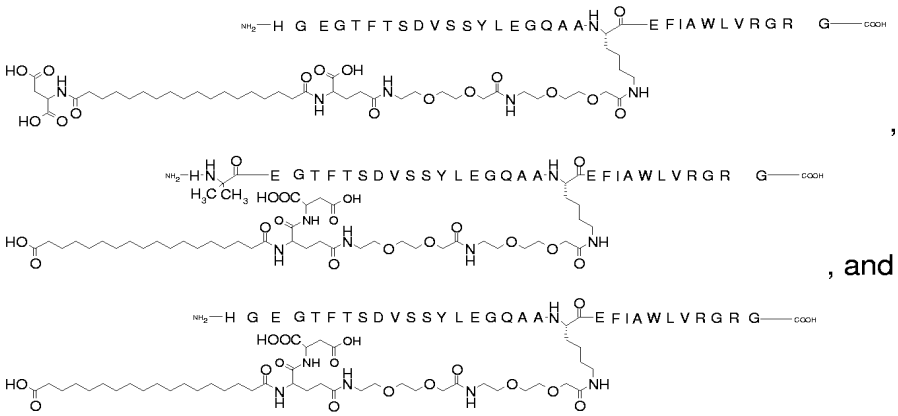

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An embodiment provides a method for increasing the time of action in a patient of a GLP-1 analog, characterised in acylating said GLP-1 analog with a moiety B-U as disclosed in any of the preceding embodiments, on the lysine residue in position 26 of said GLP-1 analog.

An embodiment provides a method for increasing the time of action in a patient of a GLP-1 analog to more than about 40 hours, characterised in modifying at least one of the amino acid residues in positions 7 and 8 of a GLP-1(7-37) peptide or an analog thereof, and acylating said GLP-1 analog with a moiety B-U'- as disclosed in any of the preceding embodiments on the lysine residue in position 26 of said GLP-1 analog.

An embodiment provides a pharmaceutical composition comprising a compound according to any one the embodiments above, and a pharmaceutically acceptable excipient.

An embodiment provides a pharmaceutical composition according to the embodiment above, which is suited for parenteral administration.

An embodiment provides the use of a compound according to any one of the embodiments above for the preparation of a medicament.

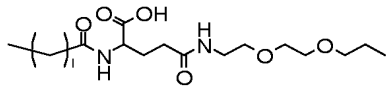
An embodiment provides the use of a compound according to any one of the embodiments above for the preparation of a medicament for the treatment or prevention of hyperglycemia, type 2 diabetes, impaired glucose tolerance, type 1 diabetes, obesity, hypertension, syndrome X, dyslipidemia, cognitive disorders, atheroschlerosis, myocardial infarction, coronary heart disease and other cardiovascular disorders, stroke, inflammatory bowel syndrome, dyspepsia and gastric ulcers.

An embodiment provides the use of a compound according to any one of the embodiments above for the preparation of a medicament for delaying or preventing disease progression in type 2 diabetes.

An embodiment provides the use of a compound according to any one of the embodiments above for the preparation of a medicament for decreasing food intake, decreasing β-cell apoptosis, increasing β-cell function and β-cell mass, and/or for restoring glucose sensitivity to β-cells.

In an embodiment the invention provides a compound according to the embodiments above, wherein said GLP-1 analog is Aib^{8},Arg^{34}-GLP-1(7-37) or $Aib^{8,22}$,Arg^{34}-GLP-1(7-37) attached to a linker B-U';

In an embodiment of Formula II, B-U represents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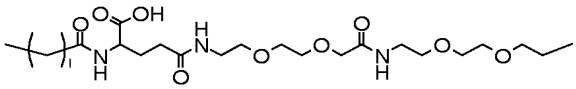
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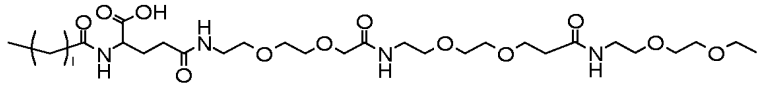
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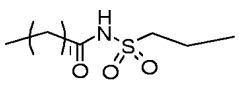
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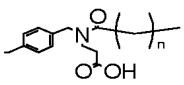
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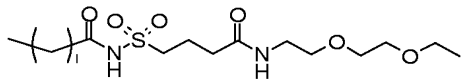
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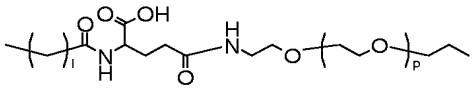
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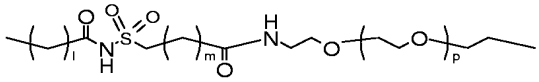


where l is 14, 15 or 16;
n is 15, 16, 17 or 18;
p is 3, 7, 11 or 24.

In embodiments the invention provides a compound according to any one of the embodiments above, wherein said diacid comprises a dicarboxylic acid.

In embodiments the invention provides a compound according to any one of the embodiments above, wherein the acylation group comprises a straight-chain or branched alkane α,ω-dicarboxylic acid.

In embodiments the invention provides compound according to the embodiment above, wherein the acylation group comprises the structure HOOC-$(CH_2)_n$CO-, wherein n is 12 to 20.

In embodiments the invention provides a compound according to the embodiment above, wherein the acylation group comprises a structure selected from HOOC-$(CH_2)_{14}$CO-, HOOC-$(CH_2)_{15}$CO-, HOOC-$(CH_2)_{16}$CO-, HOOC-$(CH_2)_{17}$CO-, and HOOC-$(CH_2)_{18}$CO-.

In embodiments the invention provides a compound according to the embodiment above, wherein the acylation group comprises the structure HOOC-$(CH_2)_{16}$CO-.

Another object of the present invention is to provide a pharmaceutical formulation comprising a compound according to the present invention which is present in a concentration from 0.1 mg/ml to 25 mg/ml, and wherein said formulation has a pH from 3.0 to 9.0. The formulation may further comprise a buffer system, preservative(s), tonicity agent(s), chelating agent(s), stabilizers and surfactants. In one embodiment of the invention the pharmaceutical formulation is an aqueous formulation, i.e. formulation comprising water. Such formulation is typically a solution or a suspension. In a further embodiment of the invention the pharmaceutical formulation is an aqueous solution. The term “aqueous formulation” is defined as a formulation comprising at least 50 %w/w water. Likewise, the term “aqueous solution” is defined as a solution comprising at least 50 %w/w water, and the term “aqueous suspension” is defined as a suspension comprising at least 50 %w/w water.

In another embodiment the pharmaceutical formulation is a freeze-dried formulation, whereto the physician or the patient adds solvents and/or diluents prior to use.

In another embodiment the pharmaceutical formulation is a dried formulation (e.g. freeze-dried or spray-dried) ready for use without any prior dissolution.

In a further aspect the invention relates to a pharmaceutical formulation comprising an aqueous solution of a compound according to the present invention, and a buffer, wherein said compound is present in a concentration from 0.1 mg/ml or above, and wherein said formulation has a pH from about 3.0 to about 9.0.

In another embodiment of the invention the pH of the formulation is from about 7.0 to about 9.5. In another embodiment of the invention the pH of the formulation is from about 3.0 to about 7.0. In another embodiment of the invention the pH of the formulation is from about 5.0 to about 7.5. In another embodiment of the invention the pH of the formulation is from about 7.5 to about 9.0. In another embodiment of the invention the pH of the formulation is from about 7.5 to about 8.5. In another embodiment of the invention the pH of the formulation is from about 6.0 to about 7.5. In another embodiment of the invention the pH of the formulation

is from about 6.0 to about 7.0. In another embodiment the pharmaceutical formulation is from 8.0 to 8.5.

In a further embodiment of the invention the buffer is selected from the group consisting of sodium acetate, sodium carbonate, citrate, glycylglycine, histidine, glycine, lysine, arginine, sodium dihydrogen phosphate, disodium hydrogen phosphate, sodium phosphate, and tris(hydroxymethyl)-aminomethan, bicine, tricine, malic acid, succinate, maleic acid, fumaric acid, tartaric acid, aspartic acid or mixtures thereof. Each one of these specific buffers constitutes an alternative embodiment of the invention.

In a further embodiment of the invention the formulation further comprises a pharmaceutically acceptable preservative. In a further embodiment of the invention the preservative is selected from the group consisting of phenol, o-cresol, m-cresol, p-cresol, methyl p-hydroxybenzoate, propyl p-hydroxybenzoate, 2-phenoxyethanol, butyl p-hydroxybenzoate, 2-phenylethanol, benzyl alcohol, chlorobutanol, and thiomerosal, bronopol, benzoic acid, imidurea, chlorohexidine, sodium dehydroacetate, chlorocresol, ethyl p-hydroxybenzoate, benzethonium chloride, chlorphenesine (3p-chlorphenoxypropane-1,2-diol) or mixtures thereof. In an embodiment the preservative is phenol or m-cresol. In a further embodiment of the invention the preservative is present in a concentration from 0.1 mg/ml to 20 mg/ml. In a further embodiment of the invention the preservative is present in a concentration from 0.1 mg/ml to 5 mg/ml. In a further embodiment of the invention the preservative is present in a concentration from 5 mg/ml to 10 mg/ml. In a further embodiment of the invention the preservative is present in a concentration from 10 mg/ml to 20 mg/ml. Each one of these specific preservatives constitutes an alternative embodiment of the invention. The use of a preservative in pharmaceutical compositions is well-known to the skilled person. For convenience reference is made to Remington: *The Science and Practice of Pharmacy*, 19th edition, 1995.

In a further embodiment of the invention the formulation further comprises an isotonic agent. In a further embodiment of the invention the isotonic agent is selected from the group consisting of a salt (e.g. sodium chloride), a sugar or sugar alcohol, an amino acid (e.g. L-glycine, L-histidine, arginine, lysine, isoleucine, aspartic acid, tryptophan, threonine), an alditol (e.g. glycerol (glycerine), 1,2-propanediol (propyleneglycol), 1,3-propanediol, 1,3-butanediol) polyethyleneglycol (e.g. PEG400), or mixtures thereof. In an embodiment the isotoncity agent is propyleneglycol. Any sugar such as mono-, di-, or polysaccharides, or water-soluble glucans, including for example fructose, glucose, mannose, sorbose, xylose,

maltose, lactose, sucrose, trehalose, dextran, pullulan, dextrin, cyclodextrin, soluble starch, hydroxyethyl starch and carboxymethylcellulose-Na may be used. In one embodiment the sugar additive is sucrose. Sugar alcohol is defined as a C4-C8 hydrocarbon having at least one -OH group and includes, for example, mannitol, sorbitol, inositol, galactitol, dulcitol, xylitol, and arabitol. In one embodiment the sugar alcohol additive is mannitol. The sugars or sugar alcohols mentioned above may be used individually or in combination. There is no fixed limit to the amount used, as long as the sugar or sugar alcohol is soluble in the liquid preparation and does not adversely effect the stabilizing effects achieved using the methods of the invention. In one embodiment, the sugar or sugar alcohol concentration is between about 1 mg/ml and about 150 mg/ml. In a further embodiment of the invention the isotonic agent is present in a concentration from 1 mg/ml to 50 mg/ml. In a further embodiment of the invention the isotonic agent is present in a concentration from 1 mg/ml to 7 mg/ml. In an embodiment of the invention the isotonic agent is present in a concentration from 5 mg/ml to 7 mg/ml. In a further embodiment of the invention the isotonic agent is present in a concentration from 8 mg/ml to 24 mg/ml. In a further embodiment of the invention the isotonic agent is present in a concentration from 25 mg/ml to 50 mg/ml. Each one of these specific isotonic agents constitutes an alternative embodiment of the invention. The use of an isotonic agent in pharmaceutical compositions is well-known to the skilled person. For convenience reference is made to Remington: *The Science and Practice of Pharmacy*, 19th edition, 1995.

In a further embodiment of the invention the formulation further comprises a chelating agent. In a further embodiment of the invention the chelating agent is selected from salts of ethylenediaminetetraacetic acid (EDTA), citric acid, and aspartic acid, and mixtures thereof. In a further embodiment of the invention the chelating agent is present in a concentration from 0.1mg/ml to 5mg/ml. In a further embodiment of the invention the chelating agent is present in a concentration from 0.1mg/ml to 2mg/ml. In a further embodiment of the invention the chelating agent is present in a concentration from 2mg/ml to 5mg/ml. Each one of these specific chelating agents constitutes an alternative embodiment of the invention. The use of a chelating agent in pharmaceutical compositions is well-known to the skilled person. For convenience reference is made to Remington: *The Science and Practice of Pharmacy*, 19th edition, 1995.

In a further embodiment of the invention the formulation further comprises a stabilizer. The use of a stabilizer in pharmaceutical compositions is well-known to the skilled person. For convenience reference is made to Remington: *The Science and Practice of Pharmacy*, 19th edition, 1995.

More particularly, compositions of the invention are stabilized liquid pharmaceutical compositions whose therapeutically active components include a polypeptide that possibly exhibits aggregate formation during storage in liquid pharmaceutical formulations. By "aggregate formation" is intended a physical interaction between the polypeptide molecules that results in formation of oligomers, which may remain soluble, or large visible aggregates that precipitate from the solution. By "during storage" is intended a liquid pharmaceutical composition or formulation once prepared, is not immediately administered to a subject. Rather, following preparation, it is packaged for storage, either in a liquid form, in a frozen state, or in a dried form for later reconstitution into a liquid form or other form suitable for administration to a subject. By "dried form" is intended the liquid pharmaceutical composition or formulation is dried either by freeze drying (i.e., lyophilization; see, for example, Williams and Polli (1984) J. Parenteral Sci. Technol. 38:48-59), spray drying (see Masters (1991) in Spray-Drying Handbook (5th ed; Longman Scientific and Technical, Essez, U.K.), pp. 491-676; Broadhead et al. (1992) Drug Devel. Ind. Pharm. 18:1169-1206; and Mumenthaler et al. (1994) Pharm. Res. 11:12-20), or air drying (Carpenter and Crowe (1988) Cryobiology 25:459-470; and Roser (1991) Biopharm. 4:47-53). Aggregate formation by a polypeptide during storage of a liquid pharmaceutical composition can adversely affect biological activity of that polypeptide, resulting in loss of therapeutic efficacy of the pharmaceutical composition. Furthermore, aggregate formation may cause other problems such as blockage of tubing, membranes, or pumps when the polypeptide-containing pharmaceutical composition is administered using an infusion system.

The pharmaceutical compositions of the invention may further comprise an amount of an amino acid base sufficient to decrease aggregate formation by the polypeptide during storage of the composition. By "amino acid base" is intended an amino acid or a combination of amino acids, where any given amino acid is present either in its free base form or in its salt form. Where a combination of amino acids is used, all of the amino acids may be present in their free base forms, all may be present in their salt forms, or some may be present in their free base forms while others are present in their salt forms. In one embodiment, amino acids to use in preparing the compositions of the invention are those carrying a charged side chain, such as arginine, lysine, aspartic acid, and glutamic acid. Any stereoisomer (i.e., L, D, or a mixture thereof) of a particular amino acid (e.g. methionine, histidine, imidazole, arginine, lysine, isoleucine, aspartic acid, tryptophan, threonine and mixtures thereof) or combinations of these stereoisomers, may be present in the pharmaceutical compositions of the invention so long as the particular amino acid is present either in its free base form or its salt form. In one embodiment the L-stereoisomer is used. Compositions of the invention may also be formulated with analogues of these amino acids. By "amino acid analogue" is intended a

derivative of the naturally occurring amino acid that brings about the desired effect of decreasing aggregate formation by the polypeptide during storage of the liquid pharmaceutical compositions of the invention. Suitable arginine analogues include, for example, aminoguanidine, ornithine and N-monoethyl L-arginine, suitable methionine analogues include ethionine and buthionine and suitable cysteine analogues include S-methyl-L cysteine. As with the other amino acids, the amino acid analogues are incorporated into the compositions in either their free base form or their salt form. In a further embodiment of the invention the amino acids or amino acid analogues are used in a concentration, which is sufficient to prevent or delay aggregation of the protein.

In a further embodiment of the invention methionine (or other sulphuric amino acids or amino acid analogous) may be added to inhibit oxidation of methionine residues to methionine sulfoxide when the polypeptide acting as the therapeutic agent is a polypeptide comprising at least one methionine residue susceptible to such oxidation. By "inhibit" is intended minimal accumulation of methionine oxidized species over time. Inhibiting methionine oxidation results in greater retention of the polypeptide in its proper molecular form. Any stereoisomer of methionine (L or D) or combinations thereof can be used. The amount to be added should be an amount sufficient to inhibit oxidation of the methionine residues such that the amount of methionine sulfoxide is acceptable to regulatory agencies. Typically, this means that the composition contains no more than about 10% to about 30% methionine sulfoxide. Generally, this can be achieved by adding methionine such that the ratio of methionine added to methionine residues ranges from about 1:1 to about 1000:1, such as 10:1 to about 100:1.

In a further embodiment of the invention the formulation further comprises a stabilizer selected from the group of high molecular weight polymers or low molecular compounds. In a further embodiment of the invention the stabilizer is selected from polyethylene glycol (e.g. PEG 3350), polyvinyl alcohol (PVA), polyvinylpyrrolidone, carboxy-/hydroxycellulose or derivates thereof (e.g. HPC, HPC-SL, HPC-L and HPMC), cyclodextrins, sulphur-containing substances as monothioglycerol, thioglycolic acid and 2-methylthioethanol, and different salts (e.g. sodium chloride). Each one of these specific stabilizers constitutes an alternative embodiment of the invention.

The pharmaceutical compositions may also comprise additional stabilizing agents, which further enhance stability of a therapeutically active polypeptide therein. Stabilizing agents of particular interest to the present invention include, but are not limited to, methionine and EDTA, which protect the polypeptide against methionine oxidation, and a nonionic surfactant, which protects the polypeptide against aggregation associated with freeze-thawing or mechanical shearing.

In a further embodiment of the invention the formulation further comprises a surfactant. In another embodiment of the invention the pharmaceutical composition comprises two different surfactants. The term "Surfactant" as used herein refers to any molecules or ions that are comprised of a water-soluble (hydrophilic) part, the head, and a fat-soluble (lipophilic) segment. Surfactants accumulate preferably at interfaces, which the hydrophilic part is orientated towards the water (hydrophilic phase) and the lipophilic part towards the oil- or hydrophobic phase (*i.e.* glass, air, oil etc.). The concentration at which surfactants begin to form micelles is known as the critical micelle concentration or CMC. Furthermore, surfactants lower the surface tension of a liquid. Surfactants are also known as amphipathic compounds. The term "Detergent" is a synonym used for surfactants in general.

Anionic surfactants may be selected from the group of: Chenodeoxycholic acid, Chenodeoxycholic acid sodium salt, Cholic acid, Dehydrocholic acid, Deoxycholic acid, Deoxycholic acid methyl ester, Digitonin, Digitoxigenin, N,N-Dimethyldodecylamine N-oxide, Docusate sodium, Glycochenodeoxycholic acid sodium, Glycocholic acid hydrate, Glycodeoxycholic acid monohydrate, Glycodeoxycholic acid sodium salt, Glycodeoxycholic acid sodium salt, Glycolithocholic acid 3-sulfate disodium salt, Glycolithocholic acid ethyl ester, N-Lauroylsarcosine sodium salt, N-Lauroylsarcosine sodium salt, N-Lauroylsarcosine, N-Lauroylsarcosine, Lithium dodecyl sulfate, Lugol, 1-Octanesulfonic acid sodium salt, 1-Octanesulfonic acid sodium salt, Sodium 1-butanesulfonate, Sodium 1-decanesulfonate, Sodium 1-dodecanesulfonate, Sodium 1-heptanesulfonate, Sodium 1-heptanesulfonate, Sodium 1-nonanesulfonate, Sodium 1-propanesulfonate monohydrate, Sodium 2-bromoethanesulfonate, Sodium cholate hydrate, ox or sheep bile, Sodium cholate hydrate, Sodium choleate, Sodium deoxycholate, Sodium dodecyl sulfate, Sodium dodecyl sulfate, Sodium hexanesulfonate, Sodium octyl sulfate, Sodium pentanesulfonate, Sodium taurocholate, Taurochenodeoxycholic acid sodium salt, Taurodeoxycholic acid sodium salt monohydrate, Taurolithocholic acid 3-sulfate disodium salt, Tauroursodeoxycholic acid sodium salt, Trizma® dodecyl sulfate, DSS (docusate sodium, CAS registry no [577-11-7]), docusate calcium, CAS registry no [128-49-4]), docusate potassium, CAS registry no [7491-09-0]), SDS (sodium dodecyl sulfate or sodium lauryl sulfate), Dodecylphosphocholine (FOS-Choline-12), Decylphosphocholine (FOS-Choline-10), Nonylphosphocholine (FOS-Choline-9), dipalmitoyl phosphatidic acid, sodium caprylate, and/or Ursodeoxycholic acid.

Cationic surfactants may be selected from the group of: Alkyltrimethylammonium bromide

Benzalkonium chloride, Benzalkonium chloride, Benzyldimethylhexadecylammonium chloride, Benzyldimethyltetradecylammonium chloride,

Benzyltrimethylammonium tetrachloroiodate, Dimethyldioctadecylammonium bromide, Dodecylethyldimethylammonium bromide, Dodecyltrimethylammonium bromide, Dodecyltrimethylammonium bromide, Ethylhexadecyldimethylammonium bromide, Hexadecyltrimethylammonium bromide, Hexadecyltrimethylammonium bromide, Polyoxyethylene(10)-N-tallow-1,3-diaminopropane, Thonzonium bromide, and/or Trimethyl(tetradecyl)ammonium bromide.

Nonionic surfactants may be selected from the group of: BigCHAP, Bis(polyethylene glycol bis[imidazoyl carbonyl]), block copolymers as polyethyleneoxide/polypropyleneoxide block copolymers such as poloxamers, poloxamer 188 and poloxamer 407, Brij® 35, Brij® 56, Brij® 72, Brij® 76, Brij® 92V, Brij® 97, Brij® 58P, Cremophor® EL, Decaethylene glycol monododecyl ether, N-Decanoyl-N-methylglucamine, n-Dodecanoyl-N-methylglucamide, alkyl-polyglucosides, ethoxylated castor oil, Heptaethylene glycol monodecyl ether, Heptaethylene glycol monododecyl ether, Heptaethylene glycol monotetradecyl ether, Hexaethylene glycol monododecyl ether, Hexaethylene glycol monohexadecyl ether, Hexaethylene glycol monooctadecyl ether, Hexaethylene glycol monotetradecyl ether, Igepal CA-630, Igepal CA-630, Methyl-6-O-(N-heptylcarbamoyl)-beta-D-glucopyranoside, Nonaethylene glycol monododecyl ether, N-Nonanoyl-N-methylglucamine, N-Nonanoyl-N-methylglucamine, Octaethylene glycol monodecyl ether, Octaethylene glycol monododecyl ether, Octaethylene glycol monohexadecyl ether, Octaethylene glycol monooctadecyl ether, Octaethylene glycol monotetradecyl ether, Octyl-β-D-glucopyranoside, Pentaethylene glycol monodecyl ether, Pentaethylene glycol monododecyl ether, Pentaethylene glycol monohexadecyl ether, Pentaethylene glycol monohexyl ether, Pentaethylene glycol monooctadecyl ether, Pentaethylene glycol monooctyl ether, Polyethylene glycol diglycidyl ether, Polyethylene glycol ether W-1, Polyoxyethylene 10 tridecyl ether, Polyoxyethylene 100 stearate, Polyoxyethylene 20 isohexadecyl ether, Polyoxyethylene 20 oleyl ether, Polyoxyethylene 40 stearate, Polyoxyethylene 50 stearate, Polyoxyethylene 8 stearate, Polyoxyethylene bis(imidazolyl carbonyl), Polyoxyethylene 25 propylene glycol stearate, Saponin from Quillaja bark, Span® 20, Span® 40, Span® 60, Span® 65, Span® 80, Span® 85, Tergitol, Type 15-S-12, Tergitol, Type 15-S-30, Tergitol, Type 15-S-5, Tergitol, Type 15-S-7, Tergitol, Type 15-S-9, Tergitol, Type NP-10, Tergitol, Type NP-4, Tergitol, Type NP-40, Tergitol, Type NP-7, Tergitol, Type NP-9, Tetradecyl-β-D-maltoside, Tetraethylene glycol monodecyl ether, Tetraethylene glycol monododecyl ether, Tetraethylene glycol monotetradecyl ether, Triethylene glycol monodecyl ether, Triethylene glycol monododecyl ether, Triethylene glycol monohexadecyl ether, Triethylene glycol monooctyl ether, Triethylene glycol monotetradecyl ether, Triton CF-21, Triton CF-32, Triton DF-12, Triton DF-16, Triton GR-5M, Triton QS-15,

Triton QS-44, Triton X-100, Triton X-102, Triton X-15, Triton X-151, Triton X-200, Triton X-207, Triton® X-100, Triton® X-114, Triton® X-165 solution, Triton® X-305 solution, Triton® X-405, Triton® X-45, Triton® X-705-70, TWEEN® 20, TWEEN® 40, TWEEN® 60, TWEEN® 6, TWEEN® 65, TWEEN® 80, TWEEN® 81, TWEEN® 85, Tyloxapol, sphingophospholipids (sphingomyelin), and sphingoglycolipids (ceramides, gangliosides), phospholipids, and/or n-Undecyl β-D-glucopyranoside. ´

Zwitterionic surfactants may be selected from the group of: CHAPS, CHAPSO, 3-(Decyldimethylammonio)propanesulfonate inner salt, 3-(Dodecyldimethylammonio)-propanesulfonate inner salt, 3-(Dodecyldimethylammonio)propanesulfonate inner salt, 3-(N,N-Dimethylmyristylammonio)propanesulfonate, 3-(N,N-Dimethyloctadecylammonio)-propanesulfonate, 3-(N,N-Dimethyloctylammonio)propanesulfonate inner salt, 3-(N,N-Dimethylpalmitylammonio)propanesulfonate, N-alkyl-N,N-dimethylammonio-1-propanesulfonates, 3-cholamido-1-propyldimethylammonio-1-propanesulfonate, Dodecylphosphocholine, myristoyl lysophosphatidylcholine, Zwittergent 3-12 (*N*-dodecyl-*N*,*N*-dimethyl-3-ammonio-1-propanesulfonate), Zwittergent 3-10 (3-(Decyldimethylammonio)-propanesulfonate inner salt), Zwittergent 3-08 (3-(Octyldimethylammonio)pro-panesulfonate), glycerophospholipids (lecithins, kephalins, phosphatidyl serine), glyceroglycolipids (galactopyranoside), alkyl, alkoxyl (alkyl ester), alkoxy (alkyl ether)- derivatives of lysophosphatidyl and phosphatidylcholines, e.g. lauroyl and myristoyl derivatives of lysophosphatidylcholine, dipalmitoylphosphatidylcholine, and modifications of the polar head group, that is cholines, ethanolamines, phosphatidic acid, serines, threonines, glycerol, inositol, lysophosphatidylserine and lysophosphatidylthreonine, acylcarnitines and derivatives, N^{beta}-acylated derivatives of lysine, arginine or histidine, or side-chain acylated derivatives of lysine or arginine, N^{beta}-acylated derivatives of dipeptides comprising any combination of lysine, arginine or histidine and a neutral or acidic amino acid, N^{beta}-acylated derivative of a tripeptide comprising any combination of a neutral amino acid and two charged amino acids, or the surfactant may be selected from the group of imidazoline derivatives, long-chain fatty acids and salts thereof C_6-C_{12} (eg. oleic acid and caprylic acid), N-Hexadecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate, anionic (alkyl-aryl-sulphonates) monovalent surfactants, palmitoyl lysophosphatidyl-L-serine, lysophospholipids (e.g. 1-acyl-sn-glycero-3-phosphate esters of ethanolamine, choline, serine or threonine), or mixtures thereof.

The term “alkyl-polyglucosides” as used herein in relates to an straight or branched C_{5-20}-alkyl, -alkenyl or -alkynyl chain which is substituted by one or more glucoside moieties such as maltoside, saccharide etc. Embodiments of these alkyl-polyglucosides include C_{6-18}-alkyl-polyglucosides. Specific embodiments of these alkyl-polyglucosides includes the even

numbered carbon-chains such as C_6, C_8, C_{10}, C_{12}, C_{14}, C_{16}, C_{18} and C_{20} alkyl chain. Specific embodiments of the glucoside moieties include pyranoside, glucopyranoside, maltoside, maltotrioside and sucrose. In embodiments of the invention less than 6 glucosid moieties are attached to the alkyl group. In embodiments of the invention less than 5 glucosid moieties are attached to the alkyl group. In embodiments of the invention less than 4 glucosid moieties are attached to the alkyl group. In embodiments of the invention less than 3 glucosid moieties are attached to the alkyl group. In embodiments of the invention less than 2 glucosid moieties are attached to the alkyl group. Specific embodiments of alkyl-polyglucosides are alkyl glucosides such n-decyl β-D-glucopyranoside, decyl β-D-maltopyranoside, dodecyl β-D-glucopyranoside, n-dodecyl β-D-maltoside, n-dodecyl β-D-maltoside, n-dodecyl β-D-maltoside, tetradecyl β-D-glucopyranoside, decyl β-D-maltoside, hexadecyl β-D-maltoside, decyl β-D-maltotrioside, dodecyl β-D-maltotrioside, tetradecyl β-D-maltotrioside, hexadecyl β-D-maltotrioside, n-dodecyl-sucrose, n-decyl-sucrose, sucrose monocaprate, sucrose monolaurate, sucrose monomyristate, and sucrose monopalmitate.

The use of a surfactant in pharmaceutical compositions is well-known to the skilled person. For convenience reference is made to Remington: *The Science and Practice of Pharmacy*, 19th edition, 1995.

In a further embodiment of the invention the formulation further comprises protease inhibitors such as EDTA (ethylenediamine tetraacetic acid) and benzamidineHCl, but other commercially available protease inhibitors may also be used. The use of a protease inhibitor is particular useful in pharmaceutical compositions comprising zymogens of proteases in order to inhibit autocatalysis.

It is possible that other ingredients may be present in the peptide pharmaceutical formulation of the present invention. Such additional ingredients may include wetting agents, emulsifiers, antioxidants, bulking agents, tonicity modifiers, chelating agents, metal ions, oleaginous vehicles, proteins (e.g., human serum albumin, gelatine or proteins) and a zwitterion (e.g., an amino acid such as betaine, taurine, arginine, glycine, lysine and histidine). Such additional ingredients, of course, should not adversely affect the overall stability of the pharmaceutical formulation of the present invention.

Pharmaceutical compositions containing a compound according to the present invention may be administered to a patient in need of such treatment at several sites, for example, at topical sites, for example, skin and mucosal sites, at sites which bypass absorption, for example, administration in an artery, in a vein, in the heart, and at sites which involve absorption, for example, administration in the skin, under the skin, in a muscle or in the abdomen.

Administration of pharmaceutical compositions according to the invention may be through several routes of administration, for example, lingual, sublingual, buccal, in the mouth, oral, in the stomach and intestine, nasal, pulmonary, for example, through the bronchioles and alveoli or a combination thereof, epidermal, dermal, transdermal, vaginal, rectal, ocular, for examples through the conjunctiva, uretal, and parenteral to patients in need of such a treatment.

Compositions of the current invention may be administered in several dosage forms, for example, as solutions, suspensions, emulsions, microemulsions, multiple emulsion, foams, salves, pastes, plasters, ointments, tablets, coated tablets, rinses, capsules, for example, hard gelatine capsules and soft gelatine capsules, suppositories, rectal capsules, drops, gels, sprays, powder, aerosols, inhalants, eye drops, ophthalmic ointments, ophthalmic rinses, vaginal pessaries, vaginal rings, vaginal ointments, injection solution, in situ transforming solutions, for example in situ gelling, in situ setting, in situ precipitating, in situ crystallization, infusion solution, and implants.

Compositions of the invention may further be compounded in, or attached to, for example through covalent, hydrophobic and electrostatic interactions, a drug carrier, drug delivery system and advanced drug delivery system in order to further enhance stability of the compound of the present invention, increase bioavailability, increase solubility, decrease adverse effects, achieve chronotherapy well known to those skilled in the art, and increase patient compliance or any combination thereof. Examples of carriers, drug delivery systems and advanced drug delivery systems include, but are not limited to, polymers, for example cellulose and derivatives, polysaccharides, for example dextran and derivatives, starch and derivatives, poly(vinyl alcohol), acrylate and methacrylate polymers, polylactic and polyglycolic acid and block co-polymers thereof, polyethylene glycols, carrier proteins, for example albumin, gels, for example, thermogelling systems, for example block co-polymeric systems well known to those skilled in the art, micelles, liposomes, microspheres, nanoparticulates, liquid crystals and dispersions thereof, L2 phase and dispersions there of, well known to those skilled in the art of phase behaviour in lipid-water systems, polymeric micelles, multiple emulsions, self-emulsifying, self-microemulsifying, cyclodextrins and derivatives thereof, and dendrimers.

Compositions of the current invention are useful in the formulation of solids, semisolids, powder and solutions for pulmonary administration of compounds of the present invention, using, for example a metered dose inhaler, dry powder inhaler and a nebulizer, all being devices well known to those skilled in the art.

Compositions of the current invention are specifically useful in the formulation of controlled, sustained, protracting, retarded, and slow release drug delivery systems. More specifically,

but not limited to, compositions are useful in formulation of parenteral controlled release and sustained release systems (both systems leading to a many-fold reduction in number of administrations), well known to those skilled in the art. Even more preferably, are controlled release and sustained release systems administered subcutaneous. Without limiting the scope of the invention, examples of useful controlled release system and compositions are hydrogels, oleaginous gels, liquid crystals, polymeric micelles, microspheres, nanoparticles, Methods to produce controlled release systems useful for compositions of the current invention include, but are not limited to, crystallization, condensation, co-crystallization, precipitation, co-precipitation, emulsification, dispersion, high pressure homogenisation, encapsulation, spray drying, microencapsulating, coacervation, phase separation, solvent evaporation to produce microspheres, extrusion and supercritical fluid processes. General reference is made to Handbook of Pharmaceutical Controlled Release (Wise, D.L., ed. Marcel Dekker, New York, 2000) and Drug and the Pharmaceutical Sciences vol. 99: Protein Formulation and Delivery (MacNally, E.J., ed. Marcel Dekker, New York, 2000).

Parenteral administration may be performed by subcutaneous, intramuscular, intraperitoneal or intravenous injection by means of a syringe, optionally a pen-like syringe. Alternatively, parenteral administration can be performed by means of an infusion pump. A further option is a composition which may be a solution or suspension or a powder for the administration of the compound of the present invention in the form of a nasal or pulmonal liquid or powder spray. As a still further option, the pharmaceutical compositions containing the compound of the invention can also be adapted to transdermal administration, *e.g.* by needle-free injection or from a patch, optionally an iontophoretic patch, or transmucosal, *e.g.* buccal, administration.

The compounds of the present invention can be administered via the pulmonary route in a vehicle, as a solution, suspension or dry powder using any of known types of devices suitable for pulmonary drug delivery. Examples of these comprise, but are not limited to, the three general types of aerosol-generating for pulmonary drug delivery, and may include jet or ultrasonic nebulizers, metered-dose inhalers, or dry powder inhalers (Cf. Yu J, Chien YW. Pulmonary drug delivery: Physiologic and mechanistic aspects. Crit Rev Ther Drug Carr Sys 14(4) (1997) 395-453).

Based on standardised testing methodology, the aerodynamic diameter (d_a) of a particle is defined as the geometric equivalent diameter of a reference standard spherical particle of unit density (1 g/cm^3). In the simplest case, for spherical particles, d_a is related to a reference diameter (d) as a function of the square root of the density ratio as described by:

Modifications to this relationship occur for non-spherical particles (cf. Edwards DA, Ben-Jebria A, Langer R. Recent advances in pulmonary drug delivery using large, porous inhaled particles. J Appl Physiol 84(2) (1998) 379-385). The terms "MMAD" and "MMEAD" are well-described and known to the art (cf. Edwards DA, Ben-Jebria A, Langer R and represents a measure of the median value of an aerodynamic particle size distribution. Recent advances in pulmonary drug delivery using large, porous inhaled particles. J Appl Physiol 84(2) (1998) 379-385). Mass median aerodynamic diameter (MMAD) and mass median effective aerodynamic diameter (MMEAD) are used inter-changeably, are statistical parameters, and empirically describe the size of aerosol particles in relation to their potential to deposit in the lungs, independent of actual shape, size, or density (cf. Edwards DA, Ben-Jebria A, Langer R. Recent advances in pulmonary drug delivery using large, porous inhaled particles. J Appl Physiol 84(2) (1998) 379-385). MMAD is normally calculated from the measurement made with impactors, an instrument that measures the particle inertial behaviour in air.
In a further embodiment, the formulation could be aerosolized by any known aerosolisation technology, such as nebulisation, to achieve a MMAD of aerosol particles less than 10 µm, more preferably between 1-5 µm, and most preferably between 1-3 µm. The preferred particle size is based on the most effective size for delivery of drug to the deep lung, where protein is optimally absorbed (cf. Edwards DA, Ben-Jebria A, Langer A, Recent advances in pulmonary drug delivery using large, porous inhaled particles. J Appl Physiol 84(2) (1998) 379-385).
Deep lung deposition of the pulmonal formulations comprising the compound of the present invention may optional be further optimized by using modifications of the inhalation techniques, for example, but not limited to: slow inhalation flow (eg. 30 L/min), breath holding and timing of actuation.

The term "stabilized formulation" refers to a formulation with increased physical stability, increased chemical stability or increased physical and chemical stability.

The term "physical stability" of the protein formulation as used herein refers to the tendency of the protein to form biologically inactive and/or insoluble aggregates of the protein as a result of exposure of the protein to thermo-mechanical stresses and/or interaction with interfaces and surfaces that are destabilizing, such as hydrophobic surfaces and interfaces. Physical stability of the aqueous protein formulations is evaluated by means of visual inspection and/or turbidity measurements after exposing the formulation filled in suitable containers (e.g. cartridges or vials) to mechanical/physical stress (e.g. agitation) at different temperatures for various time periods. Visual inspection of the formulations is performed in a

sharp focused light with a dark background. The turbidity of the formulation is characterized by a visual score ranking the degree of turbidity for instance on a scale from 0 to 3 (a formulation showing no turbidity corresponds to a visual score 0, and a formulation showing visual turbidity in daylight corresponds to visual score 3). A formulation is classified physical unstable with respect to protein aggregation, when it shows visual turbidity in daylight. Alternatively, the turbidity of the formulation can be evaluated by simple turbidity measurements well-known to the skilled person. Physical stability of the aqueous protein formulations can also be evaluated by using a spectroscopic agent or probe of the conformational status of the protein. The probe is preferably a small molecule that preferentially binds to a non-native conformer of the protein. One example of a small molecular spectroscopic probe of protein structure is Thioflavin T. Thioflavin T is a fluorescent dye that has been widely used for the detection of amyloid fibrils. In the presence of fibrils, and perhaps other protein configurations as well, Thioflavin T gives rise to a new excitation maximum at about 450 nm and enhanced emission at about 482 nm when bound to a fibril protein form. Unbound Thioflavin T is essentially non-fluorescent at the wavelengths.
Other small molecules can be used as probes of the changes in protein structure from native to non-native states. For instance the “hydrophobic patch” probes that bind preferentially to exposed hydrophobic patches of a protein. The hydrophobic patches are generally buried within the tertiary structure of a protein in its native state, but become exposed as a protein begins to unfold or denature. Examples of these small molecular, spectroscopic probes are aromatic, hydrophobic dyes, such as antrhacene, acridine, phenanthroline or the like. Other spectroscopic probes are metal-amino acid complexes, such as cobalt metal complexes of hydrophobic amino acids, such as phenylalanine, leucine, isoleucine, methionine, and valine, or the like.

The term “chemical stability” of the protein formulation as used herein refers to chemical covalent changes in the protein structure leading to formation of chemical degradation products with potential less biological potency and/or potential increased immunogenic properties compared to the native protein structure. Various chemical degradation products can be formed depending on the type and nature of the native protein and the environment to which the protein is exposed. Elimination of chemical degradation can most probably not be completely avoided and increasing amounts of chemical degradation products is often seen during storage and use of the protein formulation as well-known by the person skilled in the art. Most proteins are prone to deamidation, a process in which the side chain amide group in glutaminyl or asparaginyl residues is hydrolysed to form a free carboxylic acid. Other

degradations pathways involves formation of high molecular weight transformation products where two or more protein molecules are covalently bound to each other through transamidation and/or disulfide interactions leading to formation of covalently bound dimer, oligomer and polymer degradation products (*Stability of Protein Pharmaceuticals, Ahern. T.J. & Manning M.C., Plenum Press, New York 1992*). Oxidation (of for instance methionine residues) can be mentioned as another variant of chemical degradation. The chemical stability of the protein formulation can be evaluated by measuring the amount of the chemical degradation products at various time-points after exposure to different environmental conditions (the formation of degradation products can often be accelerated by for instance increasing temperature). The amount of each individual degradation product is often determined by separation of the degradation products depending on molecule size and/or charge using various chromatography techniques (e.g. SEC-HPLC and/or RP-HPLC).

Hence, as outlined above, a "stabilized formulation" refers to a formulation with increased physical stability, increased chemical stability or increased physical and chemical stability. In general, a formulation must be stable during use and storage (in compliance with recommended use and storage conditions) until the expiration date is reached.

In one embodiment of the invention the pharmaceutical formulation comprising the compound of the present invention is stable for more than 6 weeks of usage and for more than 3 years of storage.
In another embodiment of the invention the pharmaceutical formulation comprising the compound of the present invention is stable for more than 4 weeks of usage and for more than 3 years of storage.
In a further embodiment of the invention the pharmaceutical formulation comprising the compound of the present invention is stable for more than 4 weeks of usage and for more than two years of storage.
In an even further embodiment of the invention the pharmaceutical formulation comprising the compound of the present invention is stable for more than 2 weeks of usage and for more than two years of storage.

In another aspect the present invention relates to the use of a compound according to the invention for the preparation of a medicament.

In one embodiment a compound according to the invention is used for the preparation of a medicament for the treatment or prevention of hyperglycemia, type 2 diabetes, impaired glucose tolerance, type 1 diabetes, obesity, hypertension, syndrome X, dyslipidemia, cognitive

disorders, atheroschlerosis, myocardial infarction, stroke, coronary heart disease and other cardiovascular disorders, inflammatory bowel syndrome, dyspepsia and gastric ulcers.

In another embodiment a compound according to the invention is used for the preparation of a medicament for delaying or preventing disease progression in type 2 diabetes.

In another embodiment a compound according to the invention is used for the preparation of a medicament for decreasing food intake, decreasing β-cell apoptosis, increasing β-cell function and β-cell mass, and/or for restoring glucose sensitivity to β-cells.

The treatment with a compound according to the present invention may also be combined with a second or more pharmacologically active substances, e.g. selected from antidiabetic agents, antiobesity agents, appetite regulating agents, antihypertensive agents, agents for the treatment and/or prevention of complications resulting from or associated with diabetes and agents for the treatment and/or prevention of complications and disorders resulting from or associated with obesity. Examples of these pharmacologically active substances are : Insulin, sulphonylureas, biguanides, meglitinides, glucosidase inhibitors, glucagon antagonists, DPP-IV (dipeptidyl peptidase-IV) inhibitors, inhibitors of hepatic enzymes involved in stimulation of gluconeogenesis and/or glycogenolysis, glucose uptake modulators, compounds modifying the lipid metabolism such as antihyperlipidemic agents as HMG CoA inhibitors (statins), Gastric Inhibitory Polypeptides (GIP analogs), compounds lowering food intake, RXR agonists and agents acting on the ATP-dependent potassium channel of the β-cells; Cholestyramine, colestipol, clofibrate, gemfibrozil, lovastatin, pravastatin, simvastatin, probucol, dextrothyroxine, neteglinide, repaglinide; β-blockers such as alprenolol, atenolol, timolol, pindolol, propranolol and metoprolol, ACE (angiotensin converting enzyme) inhibitors such as benazepril, captopril, enalapril, fosinopril, lisinopril, alatriopril, quinapril and ramipril, calcium channel blockers such as nifedipine, felodipine, nicardipine, isradipine, nimodipine, diltiazem and verapamil, and α-blockers such as doxazosin, urapidil, prazosin and terazosin; CART (cocaine amphetamine regulated transcript) agonists, NPY (neuropeptide Y) antagonists, PYY agonist, PYY2 agonists, PYY4 agonits, mixed PPY2/PYY4 agonists, MC4 (melanocortin 4) agonists, orexin antagonists, TNF (tumor necrosis factor) agonists, CRF (corticotropin releasing factor) agonists, CRF BP (corticotropin releasing factor binding protein) antagonists, urocortin agonists, β3 agonists, MSH (melanocyte-stimulating hormone) agonists, MCH (melanocyte-concentrating hormone) antagonists, CCK (cholecystokinin) agonists, serotonin re-uptake inhibitors, serotonin and noradrenaline re-uptake inhibitors, mixed serotonin and noradrenergic compounds, 5HT (serotonin) agonists, bombesin agonists, galanin antagonists, growth hormone, growth hormone releasing compounds, TRH (thyreotropin releasing hormone) agonists, UCP 2 or 3

(uncoupling protein 2 or 3) modulators, leptin agonists, DA agonists (bromocriptin, doprexin), lipase/amylase inhibitors, RXR (retinoid X receptor) modulators, TR β agonists; histamine H3 antagonists, Gastric Inhibitory Polypeptide agonists or antagonists (GIP analogs), gastrin and gastrin analogs.

The treatment with a compound according to this invention may also be combined with surgery- a surgery that influence the glucose levels and/or lipid homeostasis such as gastric banding or gastric bypass.

It should be understood that any suitable combination of the compounds according to the invention with one or more of the above-mentioned compounds and optionally one or more further pharmacologically active substances are considered to be within the scope of the present invention.

The present invention is further illustrated by the following examples which, however, are not to be construed as limiting the scope of protection. The features disclosed in the foregoing description and in the following examples may, both separately and in any combination thereof, be material for realising the invention in diverse forms thereof.

EXAMPLES

Abbreviations used:

r.t: Room temperature
DIPEA: diisopropylethylamine
H_2O: water
CH_3CN: acetonitrile
DMF: NN dimethylformamide
HBTU: 2-(1H-Benzotriazol-1-yl-)-1,1,3,3 tetramethyluronium hexafluorophosphate
Fmoc: 9 H-fluoren-9-ylmethoxycarbonyl
Boc: tert butyloxycarbonyl
OtBu: tert butyl ester
tBu: tert butyl
Trt: triphenylmethyl
Pmc: 2,2,5,7,8-Pentamethyl-chroman-6-sulfonyl
Dde: 1-(4,4-Dimethyl-2,6-dioxocyclohexylidene)ethyl
ivDde: 1-(4,4-Dimethyl-2,6-dioxocyclohexylidene)-3-methylbutyl
Mtt: 4-methyltrityl
Mmt: 4-methoxytrityl
DCM: dichloromethane

TIS: triisopropylsilane)
TFA: trifluoroacetic acid
Et_2O: diethylether
NMP: 1-Methyl-pyrrolidin-2-one
DIPEA: Diisopropylethylamine
HOAt: 1-Hydroxy-7-azabenzotriazole
HOBt: 1-Hydroxybenzotriazole
DIC: Diisopropylcarbodiimide

A: Synthesis of resin bound peptide.

The protected peptidyl resin was synthesized according to the Fmoc strategy on an Applied Biosystems 433 peptide synthesizer in 0.25 mmol or 1.0 mmol scale using the manufacturer supplied FastMoc UV protocols which employ HBTU (2-(1H-Benzotriazol-1-yl-)-1,1,3,3 tetramethyluronium hexafluorophosphate) or HATU (O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate) mediated couplings in NMP (N-methyl pyrrolidone), and UV monitoring of the deprotection of the Fmoc protection group. The starting resin used for the synthesis of the GLP-1 peptide amides was Rink-Amide resin and either Wang or chlorotrityl resin was used for GLP-1 peptides with a carboxy C-terminal. The protected amino acid derivatives used were standard Fmoc-amino acids (supplied from e.g. Anaspec, or Novabiochem) supplied in preweighed cartridges suitable for the ABI433A synthesizer with the exception of unnatural aminoacids such as Fmoc-Aib-OH (Fmoc-aminoisobutyric acid). The N terminal amino acid was Boc protected at the alpha amino group (e.g. Boc-His(Boc)OH was used for peptides with His at the N-terminal). The epsilon amino group of lysine in position 26 was either protected with Mtt, Mmt, Dde, ivDde, or Boc, depending on the route for attachment of the albumin binding moiety and spacer. The synthesis of the peptides may in some cases be improved by the use of dipeptides protected on the dipeptide amide bond with a group that can be cleaved under acidic conditions such but not limited to 2-Fmoc-oxy-4-methoxybenzyl or 2,4,6-trimethoxybenzyl. In cases where a serine or a threonine is present in the peptide, the use of pseudoproline dipeptides may be used (see e.g. catalogue from Novobiochem 2002/2003 or newer version, or W.R. Sampson (1999), J. Pep. Sci. 5, 403.

<u>Procedure for removal of ivDde or Dde-protection</u>.

The resin (0.25 mmol) was placed in a manual shaker/filtration apparatus and treated with 2% hydrazine in N-methyl pyrrolidone (20 ml, 2x12 min) to remove the Dde or

ivDde group and wash with N-methyl pyrrolidone (4x20 ml).

Procedure for removal of Mtt or Mmt-protection.

The resin (0.25 mmol) was placed in a manual shaker/filtration apparatus and treated with 2% TFA and 2-3% TIS in DCM (20 ml, 5-10 min repeated 6-12 times) to remove the Mtt or Mmt group and wash with DCM (2x20 ml), 10%MeOH and 5% DIPEA in DCM (2x20ml) and N-methyl pyrrolidone (4x20 ml).

Procedure for attachment of sidechains to Lysine residue.

The albumin binding residue (B-U- sidechain of formula I) can be attached to the GLP-1 peptide either by acylation to resin bound peptide or acylation in solution to the unprotected peptide using standard acylating reagent such as but not limited to DIC, HOBt/DIC, HOAt/DIC, or HBTU.

Attachment to resin bound peptide:

Route I

Activated (active ester or symmetric anhydride) albumin binding residue (B-U- sidechain of formula I) such as octadecanedioic acid mono-(2,5-dioxo-pyrrolidin-1-yl) ester (Ebashi *et al.* EP511600, 4 molar equivalents relative to resin bound peptide) was dissolved in NMP (25 mL), added to the resin and shaken overnight at room temperature. The reaction mixture was filtered and the resin was washed extensively with NMP, dichloromethane, 2-propanol, methanol and diethyl ether.

Route II

The albumin binding residue (B-U- sidechain of formula I) was dissolved in N-methyl pyrrolidone/methylene chloride (1:1, 10 ml). The activating reagent such as hydroxybenzotriazole (HOBt) (4 molar equivalents relative to resin) and diisopropylcarbodiimide (4 molar equivalents relative to resin) was added and the solution was stirred for 15 min. The solution was added to the resin and diisopropyethylamine (4 molar equivalents relative to resin) was added. The resin was shaken 2 to 24 hours at room temperature. The resin was washed with N-methyl pyrrolidone (2x20 ml), N-methyl pyrrolidone/Methylene chloride (1:1) (2x20ml) and methylene chloride (2x20 ml).

Route III

Activated (active ester or symmetric anhydride) albumin binding residue (B-U- sidechain of formula I) such as octadecanedioic acid mono-(2,5-dioxo-pyrrolidin-1-yl) ester (Ebashi *et al.*

EP511600, 1-1.5 molar equivalents relative to the GLP-1 peptide was dissolved in an organic solvent such as acetonitrile, THF, DMF, DMSO or in a mixture of water/organic solvent (1-2 ml) and added to a solution of the peptide in water (10-20ml) together with 10 molar equivalents of DIPEA. In case of protecting groups on the albumin binding residue such as tert.-butyl, the reaction mixture was lyophilized O/N and the isolated crude peptide deprotected afterwards – in case of a *tert*-butyl group the peptide was dissolved in a mixture of trifluoroacetic acid, water and triisopropylsilane (90:5:5). After for 30min the mixture was, evaporated in vacuo and the finale petide purified by preparative HPLC.

Procedure for removal of Fmoc-protection: The resin (0.25 mmol) was placed in a filter flask in a manual shaking apparatus and treated with N-methyl pyrrolidone/methylene chloride (1:1) (2x20 ml) and with N-methyl pyrrolidone (1x20 ml), a solution of 20% piperidine in N-methyl pyrrolidone (3x20 ml, 10 min each). The resin was washed with N-methyl pyrrolidone (2x20 ml), N-methyl pyrrolidone/Methylene chloride (1:1) (2x20ml) and methylene chloride (2x20 ml).

Procedure for cleaving the peptide off the resin:
The peptide was cleaved from the resin by stirring for 180 min at room temperature with a mixture of trifluoroacetic acid, water and triisopropylsilane (95:2.5:2.5 to 92:4:4). The cleavage mixture was filtered and the filtrate was concentrated to an oil by a stream of nitrogen. The crude peptide was precipitated from this oil with 45 ml diethyl ether and washed 1 to 3 times with 45 ml diethyl ether.

Purification: The crude peptide was purified by semipreparative HPLC on a 20 mm x 250 mm column packed with either 5μ or 7μ C-18 silica. Depending on the peptide one or two purification systems were used.
TFA: After drying the crude peptide was dissolved in 5 ml 50% acetic acid H_2O and diluted to 20 ml with H_2O and injected on the column which then was eluted with a gradient of 40-60 % CH_3CN in 0.1% TFA 10 ml/min during 50 min at 40 °C. The peptide containing fractions were collected. The purified peptide was lyophilized after dilution of the eluate with water.
Ammonium sulphate: The column was equilibrated with 40% CH_3CN in 0.05M $(NH_4)_2SO_4$, which was adjusted to pH 2.5 with concentrated H_2SO_4. After drying the crude peptide was dissolved in 5 ml 50% acetic acid H_2O and diluted to 20 ml with H_2O and injected on the column which then was eluted with a gradient of 40% - 60% CH_3CN in 0.05M $(NH_4)_2SO_4$, pH 2.5 at 10 ml/min during 50 min at 40 °C. The peptide containing fractions were collected and diluted with 3 volumes of H_2O and passed through a Sep-Pak® C18 cartridge (Waters part.

#:51910) which has been equilibrated with 0.1% TFA. It was then eluted with 70% CH_3CN containing 0.1% TFA and the purified peptide was isolated by lyophilisation after dilution of the eluate with water.

The final product obtained was characterised by analytical RP-HPLC (retention time) and by LCMS

The RP-HPLC analysis was performed using UV detection at 214 nm and a Vydac 218TP54 4.6mm x 250mm 5µ C-18 silica column (The Separations Group, Hesperia, USA) which was eluted at 1 ml/min at 42 °C. Two different elution conditions were used:

A1: Equilibration of the column with in a buffer consisting of 0.1M $(NH_4)_2SO_4$, which was adjusted to pH 2.5 with concentrated H_2SO_4 and elution by a gradient of 0% to 60% CH_3CN in the same buffer during 50 min.

B1: Equilibration of the column with 0.1% TFA / H_2O and elution by a gradient of 0% CH_3CN / 0.1% TFA / H_2O to 60% CH_3CN / 0.1% TFA / H_2O during 50 min.

B6: Equilibration of the column with 0.1% TFA / H_2O and elution by a gradient of 0% CH_3CN / 0.1% TFA / H_2O to 90% CH_3CN / 0.1% TFA / H_2O during 50 min.

Alternative the RP-HPLC analysis was performed using UV detection at 214 nm and a Symmetry300, 3.6mm x 150mm, 3.5µ C-18 silica column (Waters) which was eluted at 1 ml/min at 42 °C.

B4: Equilibration of the column with 0.05% TFA / H_2O and elution by a gradient of 5% CH_3CN / 0.05% TFA / H_2O to 95% CH_3CN / 0.05% TFA / H_2O during 15 min.

The following instrumentation was used:

LCMS was performed on a setup consisting of Sciex API 100 Single quadropole mass spectrometer, Perkin Elmer Series 200 Quard pump ,Perkin Elmer Series 200 autosampler, Applied Biosystems 785A UV detector, Sedex 75 evaporative light scattering detector

The instrument control and data acquisition were done by the Sciex Sample control software running on a Windows 2000 computer.

The HPLC pump is connected to two eluent reservoirs containing:

A: 0.05% Trifluoro acetic acid in water

B: 0.05% Trifluoro acetic acid in acetonitrile

The analysis is performed at room temperature by injecting an appropriate volume of the sample (preferably 20 µl) onto the column which is eluted with a gradient of acetonitrile.

The HPLC conditions, detector settings and mass spectrometer settings used are giving in the following table.

Column : Waters Xterra MS C-18 X 3 mm id 5 μm
Gradient : 5% - 90 % acetonitrile linear during 7.5 min at 1.5ml/min
Detection : 210 nm (analogue output from DAD)
ELS (analogue output from ELS), 40 °C
MS ionisation mode API-ES

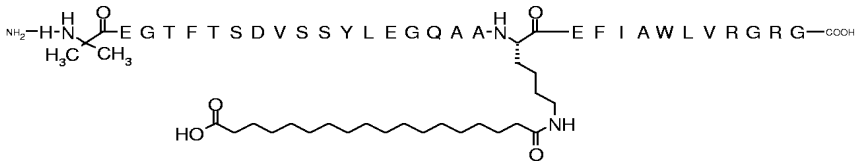
Alternatively LCMS was performed on a setup consisting of Hewlett Packard series 1100 G1312A Bin Pump, Hewlett Packard series 1100 Column compartment, Hewlett Packard series 1100 G1315A DAD diode array detector, Hewlett Packard series 1100 MSD and Sedere 75 Evaporative Light Scattering detectorcontrolled by HP Chemstation software. The HPLC pump is connected to two eluent reservoirs containing:
A: 10mM NH_4OH in water
B: 10mM NH_4OH in 90% acetonitrile
The analysis was performed at 23° C by injecting an appropriate volume of the sample (preferably 20 μl) onto the column which is eluted with a gradient of A and B.
The HPLC conditions, detector settings and mass spectrometer settings used are giving in the following table.
Column Waters Xterra MS C-18 X 3 mm id 5 m
Gradient 5% - 100% acetonitrile linear during 6.5 min at 1.5ml/min
Detection 210 nm (analogue output from DAD)
ELS (analogue output from ELS)
MS ionisation mode API-ES. Scan 100-1000 amu step 0.1 amu

Radioligand binding to plasma membranes expressing the human GLP-1 receptor

The binding assay was performed with purified plasma membranes containing the human GLP-1 receptor. The plasma membranes containing the receptors were purified from stably expressing BHK tk-ts 13 cells. The membranes were diluted in Assay Buffer (50 mM HEPES, 5 mM EGTA, 5 mM $MgCl_2$, 0.005% Tween 20, pH=7.4) to a final concentration of 0.2 mg/ml of protein and destributed to 96-well microtiter plates precoated with 0.3 % PEI. Membranes in the presence of 0.05 nM [^{125}I]GLP-1, unlabelled ligands in increasing

concentrations and different HSA concentrations (0.005%, 0.05%, and 2%) were incubated 2 hr at 30°C. After incubation, unbound ligands were separated from bound ligands by filtration through a vacuum-manifold followed by 2X100 µl washing with ice cold assaybuffer. The filters were dried overnight at RT, punched out and quantified in a γ-counter.

Example 1



N-ε^{26} (17-carboxyheptadecanoyl)-[Aib8,Arg34]GLP-1-(7-37)-peptide

A resin (Fmoc-Gly-NovaSyn TGT, 0.22 mmol/g Novabiochem 0.25 mmole) was used to produce the primary sequence on an ABI433A machine according to manufacturers guidelines. All protecting groups were acid labile with the exception of the residue used in position 26 (FmocLys(ivDde)-OH, Novabiochem) allowing specific deprotection of this lysine rather than any other lysine.

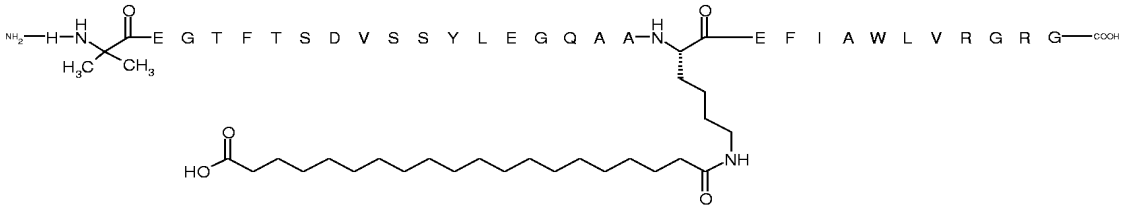
<u>Procedure</u>

The resin (0.09 mmole) was placed in a manual shaker/filtration apparatus and treated with 4% hydrazine in N-methyl pyrrolidone in (4x10 min. 4x4 ml) to remove the ivDde group. The resin was washed with N-methyl pyrrolidone (3x4 ml).Octadecanedioic acid mono-(2,5-dioxo-pyrrolidone-1-yl)ester) (4 molar equivalents relative to resin) was dissolved in DMF (4ml). The solution was added to the resin and diisopropylethylamine (8 molar equivalents relative to resin) was added. The resin was shaken 24 hours at room temperature. The resin was washed with N-methyl pyrrolidone (4x4 ml) and DCM (4x4ml). The peptide was cleaved from the resin by stirring for 180 min at room temperature with a mixture of trifluoroacetic acid, water and triisopropylsilane (92.5:5.0:2.5 4 ml).The cleavage mixture was filtered and the crude peptide was precipitated from 40 ml diethyl ether and washed 3 times with 45 ml diethyl ether. The crude peptide was purified by preparative HPLC on a 20 mm x 250 mm column packed with 7µ C-18 silica. The crude peptide was dissolved in 5 ml 50% acetic acid in water and diluted to 20 ml with H_2O and injected on the column which then was eluted with a gradient of 25-65 % (CH_3CN in water with 0.1% TFA) 20 ml/min during 40 min at RT. The peptide containing fractions were collected. The purified peptide was lyophilized after dilution of the eluate with water.

HPLC (method B4): RT= 9.94 min (91%)

LCMS: m/z = 1232 (MH_3^{3+}) Calculated for (MH_3^{3+}) = 1232

Example 2



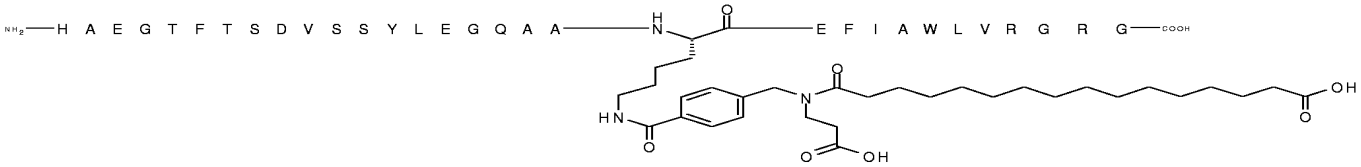
N-ε^{26}-(19-carboxynonadecanoyl)-[Aib8,Arg34]GLP-1-(7-37)-peptide

Prepared as in Example 1 and in accordance with "synthetic methods".

HPLC (method B4): RT= 10.42 min (91%)

LCMS: m/z = 1242 (MH_3^{3+}), Calculated for (MH_3^{3+}) = 1242

Example 3



N-ε^{26}-(4-{[N-(2-carboxyethyl)-N-(15-carboxypentadecanoyl)amino]methyl}benzoyl[Arg34]GLP-1-(7-37)-peptide

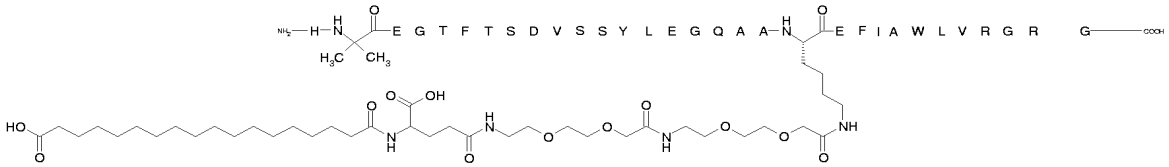
To a solution of 4-(N-(2-(*tert*-butoxycarbonyl)ethyl)-N-(15-(*tert*-butoxycarbonyl)pentadecanoyl)aminomethyl)benzoic acid (36 mg, 60 µmol) in THF (1 ml) were added DIPEA (7 µl) and O-(1-succinimidyl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (TSTU, 17 mg, 56 µl). After stirring for 1 h at room temperature, the mixture was diluted with THF (1 ml), and 1 ml of the resulting solution was added to a solution of [Arg34]GLP-1-(7-37) peptide (approx 100 mg) and DIPEA (103 µl) in water (5 ml). After 0.5 h more of the THF-solution of acylating agent (0.4 ml) was added. After stirring at room temperature for a total of 1.5 h the reaction mixture was filtered and applied to a preparative HPLC (gradient elution with 35-55% MeCN/55-35% water/10% water with 1% TFA). Fractions containing the desired product were combined and lyophylized. The product was then treated with 25 ml of a mixture of TFA and water (95/5 vol) for 15 min at room temperature, concentrated, and purified once more by HPLC. 15.4 mg of the title compound was obtained.

HPLC (method B4): RT = 9.41 min (99%)

LCMS: m/z = 1287 (MH_3^{3+}). Calculated for (MH_3^{3+}): 1287

Example 4

N-ε^{26}-[2-(2-[2-(2-[2-(2-[4-(17-Carboxyheptadecanoylamino)-4(S)-carboxybutyrylamino]ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][Aib8,Arg34]GLP-1-(7-37)peptide.

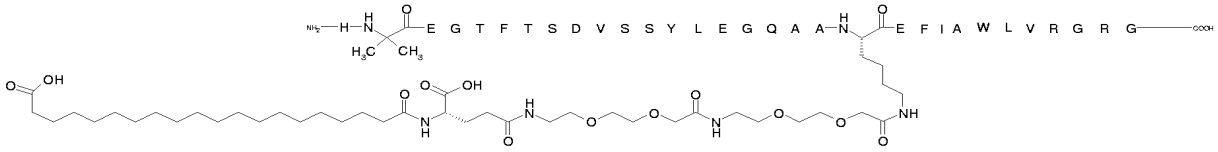


[Aib8,Arg34]GLP-1-(7-37)-peptide was prepared by standard Fmoc-solid phase peptide synthesis and purified by preparative HPLC. [Aib8,Arg34]GLP-1-(7-37)-peptide was dissolved in water (15ml) and DIPEA (50ul) was added. 17-((S)-1-tert-Butoxycarbonyl-3-{2-[2-({2-[2-(2,5-dioxopyrrolidin-1-yloxycarbonylmethoxy) ethoxy]ethylcarbamoyl}methoxy)ethoxy]ethylcarbamoyl}propylcarbamoyl)heptadecanoic acid tert-butyl ester (21 mg) was dissolved in acetonitrile/water 2:1 (1.5 ml) and added in small portions. The reaction was monitored by HPLC. When no more [Aib8,Arg34]GLP-1-(7-37)-peptide was found the reaction mixture was lyophilized O/N. To the isolated compound was added 10 ml of 90% TFA / 5% TIS/ 5% water and the reaction mixture was standing for 2 hours, evaporated *in vacuo*, and co-evaporated with heptane. The residual oil was dissolved in 15ml of water contaning 1%of NH3-aq and purified by preparative HPLC to give the title compound.

HPLC (method B4): RT = 9.60 min (100%)

LCMS: m/z = 1372 (MH_3^{3+}). Calculated for (MH_3^{3+}): 1372

Example 5



N-ε^{26}-[2-(2-[2-(2-[2-(2-[4-(19-Carboxynonadecanoylamino)-4(S)-carboxybutyrylamino]ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][Aib8,Arg34]GLP-1-(7-37)peptide.

The peptide was prepared according to: A. Synthesis of resin bound peptide in 0.25 mMol scale on a Fmoc-Gly-Wang resin (0.66 mmol/g Novabiochem) was used to produce the primary sequence on an ABI433A machine according to manufacturers guidelines. All

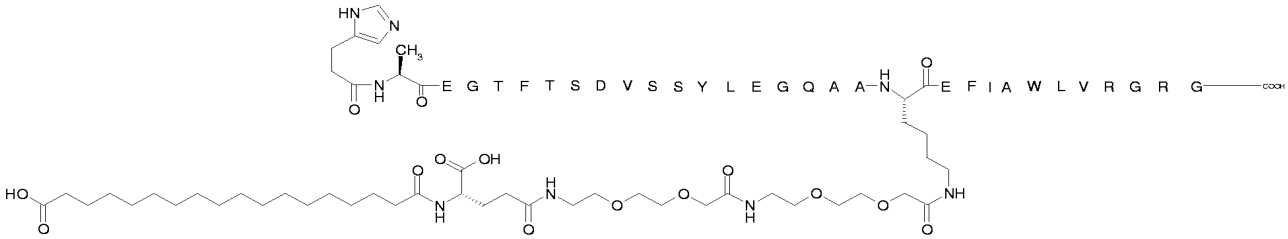
protecting groups were acid labile with the exception of the residue used in position 26 (FmocLys(Mtt)-OH, Novabiochem) which is super acid labile, allowing specific deprotection of this lysine rather than any other lysine.

Procedure for removal of Mtt-protection. The resin (0.25 mmol) was placed in a manual shaker/filtration apparatus and treated with 2% TFA, 3% TIS in DCM (20 ml, 5-10 min repeated 6-12 times) to remove the Mtt group and wash with DMF. Synthesis was continued with Procedure for attachment of sidechains to Lysine residue, following Route II, with the appropriate Procedure for removal of Fmoc-protection. Final deprotection, HPLC-purification and analysis by HPLC and LC-MS according to the procedures.

HPLC (method B6): RT = 34.56 min (100%)

LCMS: m/z = 1381.8 (MH_3^{3+}). Calculated for ($M+H^+$): 4142.7

Example 6



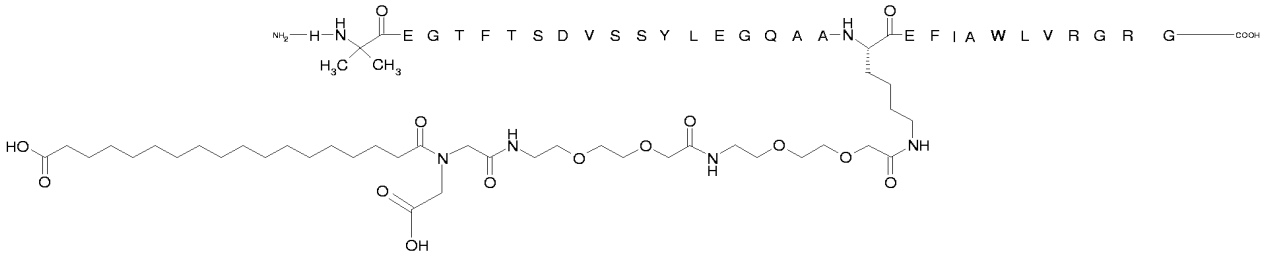
N-ε^{26}-[2-(2-[2-(2-[2-(2-[4-(17-Carboxyheptadecanoylamino)-4(S)-carboxybutyrylamino]ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][3-(4-Imidazolyl)Propionyl7,Arg34]GLP-1-(7-37)peptide

Prepared as in Example 5 and in accordance with "synthetic methods".

HPLC (method B6): RT = 32.89 min (100%)

LCMS: m/z = 1362.3 (MH_3^{3+}). Calculated for ($M+H^+$): 4085.6

Example 7



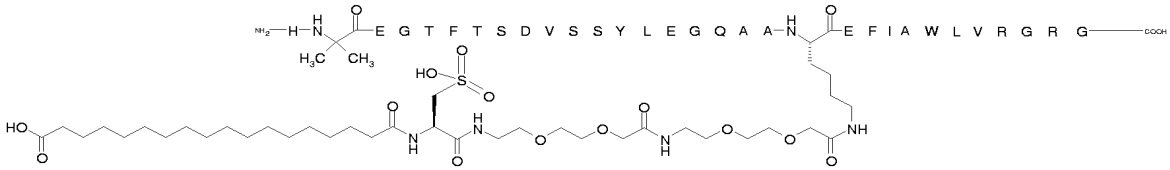
N-ε^{26}-[2-(2-[2-(2-[2-(2-[4-(17-Carboxyheptadecanoylamino)-(Carboxymethyl-amino)acetylamino]ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][Aib8,Arg34]GLP-1-(7-37)peptide

Prepared as in Example 5 and in accordance with "synthetic methods".

HPLC (method B6): RT = 32.67 min (100%)

LCMS: m/z = 1367.3 (MH_3^{3+}). Calculated for ($M+H^+$): 4100.6

Example 8



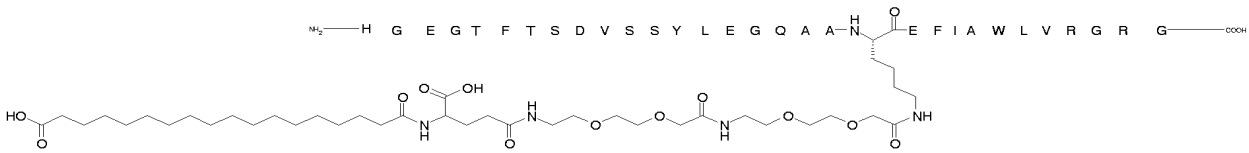
N-ε^{26}-[2-(2-[2-(2-[2-(2-[4-(17-Carboxyheptadecanoylamino)-3(S)-Sulfopropionylamino]ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][Aib8,Arg34]GLP-1-(7-37)peptide

Prepared as in Example 5 and in accordance with "synthetic methods".

HPLC (method B6): RT = 32.04 min (100%)

LCMS: m/z = 1379.8 (MH_3^{3+}). Calculated for ($M+H^+$): 4136.7

Example 9



N-ε^{26-}[2-(2-[2-(2-[2-(2-[4-(17-Carboxyheptadecanoylamino)-4(S)-carboxybutyrylamino]ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][Gly8,Arg34]GLP-1-(7-37)peptide.

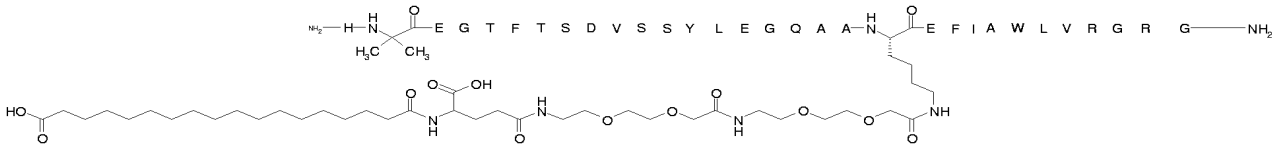
[Gly8,Arg34]GLP-1(7-37) peptide starting from 150 mg 2-chlorotrityl chloride resin (1.4 mmol/g) was prepared by Fmoc-solid phase peptide synthesis using Apex396 from Advanced Chemtech. The Lys residue at position 26 was protected as Lys(ivDde) while the functional side-chains for the other amino acids were protected with standard acid labile protecting groups. The Lys residue was deprotected with 3% hydrazine/3% piperidine in NMP for 1 hr. Then, the two units of 8-amino-3,6-dioxaoctanoic acid, γ–glutamic acid and octadecanedioic acid were coupled to the resin attached peptide using DIC/HOAt. The

peptide was finally deprotected and cleaved from the resin with TFA/TIS/H_2O/thioanisol (90/5/3/2). The peptide was isolated by LC-MS.

HPLC: Elutes at 46% acetonitrile

MALDI: 4087 (MH+)

Example 10



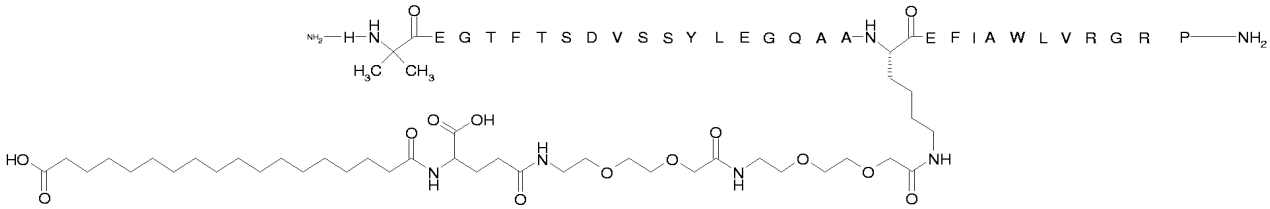
N-ε^{26}-[2-(2-[2-(2-[2-(2-[4-(17-Carboxyheptadecanoylamino)-4(S)-carboxybutyrylamino]ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][Aib8,Arg34]GLP-1-(7-37)-amide

[Aib8,34]GLP-1(7-37) amide starting from 200 mg Tentagel RAM S resin (0.26 mmol/g) was prepared by Fmoc-solid phase peptide synthesis using Apex396 from Advanced Chemtech. The Lys residue at position 26 was protected as Lys(ivDde) while the functional side-chains for the other amino acids were protected with standard acid labile protecting groups. The Lys residue was deprotected with 3% hydrazine/3% piperidine in NMP for 1 hr. Then, the two units of,8-amino-3,6-dioxaoctanoic acid, γ-glutamic acid octadecanedioic acid were coupled to the resin attached peptide using DIC/HOAt. The peptide was finally deprotected and cleaved from the resin with TFA/TIS/H_2O/thioanisol (90/5/3/2). The peptide was isolated by LC-MS.

HPLC: Elutes at 49% acetonitrile

MALDI: 4114 (MH+)

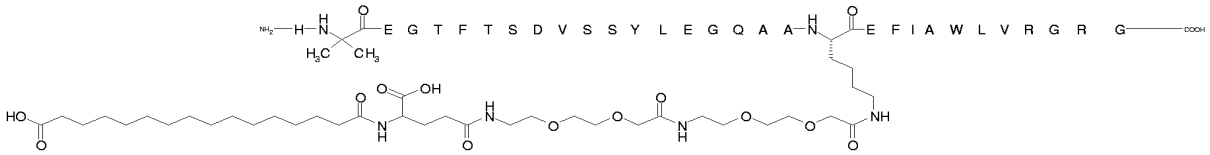
Example 11



N-ε^{26}-[2-(2-[2-(2-[2-(2-[4-(17-Carboxyheptadecanoylamino)-4(S)-carboxybutyrylamino]ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][Aib8,Arg34,Pro37]GLP-1-(7-37)amide

The peptide was prepared on a Rink amide resin (0.70 mmol/g Novabiochem) and else as in Example 5 and in accordance with "synthetic methods".
HPLC (method B6): RT = 32.13 min (100%). (method A1): RT = 44.33 min (98.4%)
LCMS: m/z = 1385.3 (MH_3^{3+}). Calculated for ($M+H^+$): 4153.8

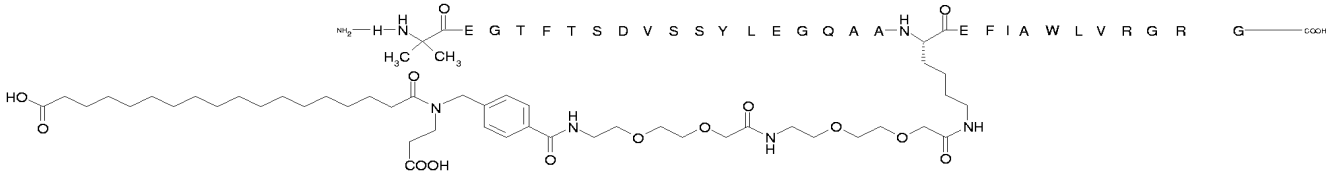
Example 12



Aib[8],Lys[26](N-ε[26]-{2-(2-(2-(2-[2-(2-(4-(pentadecanoylamino)-4-carboxybutyrylamino)ethoxy)ethoxy]acetyl)ethoxy)ethoxy)acetyl)}),Arg[34])GLP-1 H(7-37)-OH

HPLC (method B6): RT= 30.41 min
LCMS: m/z = 1362.9 (MH_3^{3+}) Calculated for (M^+) = 4085.61

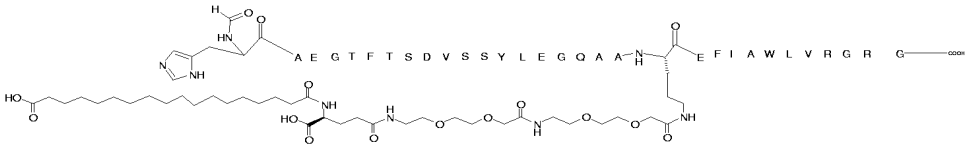
Example 13



N-ε[26]-[2-(2-[2-(2-[2-(2-[4-{[N-(2-carboxyethyl)-N-(17-carboxyheptadecanoyl)amino]methyl}benzoyl)amino]ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][Aib8,Arg34]GLP-1(7-37)
[Aib8,Arg34]GLP-1(7-37) peptide starting from 150 mg 2-chlorotrityl chloride resin (1.4 mmol/g) was prepared by Fmoc-solid phase peptide synthesis using Apex396 from Advanced Chemtech. The Lys residue at position 26 was protected as Lys(ivDde) while the functional side-chains for the other amino acids were protected with standard acid labile protecting groups. The Lys residue was deprotected with 3% hydrazine/3% piperidine in NMP for 1 hr. The two units of 8-amino-3,6-dioxaoctanoic acid and 4{[(2-tert-butoxycarbonyl-ethyl)-(17-tert-butoxycarbonyl-heptadecanoyl)-amino]-methyl}-benzoic acid were coupled to the resin attached peptide using DIC/HOAt. The peptide was finally deprotected and cleaved from the resin with TFA/TIS/H_2O/thioanisol (90/5/3/2). The peptide was isolated by preparative LC-MS.
HPLC: Elutes at 52% acetonitrile

MALDI: 4191 (MH+)

Example 14

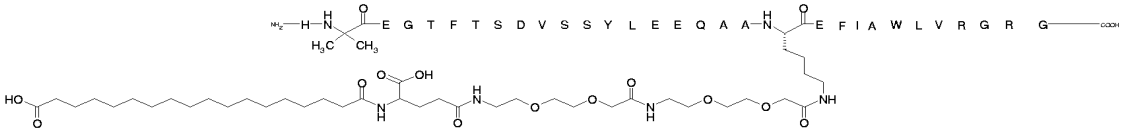


N-α^7-formyl, N-ε^{26}-[2-(2-[2-(2-[2-(2-[4-(17-Carboxyheptadecanoylamino)-4(S)-carboxybutyrylamino]ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][Arg^{34}]GLP-1-(7-37)-peptide

HPLC (method B6): RT= 32,6 min

LCMS: m/z = 1377.3 (MH_3^{3+}) Calculated for (M^+) = 4128.0

Example 15



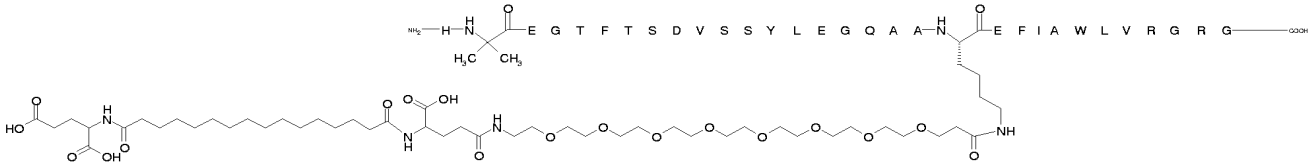
N-ε^{26}26-[2-(2-[2-(2-[2-(2-[4-(17-Carboxyheptadecanoylamino)-4(S)-carboxybutyrylamino]ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][Aib8,Glu22,Arg34]GLP-1-(7-37)peptide.

[Aib8,Glu22,Arg34]GLP-1(7-37) peptide starting from 150 mg Fmoc-Gly-Wang resin (0.66mmol/g) was prepared by Fmoc-solid phase peptide synthesis using Apex396 from Advanced Chemtech. The Lys residue at position 26 was protected as Lys(Mtt) while the functional side-chains for the other amino acids were protected with standard acid labile protecting groups. The Lys residue was deprotected with 2% TFA/2% TIS in DCM for 4 x 5 min. The two units of 8-amino-3,6-dioxaoctanoic acid, γ-glutamic and octadecanoic acid tert-butyl ester were coupled to the resin attached peptide using DIC/HOAt. The peptide was finally deprotected and cleaved from the resin with TFA/TIS/H_2O/thioanisol (90/5/3/2). The peptide was isolated by LC-MS.

HPLC: Elutes at 50% acetonitrile

MALDI: 4187 (MH+)

Example 16



N-ε^{26}\{3-[2-(2-\{2-[2-(2-\{2-[2-(2-[4-(15-(N-((S)-1,3-dicarboxypropyl)carbamoyl)pentadecanoylamino)-(S)-4-carboxybutyrylamino]ethoxy)ethoxy]ethoxy\}ethoxy)ethoxy]ethoxy\}ethoxy)ethoxy]propionyl\}[Aib8,Arg34]GLP-1-(7-37)-peptide

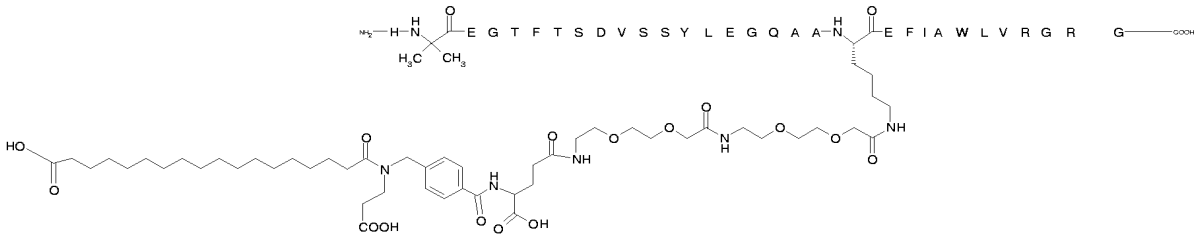
Method and analysis

Prepared as in Example 3 and in accordance with "synthetic methods".

HPLC (method B4): RT = 10.29 min (92%)

LCMS: m/z = 1450 (MH_3^{3+}). Calculated for (MH_3^{3+}): 1450

Example 17



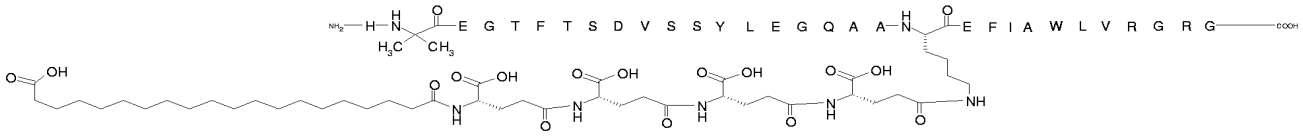
N-ε^{26}-[2-(2-[2-(2-[2-(2-[4-\{[N-(2-carboxyethyl)-N-(17-carboxyheptadecanoyl)amino]methyl\}benzoyl)amino](4(S)-carboxybutyrylamino)ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][Aib8,Arg34]GLP-1(7-37)

[Aib8,Arg34]GLP-1(7-37) peptide starting from 150 mg Fmoc-Gly Wang resin (0.66mmol/g) was prepared by Fmoc-solid phase peptide synthesis using Apex396 from Advanced Chemtech. The Lys residue at position 26 was protected as Lys(Mtt) while the functional side-chains for the other amino acids were protected with standard acid labile protecting groups. The Lys residue was deprotected with 2% TFA/2% TIS in DCM for 4 x 5 min. The two units of 8-amino-3,6-dioxaoctanoic acid, γ-glutamic acid and 4\{[(2-tert-butoxycarbonyl-ethyl)-(17-tert-butoxycarbonyl-heptadecanoyl)-amino]-methyl\}-benzoic acid were coupled to the resin attached peptide using DIC/HOAt. The peptide was finally deprotected and cleaved from the resin with TFA/TIS/H_2O/thioanisol (90/5/3/2). The peptide was isolated by preparative HPLC.

HPLC: Elutes at 51% acetonitrile

MALDI: 4320 (MH+)

Example 18



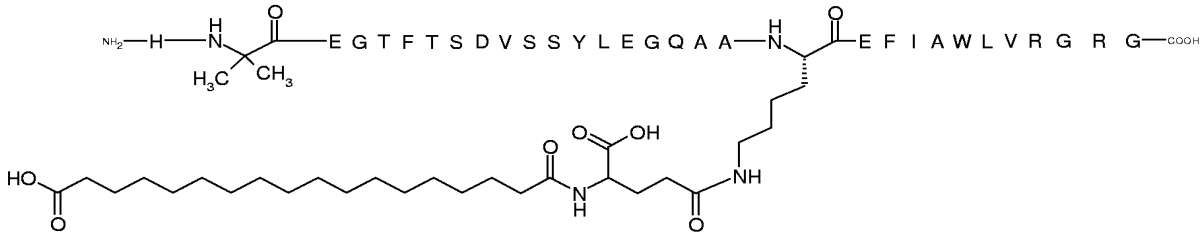
N-ε^{26}-{(S)-4-carboxy-4-((S)-4-carboxy-4-((S)-4-carboxy-4-((S)-4-carboxy-4-(19-carboxynonadecanoylamino)butyrylamino)butyrylamino)butyrylamino)butyrylamino}[Aib8,Arg 34]GLP-1-(7-37)

The peptide was synthesized using Fmoc chemistry on a Liberty Microwave Peptide Synthesizer (CEM Corporation). The synthesis was performed on a Gly-Wang resin (Novabiochem) with a loading of 0.66 mmol/g using 4 fold excess of amino acids and DIC/HOAt for coupling. The N-terminal histidine was Boc-protected and the lysine to be modified was Mtt-protected. After synthesis of the peptide backbone, the Mtt group was removed with 3% TFA in DCM and the side chain was built on the Liberty using standard peptide synthesis protocols. In the last step the fatty diacid was added as a mono-t-butyl-ester.

After cleavage with TFA/TIS/water (95:2.5:2.5), the peptide was dissolved in 50% acetonitrile by addition of DIPEA and purified on a Waters LC-MS system using a 7.8 x 300 mm X-Terra Prep MS C18 10 µm column running at room temperature. After 5 minutes at 30% CH_3CN, 0.08% TFA, 4 ml/min, the column was eluted with a linear gradient of 30 to 70% CH_3CN over 40 minutes. The fractions containing the desired compound were collected and the concentration of the peptide in the eluate was determined by measurement of the UV absorption at 280 nm assuming molar extinction coefficients of 1280 and 3690 for tyrosine and tryptophan respectively. The identity and purity was confirmed by MALDI. After the concentration determination the eluate was aliquotted into vials containing the desired amount and dried by vacuum centrifugation.

HPLC: Elutes at 52% acetonitrile
MALDI: 4239 (MH+)

Example 19



N-ε^{26}-4-(17-Carboxyheptadecanoylamino)-4(S)-carboxybutyryl-[Aib8,Arg34]GLP-1-(7-37)-peptide

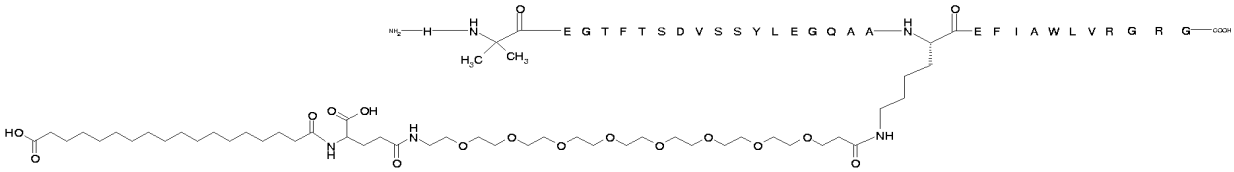
Method and analysis

Prepared as in Example 4 and in accordance with "synthetic methods".

HPLC (method B4): Rt = 9.64 min (97 %)

LCMS: m/z: = 1276 (MH_3^{3+}), Calculated for (MH_3^{3+}) 1276

Example 20

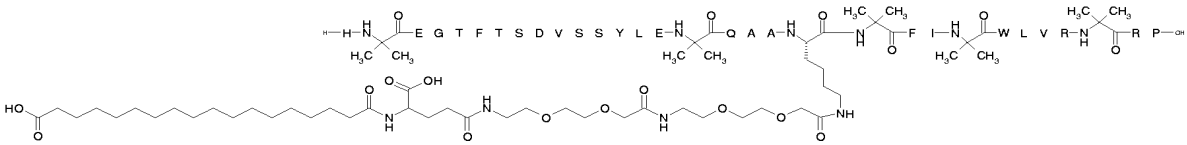


N-ε^{26}-{3-[2-(2-{2-[2-(2-{2-[2-(2-[4-(17-carboxyheptadecanoylamino)-4(S)-carboxybutyrylamino]
ethoxy)ethoxy]ethoxy}ethoxy)ethoxy]ethoxy}ethoxy)ethoxy]propionyl}[Aib8,Arg34]GLP-1-(7-37)-peptide.

LCMS*: m/z: = 1417 (MH_3^{3+}), Calculated for (MH_3^{3+}) 1417

*HPLC (Eluted at 0.5 mL/min at 42°C by a linear gradient from 5 ---->80% acetonitrile, 85----->10% water and 10% of a solution of 1.0% trifluoroacetic acid over 50min. UV detection at 214 on a Symmetry300, 5um, 3.9 mm x 150 mm C-18 silica column.)method B4): Rt = 32.09 min (95 %)

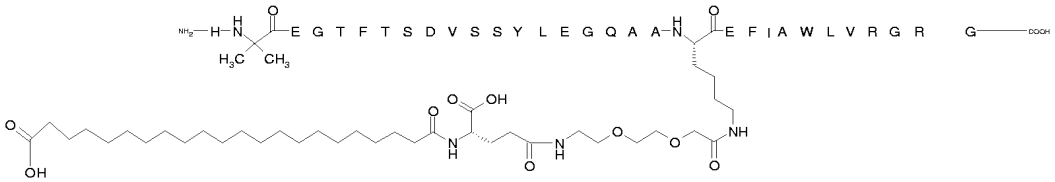
Example 21



N-ε^{26}-{2-(2-(2-(2-[2-(2-(4-(17-carboxyheptadecanoylamino)-4-carboxybutyrylamino)ethoxy)ethoxy]acetyl)ethoxy)ethoxy)acetyl)}-[Aib^{8,22,27,30,35},Arg^{34},Pro^{37}, Lys^{26}] GLP-1 (7-37)amide

HPLC (method B6): RT= 35.0 min
LCMS: m/z = 1394.0 (MH_3^{3+}) Calculated for (M^+) = 4180.0

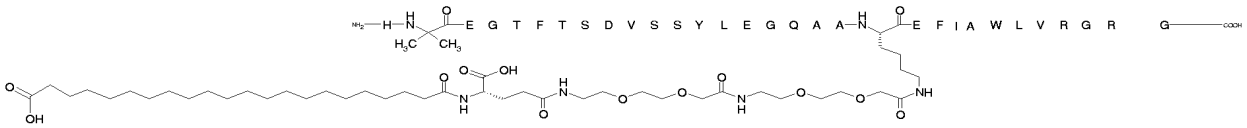
Example 22



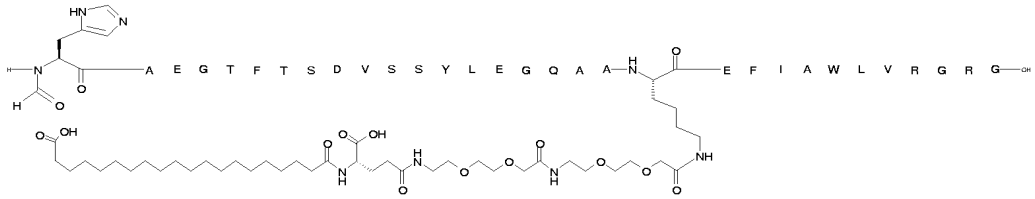
N-ε^{26}-[2-(2-[2-[4-(21-Carboxyuneicosanoylamino)-4(S)-carboxybutyrylamino]ethoxy]ethoxy)acetyl][Aib8,Arg34]GLP-1-(7-37)
Prepared using the same method as in Example 19.

HPLC: Elutes at 53.4% acetonitrile
MALDI: 4025 (MH+)

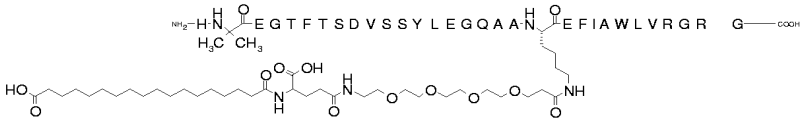
Other compounds of this invention include:

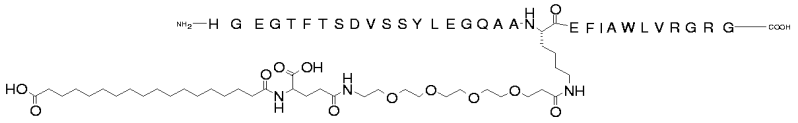


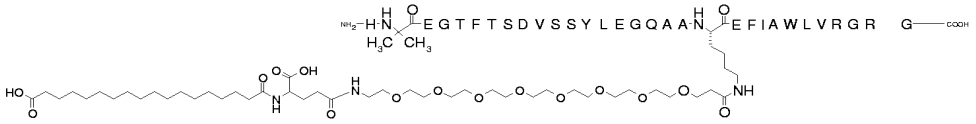
N-ε^{26}-[2-(2-[2-(2-[2-(2-[4-(21-Carboxyuneicosanoylamino)-4(S)-carboxybutyrylamino]ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][Aib8,Arg34]GLP-1-(7-37)peptide.

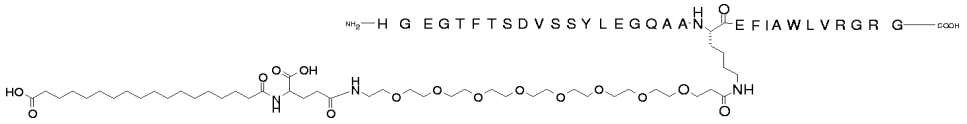


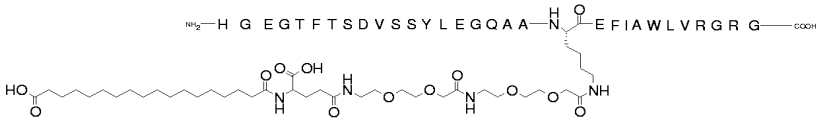
N-α1-formyl-N-ε^{26}-[2-(2-[2-(2-[2-(2-[4-(19-Carboxynonadecanoylamino)-4(S)-carboxybutyrylamino]ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][Arg34]GLP-1-(7-37)peptide.

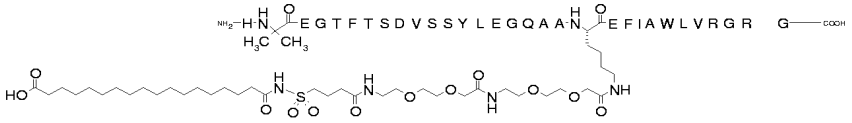


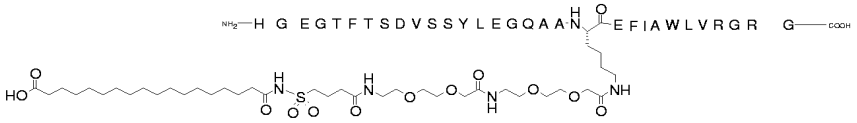


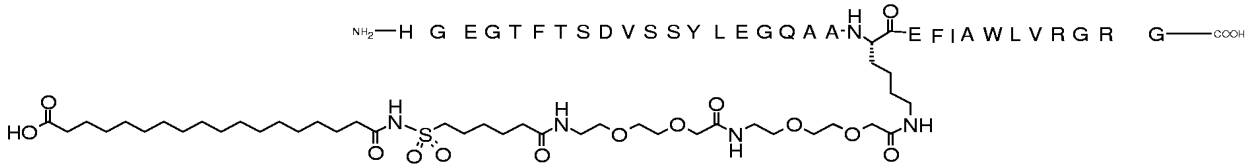


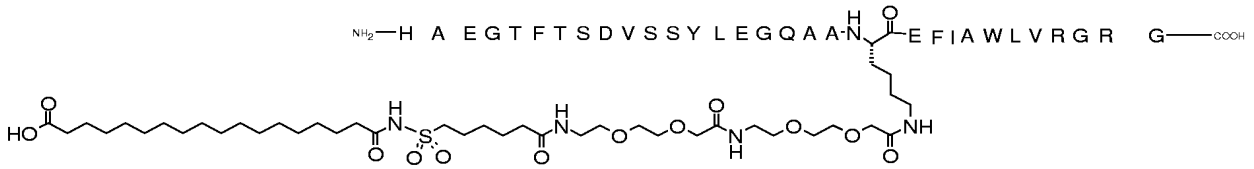


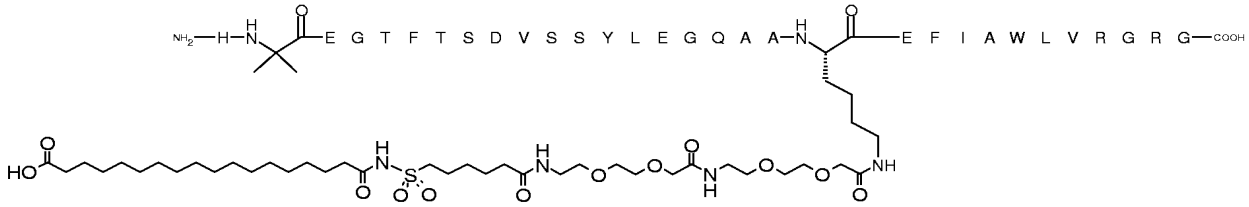


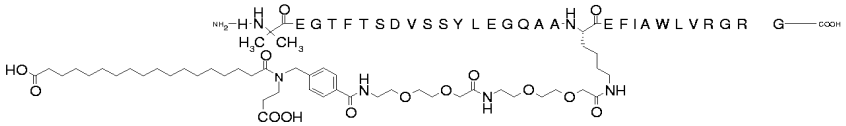


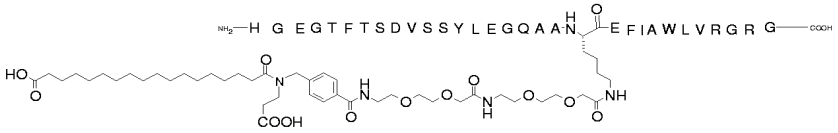


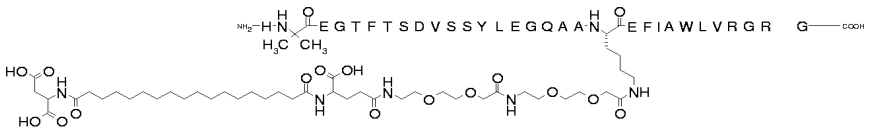


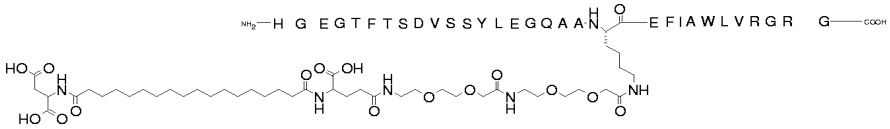


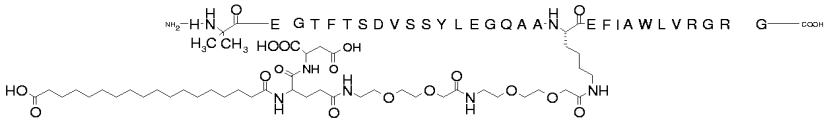


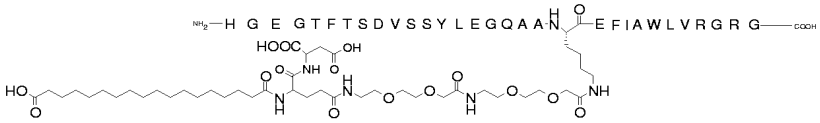












Pharmacodynamic study using db/db mice

In one aspect of this invention the GLP-1 agonists have a duration of action of at least 24hrs after dosing of 30nmol/kg to db/db mice

The efficacy and duration of action are measured in db/db mice.

Male db/db mice are shipped from Taconic, Denmark at the age of 8-10 weeks. From the time of arrival, the mice are housed under standard conditions but at 24 °C. The mice are kept 10 per cage until experimentation with free access to standard chow (Altromin,

Brogaarden APS., Denmark) and tap water at a normal day: light cycle (light on at 6 am). The mice are used for 1 experiment per week for 3 weeks. After this, the mice are euthanized.

After an acclimatisation period of 1 week, the blood glucose is measured by sampling from the tail tip capillary. In brief, 5 µl blood is sampled in heparinised glass capillary tubes and immediately suspended in 250 µl EBIO buffer solution (Eppendorf, Germany) in an 1.5 ml Eppendorf tube. The blood glucose concentration is measured by the glucose oxidase method at the EBIO Plus Auto analyser (Eppendorf, Germany).

The cut of value for blood glucose is 10 mM. When evaluating the mice, it is essential, that all 42 mice entering the experiment have blood glucose values above 10 mM, but also that the inter-mice variance is small. Therefore, if many mice are not severely diabetic, whereas some are, the start up of experiments should be postponed one week and new basal blood glucose measurements be made.

Based on the basal blood glucose values, the mice are allocated to 7 groups of n=6 with matching group mean blood glucose values.

On the day of testing the basal blood glucose morning values are assessed as described above and the basal body weight of each mouse is assessed. A time 0, the compound is dosed subcutaneously in the scruff of the neck (dosing volume app. 300 µl/50 g mouse).

The blood glucose values are followed up to 48 hours (time 1, 3, 6, 24 and 48 h) and the terminal body weight is assessed.

All data are entered into Graphpad Prism where mean blood glucose and mean delta body weights are calculated.

One aspect of this invention is to prepare GLP-1 analogues/derivatives with extended plasma half-lives that are suitable for once weekly administration. The pharmaco kinetic properties can be evaluated in mini pigs or domestic pigs as described below

Pharmacokinetic screening of once weekly GLP-1 analogues

Pharmacokinetic screening of GLP-1 analogues for identification of suitable once weekly candidates were performed on candidates that according to the project screenings plan were shown to be sufficiently potent with respect to glucose lowering potential in a diabetic mouse model (db/db mice) and subsequently had a time of duration of 48 hours or more in the db/db mouse model.

Primary screening

The first part of the pharmacokinetic screening consisted of a single dose subcutaneous administration of 2 nmol/kg to three minipigs weighing 8-12 kg. Blood samples were drawn from each animal at predose, 0.5, 1, 2, 4, 6, 8, 12, 24, 48, 72, 96 and 120 hours post-injection. All blood samples were stabilised with a special stabilisation buffer consisting of: EDTA (di-sodium) 0.18 M, Aprotenin 15000 KIE/ml, Val-Pyr 0.30 mM, pH adjusted to 7.4 in order to prevent enzymatic degradation of the GLP-1 analogues. Plasma was collected from each stabilised blood samples by centrifugation (4ºC, 10 min., 1270 G (4000 rpm), and analysed for the content of GLP-1 analogue by ELISA assays. Three different ELISA assays were used for the plasma analysis: "The "Total assay" using the antibody combination F1/Ra2135 detecting both the N-terminally intact 7-37GLP-1 molecule and the N-terminal enzymatically degraded 9-37GLP-1 molecule with a limit of detection (LOD) of 35 pM and a dynamic analytical range of 35-30000 pM. The "Intact assay" using the antibody combination F1/Mab26.1. This assay was detecting the N-terminally intact 7-37GLP-1 molecule only. The LOD was 35 pM and a dynamic analytical range of 35-30000 pM. The "Aib-intact assay" using the antibody combination F1/GLP162-3F15. This assay was detecting the Aib stabilised N-terminal of the GLP-1 molecule enabling detection of stabilised GLP-1 analogues. The LOD was 45 pM and the dynamic analytical range 45-30000 pM.

All plasma concentration-time profiles were analysed pharmacokinetically by a non-compartmental analysis. The following pharmacokinetic parameters were calculated if data permitted: t_{max}, C_{max}, AUC, AUC/Dose, $AUC_{\%Extrapol}$, λ_z, $t_{½}$, CL/F, V_z/F and MRT.

Secondary screening

A second part of the pharmacokinetic screening was conducted on those compounds with an initial terminal half-life of 60-70 hours or more. This screening consisted of a single dose intravenous and subcutaneous administration of 2 nmol/kg to six minipigs for each route of administration. The blood sampling schedule was extended from 0-120 hours to 0-432 and 0-504 hours after intravenous and subcutaneous administration respectively. This was done in order to increase the precision and accuracy of the pharmacokinetic parameter estimates, especially the terminal half-life, AUC and the derived parameters clearance and volume of distribution, and to estimate the bioavailability after subcutaneous administration.

GLP-1 (AIB8- INTACT) ASSAY

The assay was a two-site assay with simultaneous incubation of the analyte with catcher and detector antibody. A ready to use chemiluminescent substrate was used to maximize signal. The assay neither recognizes endogen GLP-1 (7-37) nor the DPPIV cleaved GLP-1 (9-37).

Reference plasma for GLP-1 assays

0-plasma was prepared from pooled EDTA plasma without Valine Pyrrolidide and Aprotinin from fasting animals. The pooled EDTA plasma was incubated at 37°C for 4 hours to remove traces of GLP-1 and after incubation Valine Pyrrolidide and Aprotinin were added.

Buffers

Coating buffer

PBS was used as coating buffer: 10mM sodium phosphate and 145mM sodium chloride adjusted to pH 7.4.

Washing buffer

PBS with 0.05% (v/v) Tween 20

Assay buffer

PBS with 0.05% (v/v) Tween 20, 10g/L BSA and 10mg/L anti-TNP.

Streptavidin buffer

Washing buffer with an additional 0.5M NaCl.

Substrate

Ready to use substrate SuperSignal ELISA Femto (Pierce, cat.no. 37075).

Standards

Standards were prepared from a 25 µM stock solution of 0113-0000-0217. The peptide was serially diluted into reference plasma to make standards with final concentrations of 30000-10000-3333-1111-370-123-41 and 0 pM. Standards were stored in Micronic tubes in 100µL aliquots at -20°C.

Assay procedure

Crystal 2000 Microplates (black) were coated with monoclonal antibody GLPb1-7F1, 100µL of 5 µg/mL in PBS overnight at 4°C.

Plates were washed 5 times with washing buffer in an automated plate washer (SkanWasher, Skatron) and allowed to stand for at least 30min. with washing buffer to block remaining sites.

20µL of sample or standard was added to each well in duplicate immediately followed by 100µL GLP162-3F15 biotinylated, 1µg/mL in assay buffer. Plates were incubated for 2 hours at room temperature on a plate shaker followed by 5 wash cycles as previously described.

100µL of streptavidin-peroxidase solution (KPL, code 14-30-00, 1:20000 in streptavidin buffer) was added to each well and incubated for 1 hour at room temperature on a plate shaker. Plates were washed as previously described and after emptying 100µL of SuperSignal femto was added. Plates were put on a shaker for 1 minute and measured in Orion Luminometer (Berthold). Data were transferred to MultiCalc and standard curves

calculated using the weighted 4PL method. Sample concentrations were calculated from the standard curve.

GLP-1 (TOTAL) ASSAY

The assay was a two-site assay with simultaneous incubation of the analyte with catcher and detector antibody. The assay recognizes N-terminally cleaved GLP-1 up to GLP-1(12-37).

Buffers

Coating buffer

PBS was used as coating buffer: 10mM sodium phosphate and 145mM sodium chloride adjusted to pH 7.4.

Washing buffer

PBS with 0.05% (v/v) Tween 20

Assay buffer

PBS with 0.05% (v/v) Tween 20, 10g/L BSA and 10mg/L anti-TNP.

Streptavidin buffer

Washing buffer with an additional 0.5M NaCl.

Substrate

Ready-to-use substrate TMB (KemEnTec code 4380A)

Stop buffer

4 M H_3PO_4

Standards

Standards were prepared from a 25 µM stock solution of 0113-0000-0217. The peptide was serially diluted into reference plasma to make standards with final concentrations of 30000-10000-3333-1111-370-123-41 and 0 pM. Standards were stored in Micronic tubes in 100µL aliquots at -20°C.

Assay procedure

Maxisorp microtiter plates (NUNC) were coated with monoclonal antibody GLPb1-7F1, 100µL of 5 µg/mL in PBS overnight at 4°C.

Plates were washed 5 times with washing buffer in an automated plate washer (SkanWasher, Skatron) and allowed to stand for at least 30min. with washing buffer to block remaining sites.

20µL of sample or standard was added to each well immediately followed by 100µL Ra2135-biotinylated, 1µg/mL in assay buffer. Plates were incubated for 2 hours at room temperature on a plate shaker followed by 5 wash cycles as previously described.

100µL of streptavidin-peroxidase solution (Amersham Bioscinces code RPN4401V, 1:8000 in assay buffer) was added to each well and incubated for 1 hour at room temperature on a

plate shaker. Plates were washed as previously described and after emptying 100µL of TMB was added and after 5 minutes stopped with 100 µL H_3PO_4 .
Plates were measured in Victor Multilabel Reader (Wallac). Data were transferred to MultiCalc and standard curves calculated using the weighted 4PL method. Sample concentrations were calculated from the standard curve.

The in-life experimental procedures, plasma analysis and pharmacokinetic analysis were identical to that described under the primary screening.

Pharmaceutical formulation:

A compound of the invention may be formulated as:

Compound of example 4	6,25 mg/ml
Propyleneglycol	14,0 mg/ml
Phenol	5.5 mg/ml
Phosphate Buffer pH 8.15	

Optionally the compound is treated with heat and/or base before formulation as described in PCT/ EP2005/055946.

Claims

1. A GLP-1 analog having a modification of at least one non-proteogenic amino acid residue in positions 7 and/or 8 relative to the sequence GLP-1(7-37) (SEQ ID No 1), which is acylated with a moiety to the lysine residue in position 26, and where said moiety comprises at least two acidic groups, wherein one acidic group is attached terminally.

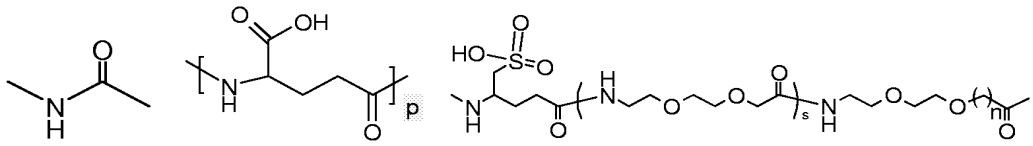
2. A GLP-1 analog according to claim 1, wherein the moiety attached in position 26 comprises a hydrophilic linker.

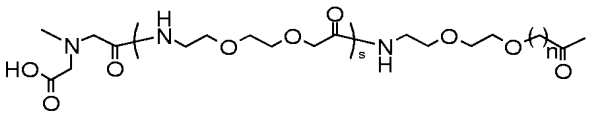
3. A GLP-1 analog according to claim 2, wherein the hydrophilic linker comprises at least 5 non-hydrogen atoms where 30-50% of these are either N or O.

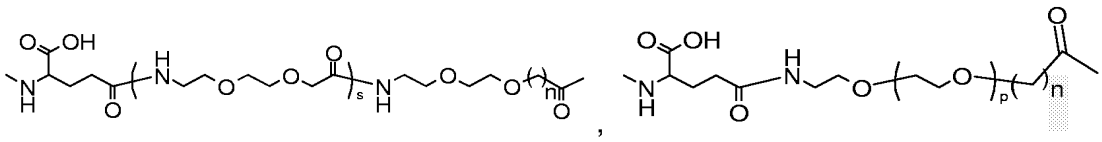
4. A GLP-1 analog according to any of the above claims, wherein the moiety attached in position 26 comprises an albumin binding moiety seperated from the peptide by the hydrophilic linker.

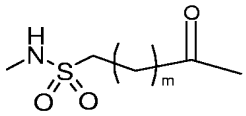
5. A GLP-1 analog according to claim 4 wherein the albumin binding moiety is a linear or branched lipophilic moiety containing 4-40 carbon atoms having a distal acidic group.

6. A GLP-1 analog according to any of the above claims, wherein the acylated moiety is B-U', where U' is selected from





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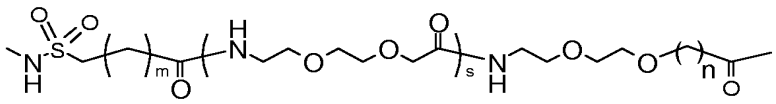
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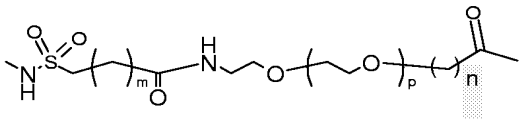

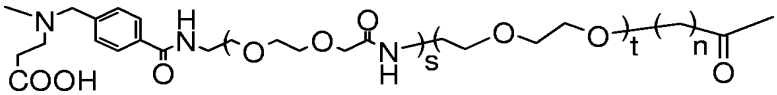
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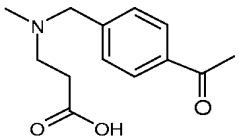
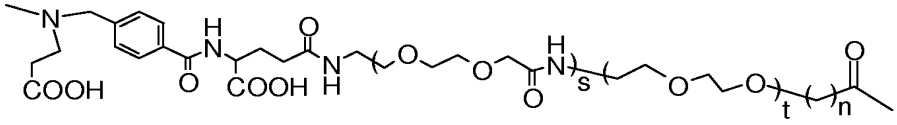
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m is 0, 1, 2, 3, 4, 5, or 6,

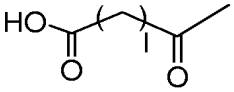
n is 1, 2 or 3

s is 0, 1, 2, or 3,

t is 0, 1, 2, 3, or 4

p is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17,18, 19, 20, 21, 22, or 23;

and where B is an acidic group selected from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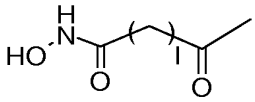
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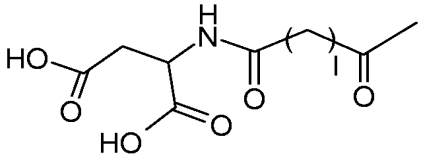
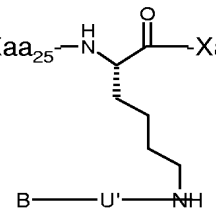


where l is 12, 13, 14, 15, 16, 17, 18, 19 or 20

7. A GLP-1 analog which is a compound of formula I (SEQ ID No. 2) :

Xaa_7-Xaa_8-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Xaa_{16}-Ser-Xaa_{18}-Xaa_{19}-Xaa_{20}-Glu-Xaa_{22}-

Xaa_{23}-Ala-Xaa_{25}-  -Xaa_{27}-Phe-Ile-Xaa_{30}-Trp-Leu-Xaa_{33}-Xaa_{34}-Xaa_{35}-Xaa_{36}-Xaa_{37}

Formula I

wherein

Xaa_7 is L-histidine, imidazopropionyl, α-hydroxy-histidine, D-histidine, desamino-histidine, 2-amino-histidine, β-hydroxy-histidine, homohistidine, N^{α}-acetyl-histidine, N^{α}-formyl-histidine, α-

fluoromethyl-histidine, α-methyl-histidine, 3-pyridylalanine, 2-pyridylalanine or 4-pyridylalanine

Xaa_8 is Ala, Gly, Val, Leu, Ile, Thr, Ser, Lys, Aib, (1-aminocyclopropyl) carboxylic acid, (1-aminocyclobutyl) carboxylic acid, (1-aminocyclopentyl) carboxylic acid, (1-aminocyclohexyl) carboxylic acid, (1-aminocycloheptyl) carboxylic acid, or (1-aminocyclooctyl) carboxylic acid;

Xaa_{16} is Val or Leu;

Xaa_{18} is Ser, Lys or Arg;

Xaa_{19} is Tyr or Gln;

Xaa_{20} is Leu or Met;

Xaa_{22} is Gly, Glu or Aib;

Xaa_{23} is Gln, Glu, Lys or Arg;

Xaa_{25} is Ala or Val;

Xaa_{27} is Glu or Leu;

Xaa_{30} is Ala, Glu or Arg;

Xaa_{33} is Val or Lys;

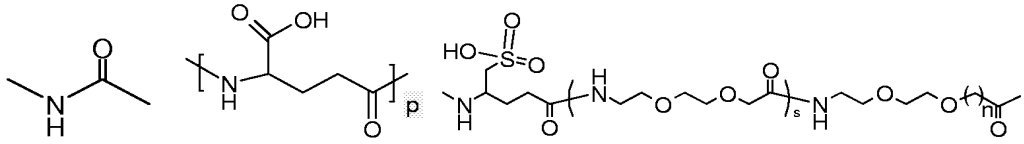
Xaa_{34} is Lys, Glu, Asn or Arg;

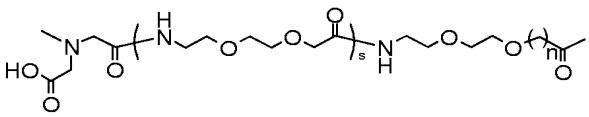
Xaa_{35} is Gly or Aib;

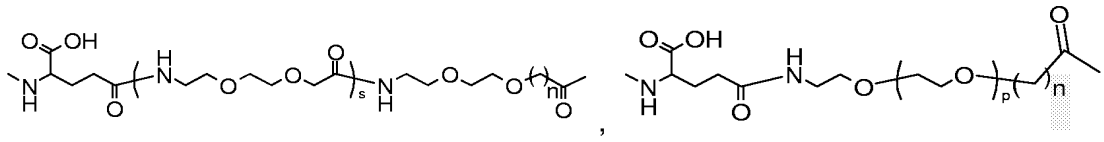
Xaa_{36} is Arg, Gly or Lys, or is absent;

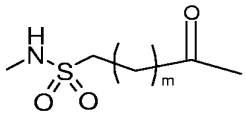
Xaa_{37} is Gly, Ala, Glu, Pro, Lys, or is absent;

and B and U' together is the acylated moiety, where U' is selected from





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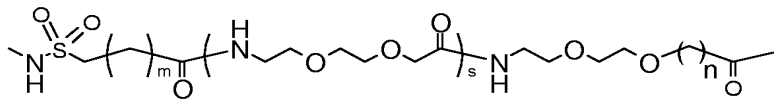
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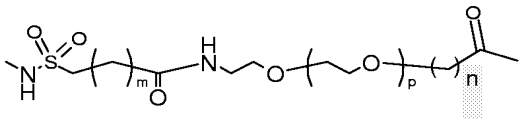

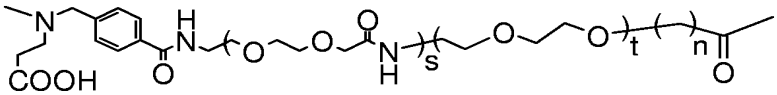
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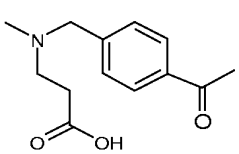
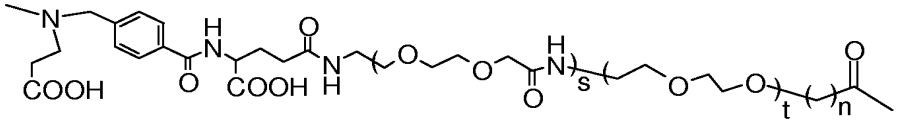
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m is 0, 1, 2, 3, 4, 5, or 6,

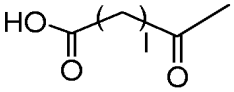
n is 1, 2 or 3

s is 0, 1, 2, or 3,

t is 0, 1, 2, 3, or 4

p is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17,18, 19, 20, 21, 22, or 23;

and where B is an acidic group selected from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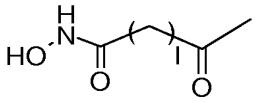
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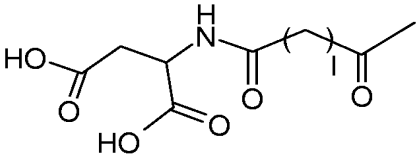
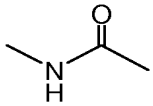


where l is 12, 13, 14, 15, 16, 17, 18, 19 or 20;

8. A GLP-1 analog according to claims 6-7, wherein U' is selected from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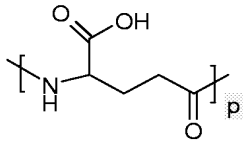
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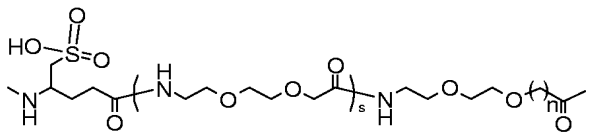

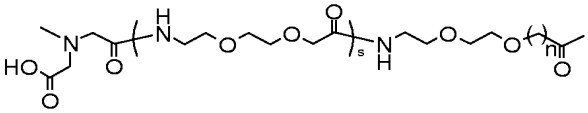

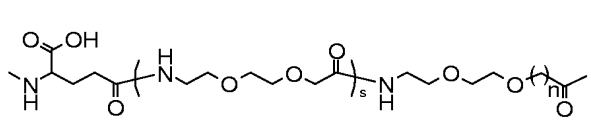
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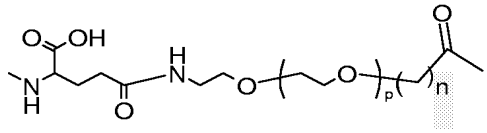

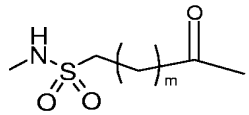
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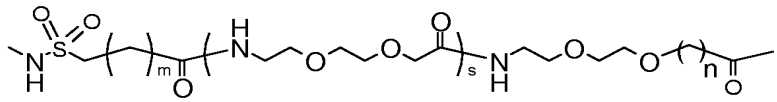
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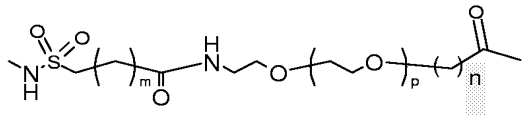

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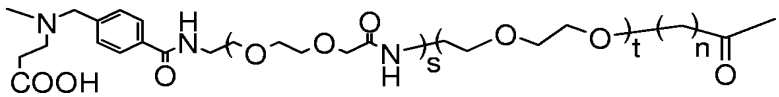
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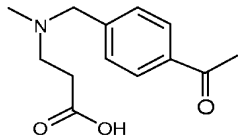
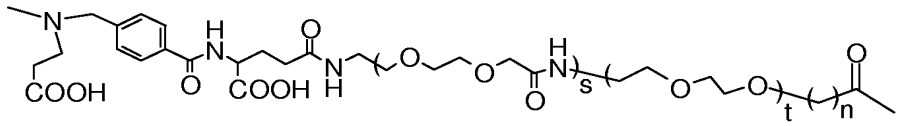
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m is 2, 3, 4 or 5,

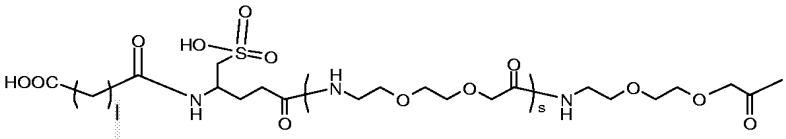
n is 1 or 2

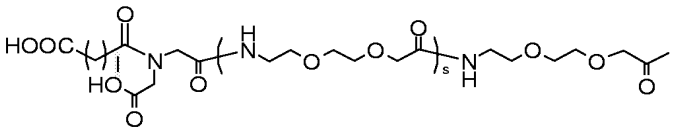
s is 0, 1, or 2,

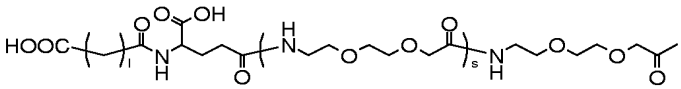
t is 0, 1, 2, or 3

p is 1, 2, 3, 4, 7, 11 or 23

9. A GLP-1 analog according to claims 6-8 wherein B-U'- is



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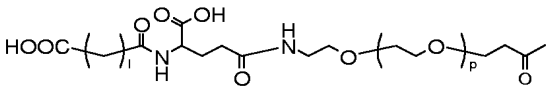

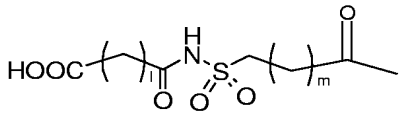
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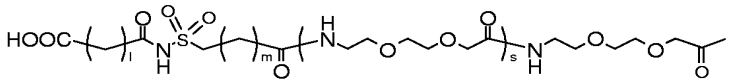
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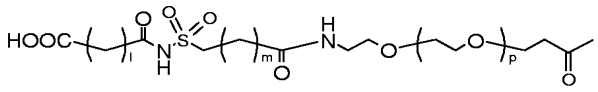
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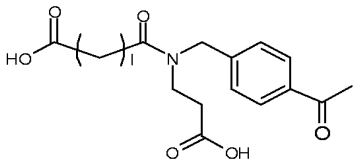
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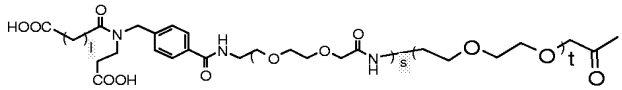
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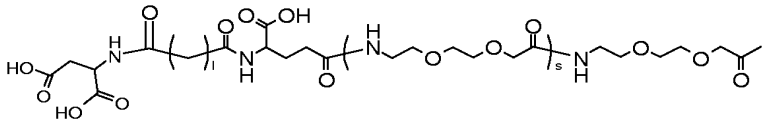
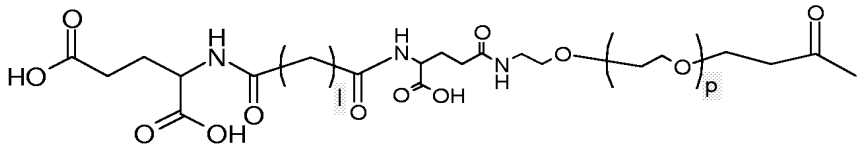



where l is 14, 15, 16, 17, 18, 19 or 20;
p is 1, 2, 3, 4, 7, 8 , 9, 10, 11 or 12.
s is 0, 1 or 2
t is 0 or 1;
m is 2, 3 or 4;

10. A GLP-1 analog according to claim 9, wherein
where l is 14, 15, 16, 17 or 18
p is 1, 2, 3, 4 or 11;
s is 0, 1 or 2;
t is 0 or 1;

11. A GLP-1 analog according to any of the claims 6-10, wherein s is 1.

12. A GLP-1 analog according any of the claims 6-10 wherein l is 16

13. A GLP-1 analog according to any of the claims 6-10 wherein p is 3 or 4.

14. A GLP-1 analog according to any of the claims 6-10 wherein n is 1.

15. A GLP-1 analog according to any of the claims 7-14, wherein
Xaa_7 is His or desamino-histidine;
Xaa_8 is Ala, Gly, Val, Leu, Ile, Lys or Aib;
Xaa_{16} is Val;
Xaa_{18} is Ser;
Xaa_{19} is Tyr;
Xaa_{20} is Leu;
Xaa_{22} is Gly, Glu or Aib;
Xaa_{23} is Gln or Glu;
Xaa_{25} is Ala;
Xaa_{27} is Glu;
Xaa_{30} is Ala or Glu;
Xaa_{33} is Val;
Xaa_{34} is Lys or Arg;
Xaa_{35} is Gly or Aib;
Xaa_{36} is Arg or Lys
Xaa_{37} is Gly, amide or is absent;

16. A GLP-1 analog according to claim 15, wherein
Xaa_7 is His
Xaa_8 is Gly, or Aib;
Xaa_{16} is Val;
Xaa_{18} is Ser;
Xaa_{19} is Tyr;
Xaa_{20} is Leu;
Xaa_{22} is Glu or Aib;
Xaa_{23} is Gln;
Xaa_{25} is Ala;
Xaa_{27} is Glu;
Xaa_{30} is Ala;
Xaa_{33} is Val;
Xaa_{34} is Lys or Arg;
Xaa_{35} is Gly or Aib;
Xaa_{36} is Arg

Xaa_{37} is Gly

17. A GLP-1 analog according to any one of the claims 1-15, wherein said GLP-1 analog comprises a modification of the N-terminal L-histidine in position 7 of the GLP-1(7-37) sequence.

18. A GLP-1 analog according to claim 17, wherein said GLP-1 analog comprises imidazopropionyl^7, α-hydroxy-histidine^7 or N-methyl-histidine^7, D-histidine^7, desamino-histidine^7, 2-amino-histidine^7, β-hydroxy-histidine^7, homohistidine^7, N^{α}-acetyl-histidine^7, α-fluoromethyl-histidine^7, α-methyl-histidine^7, 3-pyridylalanine^7, 2-pyridylalanine^7 or 4-pyridylalanine^7.

19. A GLP-1 analog according to any one of the claims 1-18, wherein said GLP-1 analog comprises a substitution of the L-alanine in position 8 of the GLP-1(7-37) sequence for another amino acid residue.

20. A GLP-1 analog according to claim 19, wherein said GLP-1 analog comprises Aib^8, Gly^8, Val^8, Ile^8, Leu^8, Ser^8, Thr^8, (1-aminocyclopropyl) carboxylic acid, (1-aminocyclobutyl) carboxylic acid, (1-aminocyclopentyl) carboxylic acid, (1-aminocyclohexyl) carboxylic acid, (1-aminocycloheptyl) carboxylic acid, or (1-aminocyclooctyl) carboxylic acid.

21. A GLP-1 analog according to claim 20, wherein said GLP-1 analog comprises Aib^8;

22. A GLP-1 analog according to any one of the preceding claims, wherein said GLP-1 analog comprises no more than fifteen amino acid residues which have been exchanged, added or deleted as compared to GLP-1(7-37) (SEQ ID No. 1),

23. A GLP-1 analog according to claim 22, wherein no more than ten amino acid residues which have been exchanged, added or deleted as compared to GLP-1(7-37) (SEQ ID No. 1).

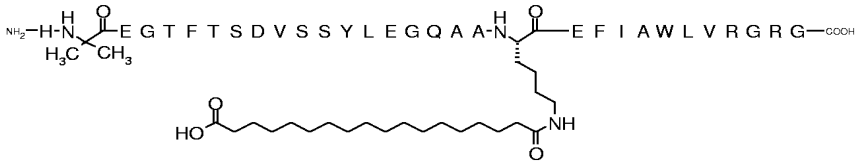
24. A GLP-1 analog according to claim 23, wherein said GLP-1 analog comprises no more than six amino acid residues which have been exchanged, added or deleted as compared to GLP-1(7-37) (SEQ ID No. 1).

25. A GLP-1 analog according to any of the above claims, wherein said GLP-1 analog comprises no more than 3 amino acid residues which are not encoded by the genetic code.

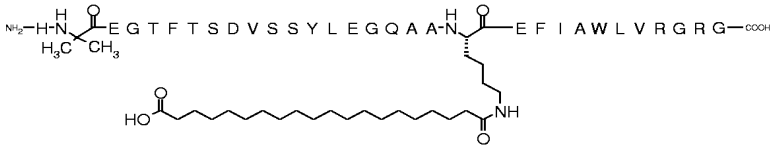
26. A GLP-1 analog according to any one of the preceding claims, wherein said GLP-1 analog comprises only one lysine residue.

27. A GLP-1 analog according to any of the above claims, which is
Aib^{8},Arg^{34}-GLP-1(7-37)
Aib^{8,22},Arg^{34}-GLP-1(7-37).
Arg^{34}-GLP-1(7-37).
[3-(4-Imidazolyl)Propionyl7,Arg34]GLP-1-(7-37)peptide
Gly^{8},Arg^{34}-GLP-1(7-37)
Aib^{8},Arg^{34}, Pro^{37}-GLP-1(7-37)
Aib^{8,22,27,30,35},Arg^{34},Pro^{37}- GLP-1 (7-37)amide,
all of which are substituted by B-U' in position 26.

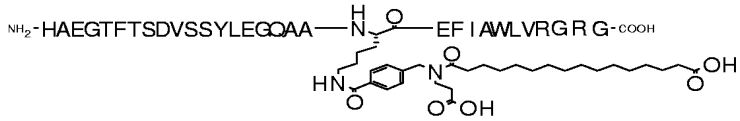
28. A compound according to any one of the preceding claims, which is selected from



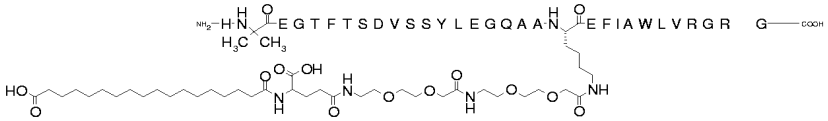
N-ε^{26}--(17-carboxyheptadecanoyl)-[Aib8,Arg34]GLP-1-(7-37)-peptide,



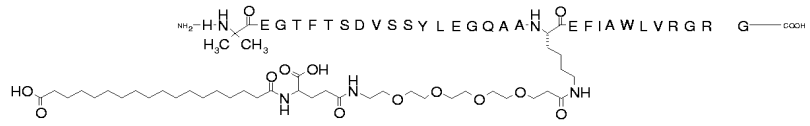
N-ε^{26}-(19-carboxynonadecanoyl)-[Aib8,Arg34]GLP-1-(7-37)-peptide,



N-ε^{26}- (4-{[N-(2-carboxyethyl)-N-(15-carboxypentadecanoyl)amino]methyl}benzoyl)[Arg34]GLP-1-(7-37),



N-ε^{26}-[2-(2-[2-(2-[2-(2-[4-(17-Carboxyheptadecanoylamino)-4(S)-carboxybutyrylamino]ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl][Aib8,Arg34]GLP-1-(7-37)peptide,



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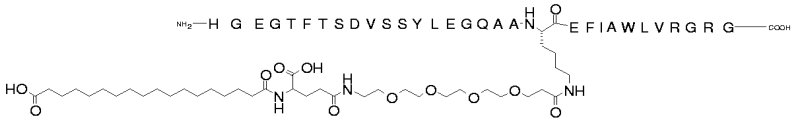

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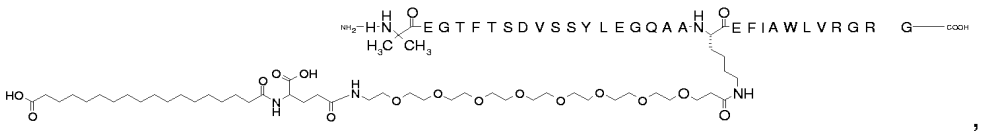

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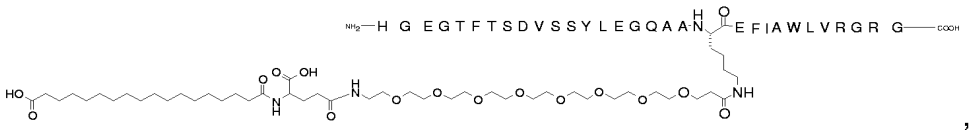

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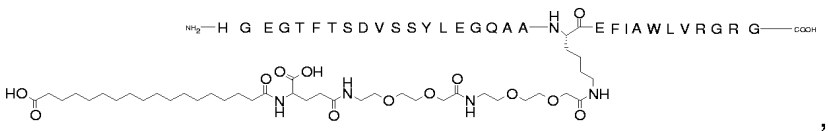

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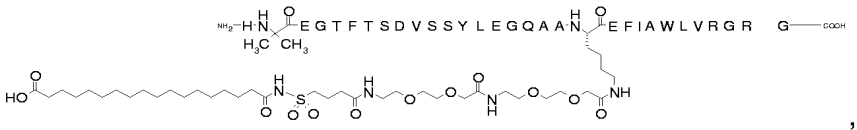

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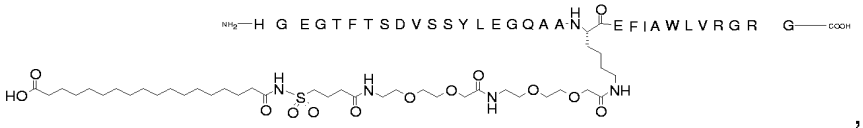

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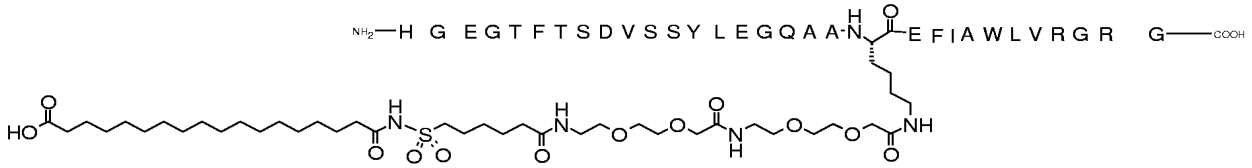

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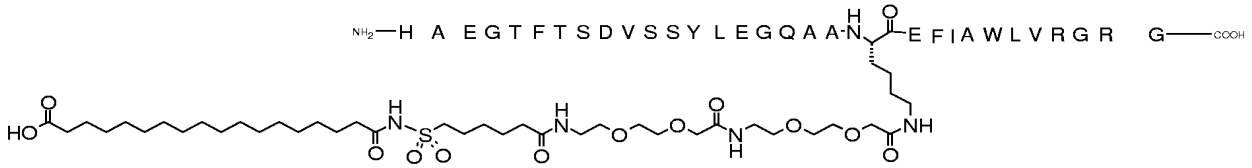

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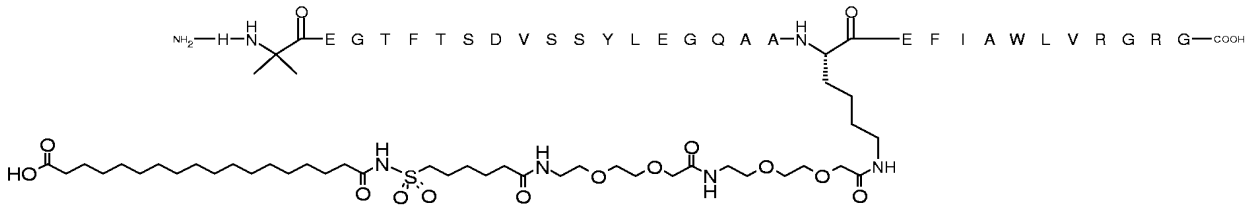

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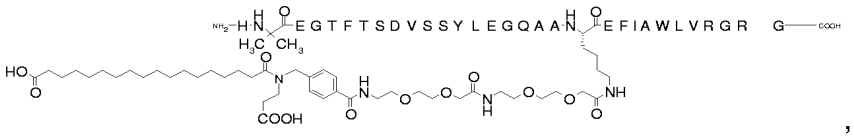

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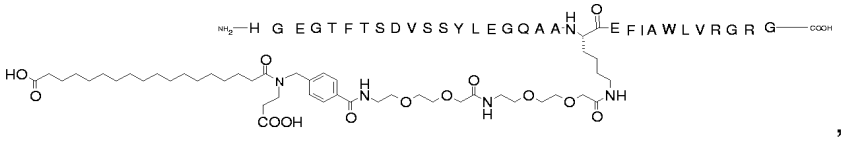

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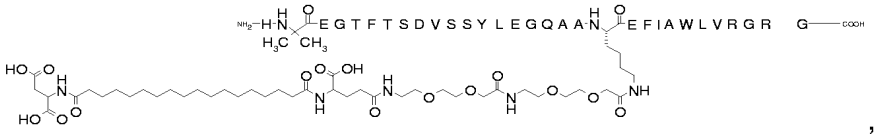

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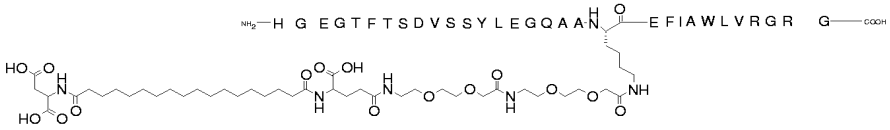

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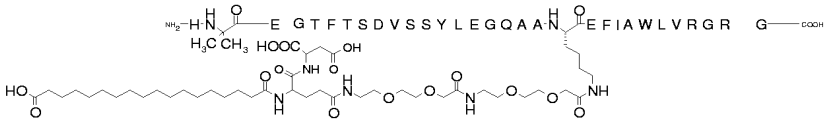

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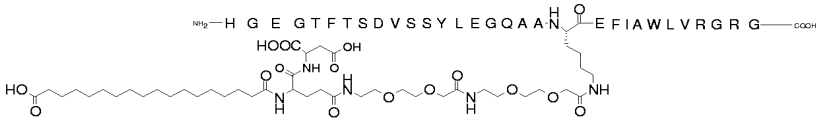

29. A method for increasing the time of action in a patient of a GLP-1 analog, characterised in acylating said GLP-1 analog with a moiety B-U' as disclosed in any of the preceding claims, on the lysine residue in position 26 of said GLP-1 analog.

30. A method for increasing the time of action in a patient of a GLP-1 analog to more than about 40 hours, characterised in modifying at least one of the amino acid residues in positions 7 and 8 of a GLP-1(7-37) peptide or an analog thereof, and acylating said GLP-1 analog with a moiety B-U'- as disclosed in any of the preceding claims on the lysine residue in position 26 of said GLP-1 analog.

31. A pharmaceutical composition comprising a compound according to any one of claims 1-28, and a pharmaceutically acceptable excipient.

32. The pharmaceutical composition according to claim 31, which is suited for parenteral administration.

33. Use of a compound according to any one of the claims 1-28 for the preparation of a medicament.

34. Use of a compound according to any one of the claims 1-28 for the preparation of a medicament for the treatment or prevention of hyperglycemia, type 2 diabetes, impaired glucose tolerance, type 1 diabetes, obesity, hypertension, syndrome X, dyslipidemia, cognitive disorders, atheroschlerosis, myocardial infarction, coronary heart disease and other cardiovascular disorders, stroke, inflammatory bowel syndrome, dyspepsia and gastric ulcers.

35. Use of a compound according to any one of the claims 1-28 for the preparation of a medicament for delaying or preventing disease progression in type 2 diabetes.

36. Use of a compound according to any one of the claims 1-28 for the preparation of a medicament for decreasing food intake, decreasing β-cell apoptosis, increasing β-cell function and β-cell mass, and/or for restoring glucose sensitivity to β-cells.

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(74) Common Representative: **NOVO NORDISK A/S**; Corporate Patents, Novo Allé, DK-2880 Bagsværd (DK).

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(54) Title: ACYLATED GLP-1 COMPOUNDS

(57) Abstract: Protracted GLP-1 compounds and therapeutic uses thereof.