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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
18/154,998	01/16/2023	Sang Jeon CHUNG	18896-000072-US-COA	5143

28997 7590 02/13/2024

Harness Dickey (St. Louis)
7700 Bonhomme, Suite 400
St. Louis, MO 63105

EXAMINER
XIAO, YAN

ART UNIT	PAPER NUMBER
1642	

NOTIFICATION DATE	DELIVERY MODE
02/13/2024	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):

bkamer@hdp.com
stldocket@hdp.com

Exhibit 1035
AJINOMOTO CO., INC.
IPR2025-00283

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APPLICATION NO.	ISSUE DATE	PATENT NO.
18/154,998	13-Feb-2024	11896675

Harness Dickey (St. Louis)
7700 Bonhomme, Suite 400
St. Louis, MO 63105

EGRANT NOTIFICATION

Your electronic patent grant (eGrant) is now available, which can be accessed via Patent Center at https://patentcenter.uspto.gov

The electronic patent grant is the official patent grant under 35 U.S.C. 153. For more information, please visit https://www.uspto.gov/electronicgrants

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APPLICATION NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
18/154,998	02/13/2024	11896675	18896-000072-US-COA	5143

28997 7590 01/24/2024

Harness Dickey (St. Louis)
7700 Bonhomme, Suite 400
St. Louis, MO 63105

ISSUE NOTIFICATION

The projected patent number and issue date are specified above. The patent will issue electronically. The electronically issued patent is the official patent grant pursuant to 35 U.S.C. § 153. The patent may be accessed on or after the issue date through Patent Center at https://patentcenter.uspto.gov/. The patent will be available in both the public and the private sides of Patent Center. Further assistance in electronically accessing the patent, or about Patent Center, is available by calling the Patent Electronic Business Center at 1-888-217-9197.

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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 0 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 Center (https://patentcenter.uspto.gov).

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INVENTOR(s) (Please see PATENT CENTER site https://patentcenter.uspto.gov for additional inventors):

Sang Jeon CHUNG, Gyeonggi-do, KOREA, REPUBLIC OF;
Ju Hwan KIM, Gyeonggi-do, KOREA, REPUBLIC OF;
Young Geun LEE, Gyeonggi-do, KOREA, REPUBLIC OF;
Tae Jin LEE, Gyeonggi-do, KOREA, REPUBLIC OF;
Jin Woo SEO, Gyeonggi-do, KOREA, REPUBLIC OF;

APPLICANT(s) (Please see PATENT CENTER site https://patentcenter.uspto.gov for additional applicants):

AbTis Co., Ltd., Gyeonggi-do, KOREA, REPUBLIC OF;

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ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
18/154,998	**01/10/2024 02:40:50 PM Z ET**	**18896-000072-US-COA**

Title of Invention

SITE-SPECIFIC ANTIBODY CONJUGATION AND ANTIBODY-DRUG CONJUGATE AS SPECIFIC EMBODIMENT THEREOF

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	5143	FILED BY	ERICA IMERGOOT
PATENT CENTER #	63904906	FILING DATE	01/16/2023
CUSTOMER #	28997	FIRST NAMED INVENTOR	Sang Jeon CHUNG
CORRESPONDENCE ADDRESS	-	AUTHORIZED BY	Kisuk Lee

Documents

TOTAL DOCUMENTS: 4

DOCUMENT		PAGES	DESCRIPTION	SIZE (KB)
000072uscoa_2ndReplacementDrawings.pdf		73	Drawings-other than black and white line drawings	13074 KB
000072uscoa_ResponsetoNoticetoFileCorrectedApplicationPapers.pdf		3	-	19 KB
000072uscoa_ResponsetoNoticetoFileCorrectedApplicationPapers-A.NA.pdf	(1-1)	1	Amendment after Notice of Allowance (Rule 312)	13 KB
000072uscoa_ResponsetoNoticetoFileCorrectedApplicationPapers-DRW.pdf	(2-2)	1	Drawings-only black and white line drawings	11 KB

000072uscoa_ResponsetoNoticetoFileCorrectedApplicationPapers-REM.pdf	(3-3)	1	Applicant Arguments/Remarks Made in an Amendment	13 KB

Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)
000072uscoa_2ndReplacementDrawings.pdf	60F1736913137F9502D349CE1CE5C80AAC970D8D67DE69C1C7783F7A8561B12FD00C2110903883B3B6DD6A4CE1868EFBBB29A699A7919960 6175EBB6EDCB083C
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New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application No.:	18/154,998
Filing Date:	16 January 2023
First Named Inventor:	Sang Jeon CHUNG
Group Art Unit:	1642
Examiner:	XIAO, YAN
Confirmation No.:	5143
Title:	SITE-SPECIFIC ANTIBODY CONJUGATION AND ANTIBODY-DRUG CONJUGATE AS SPECIFIC EMBODIMENT THEREOF
Attorney Docket:	18896-000072-US-COA

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

RESPONSE TO NOTICE TO FILE CORRECTED APPLICATION PAPERS

This paper is in response to the Notice to File Corrected Application Papers, dated 15 December 2023 in the above referenced application, in which a two-month shortened statutory period was set for reply. This response is timely and no fee for extension of time is believed payable.

* * *

Amendments to the Drawings begin on page **2** of this paper.

Remarks begin on page **3** of this paper.

amino acid sequence :

G—P—S—V—F—L—F—P—P—K—P—K—D—T—L—M—I

EU numbering for heavy chain :

237 238 239 240 241 242 243 244 245 246 247 248 249 250 251 252 253

FIG. 1

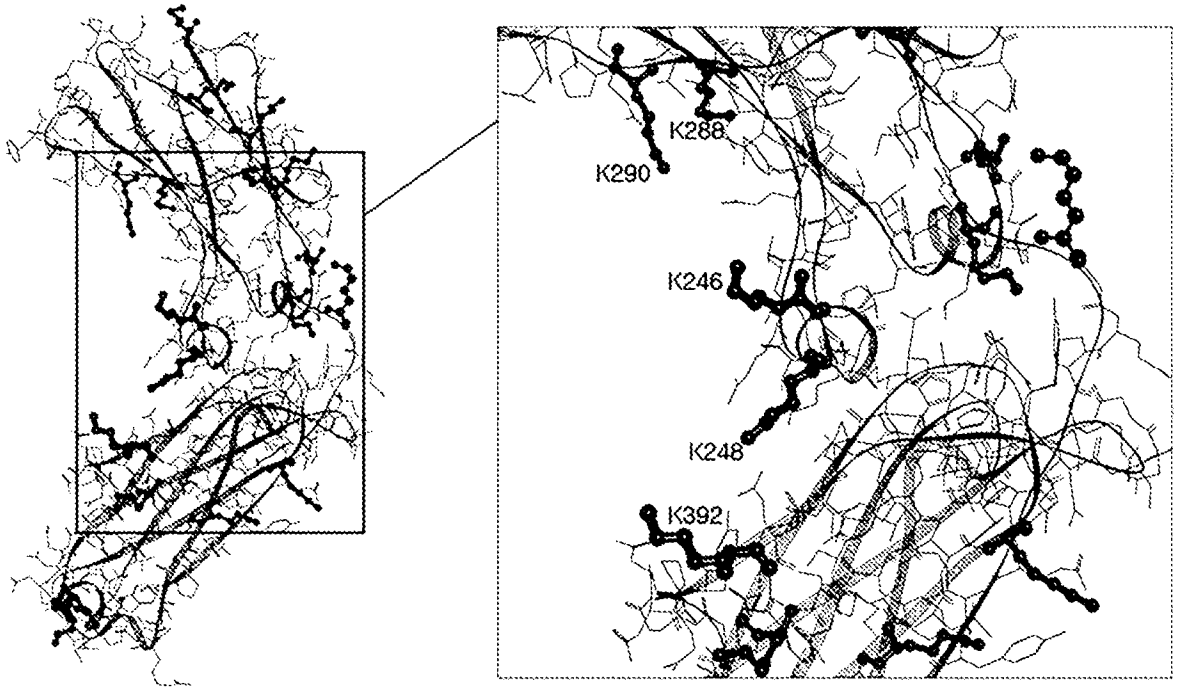


FIG. 2

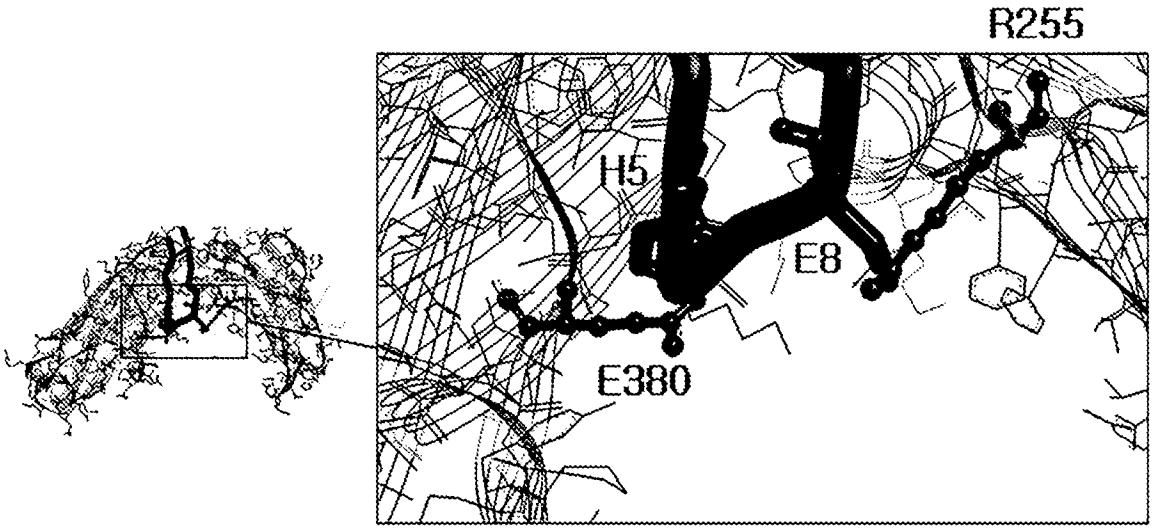


FIG. 3

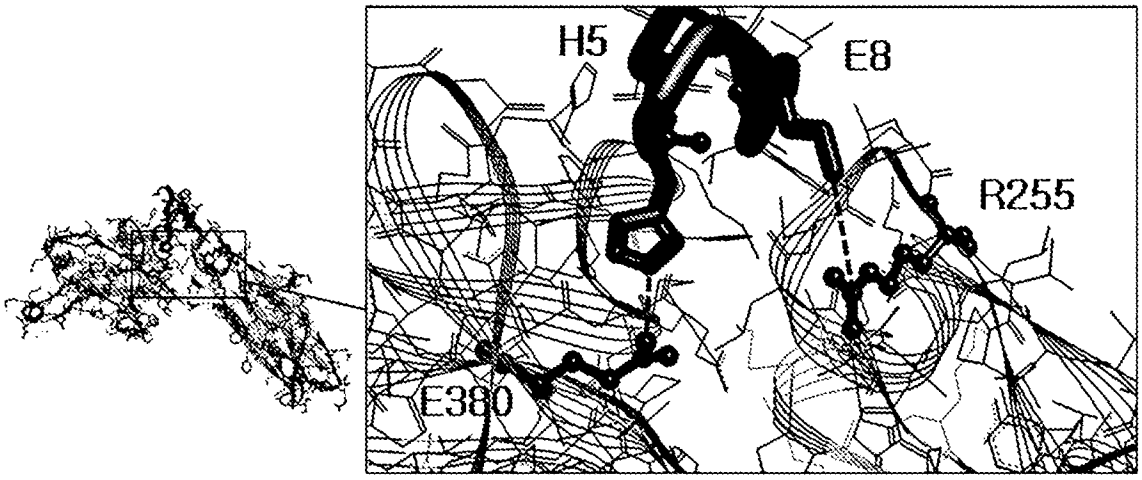


FIG. 4

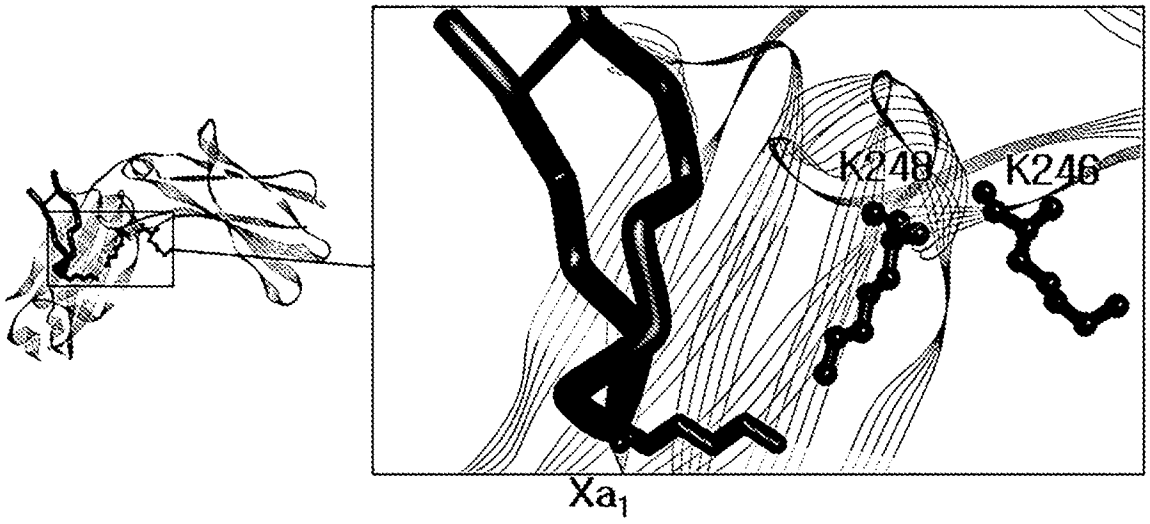


FIG. 5

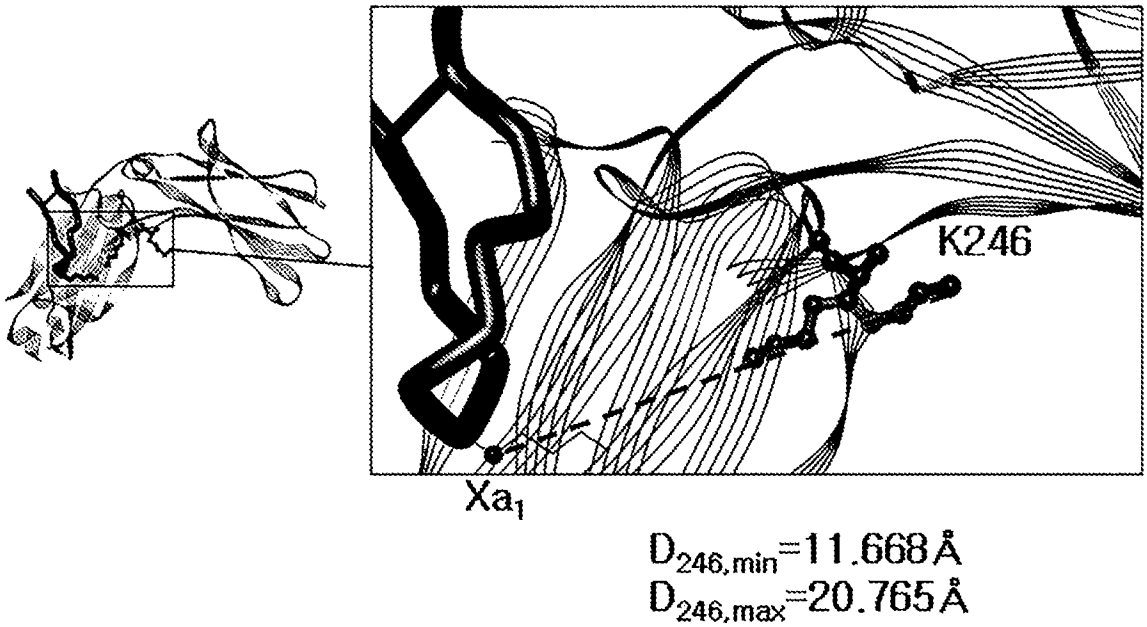


FIG. 6

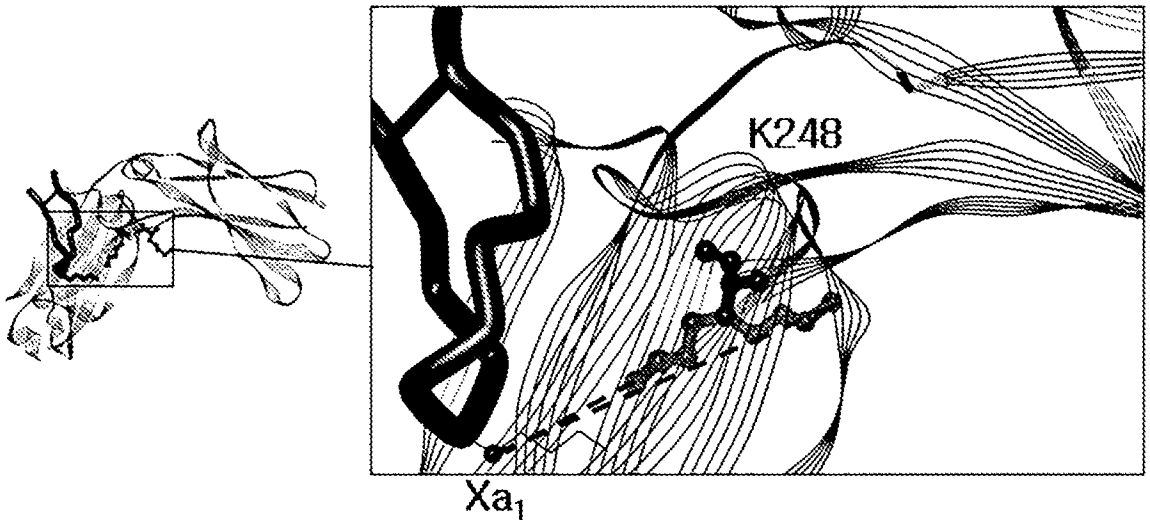


FIG. 7

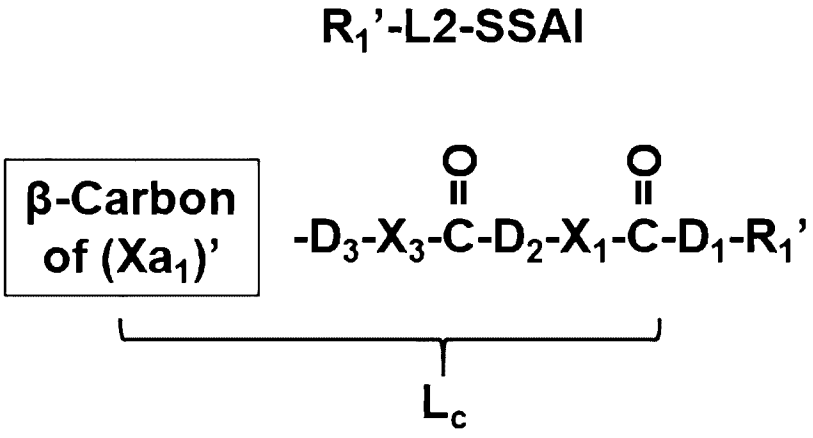


FIG. 8

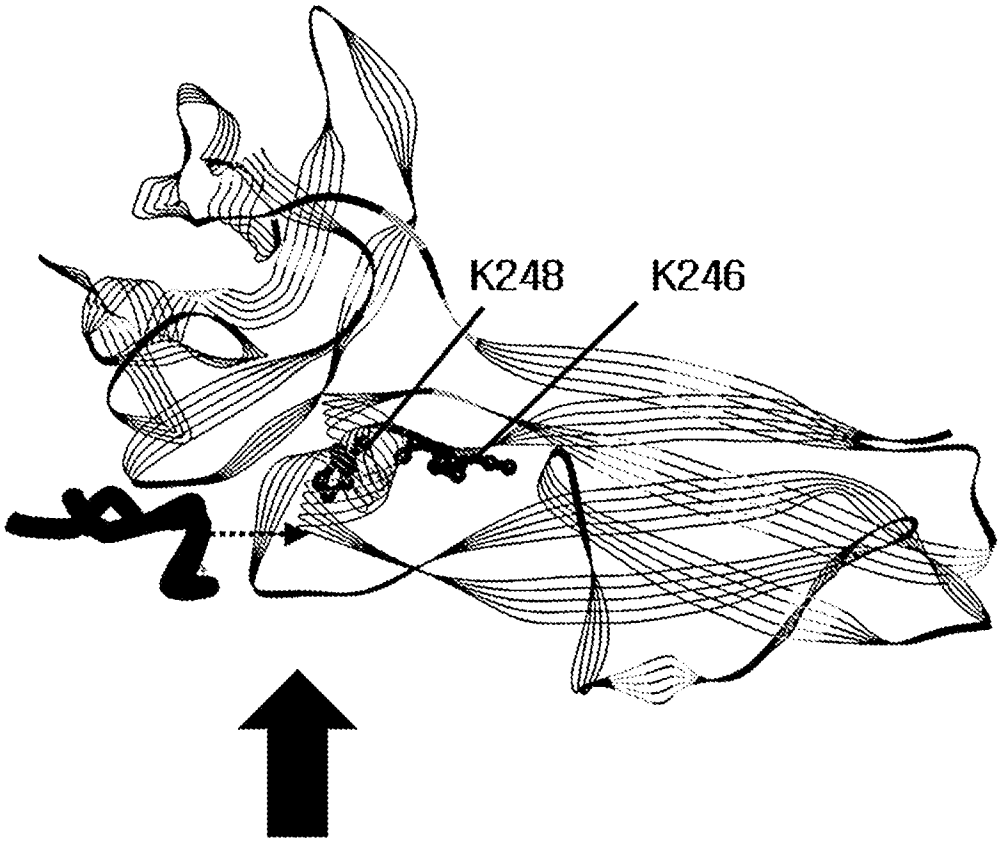


FIG. 9

If L_c is shorter than $D_{246,min}$

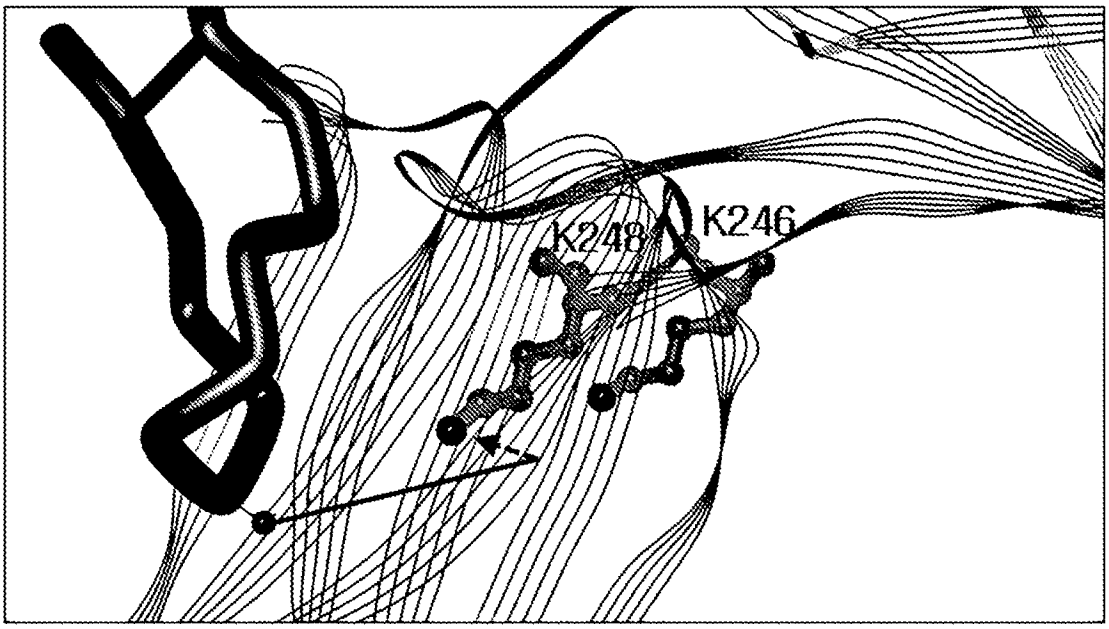


FIG. 10

If L_c is longer than $D_{248,max}$



FIG. 11

If L_c is between about $D_{246,min}$ and about $D_{248,max}$

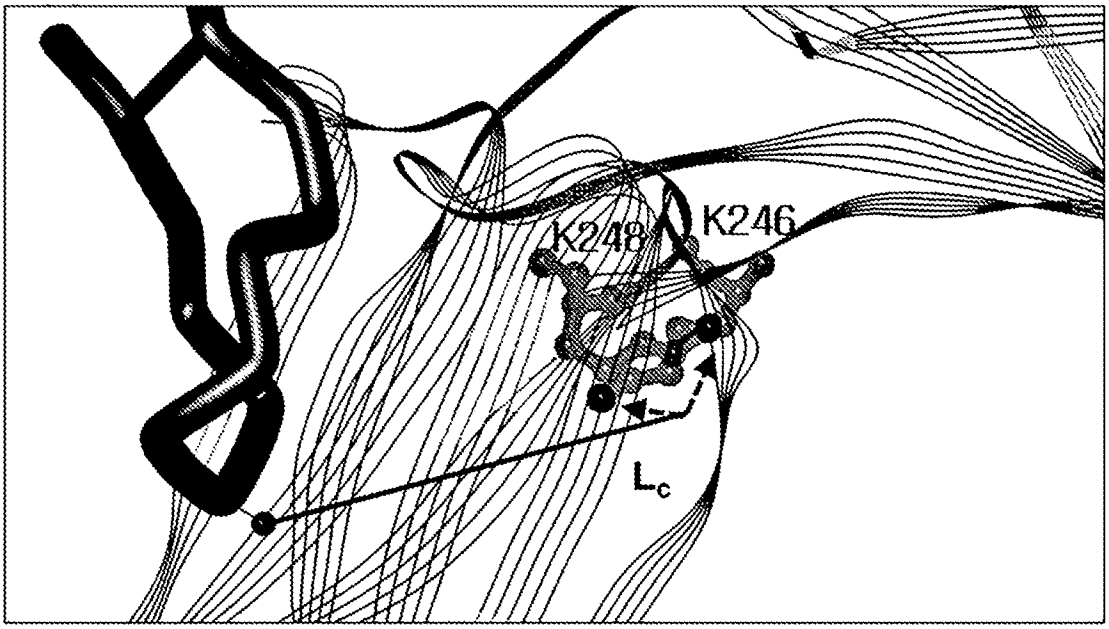


FIG. 12

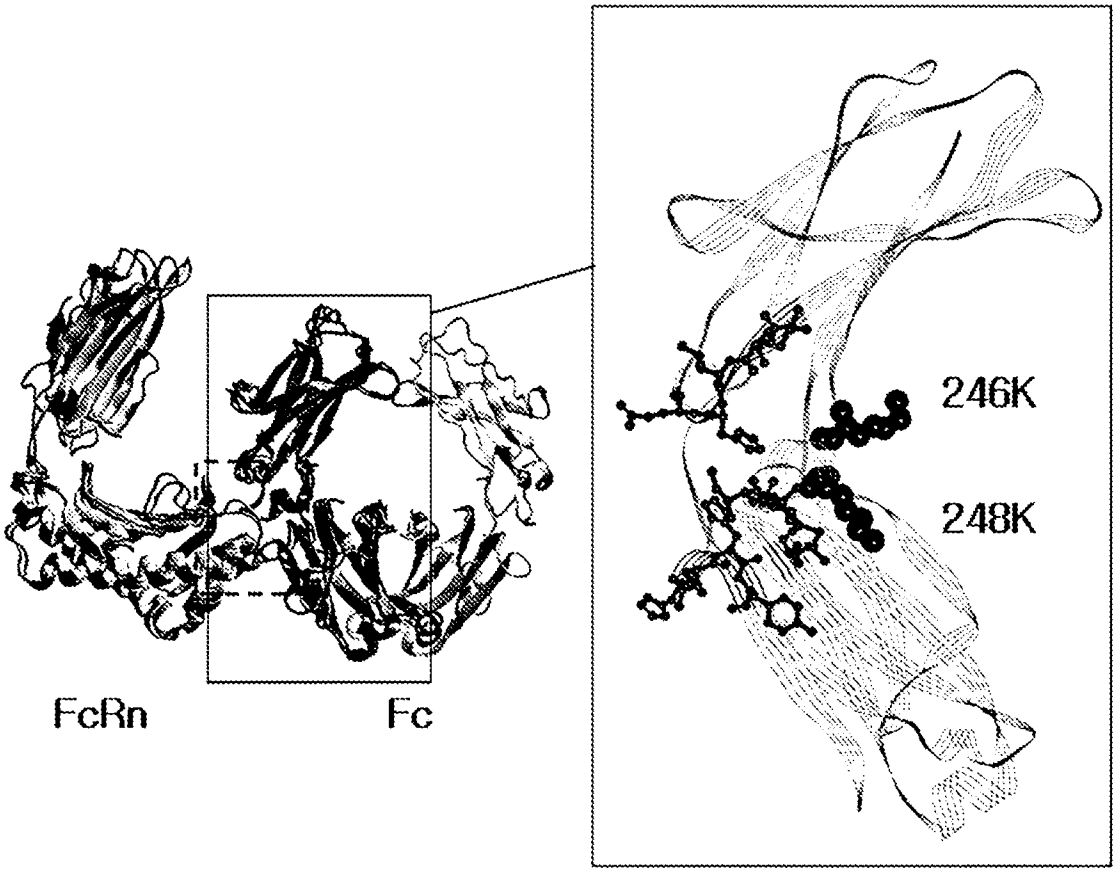


FIG. 13

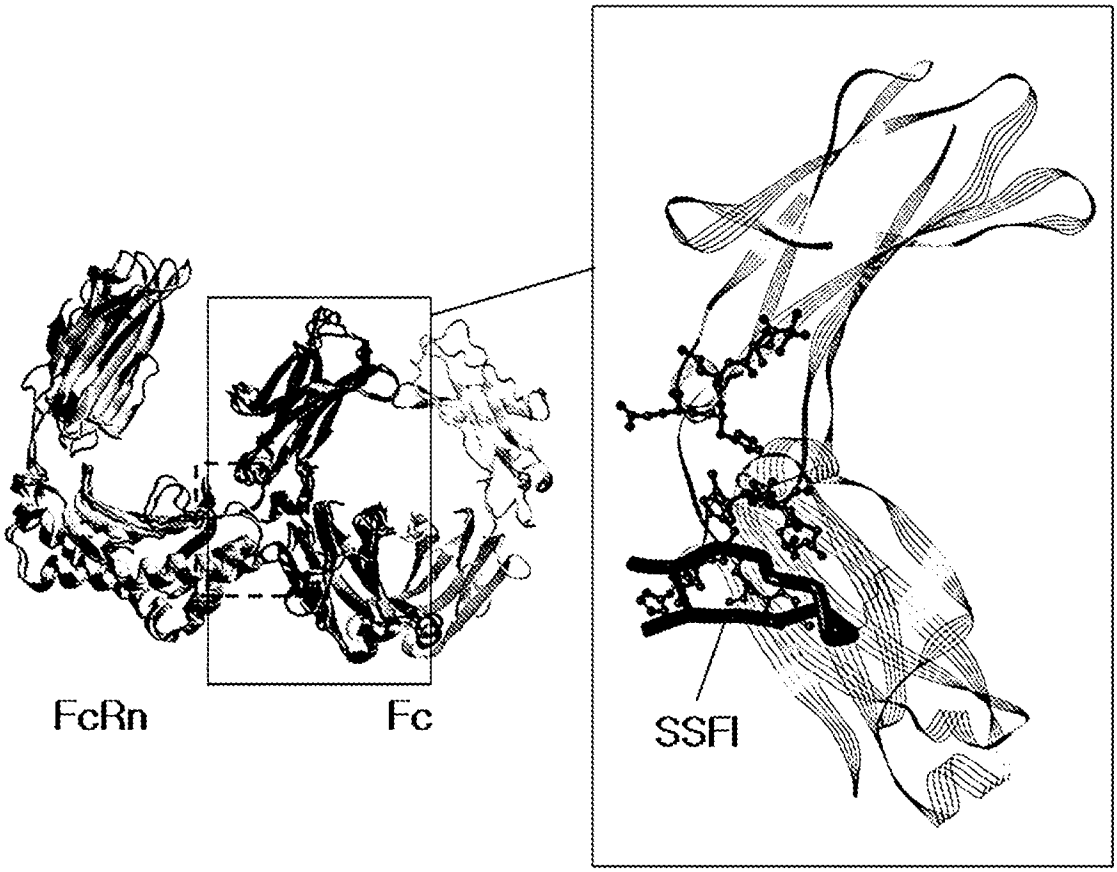


FIG. 14

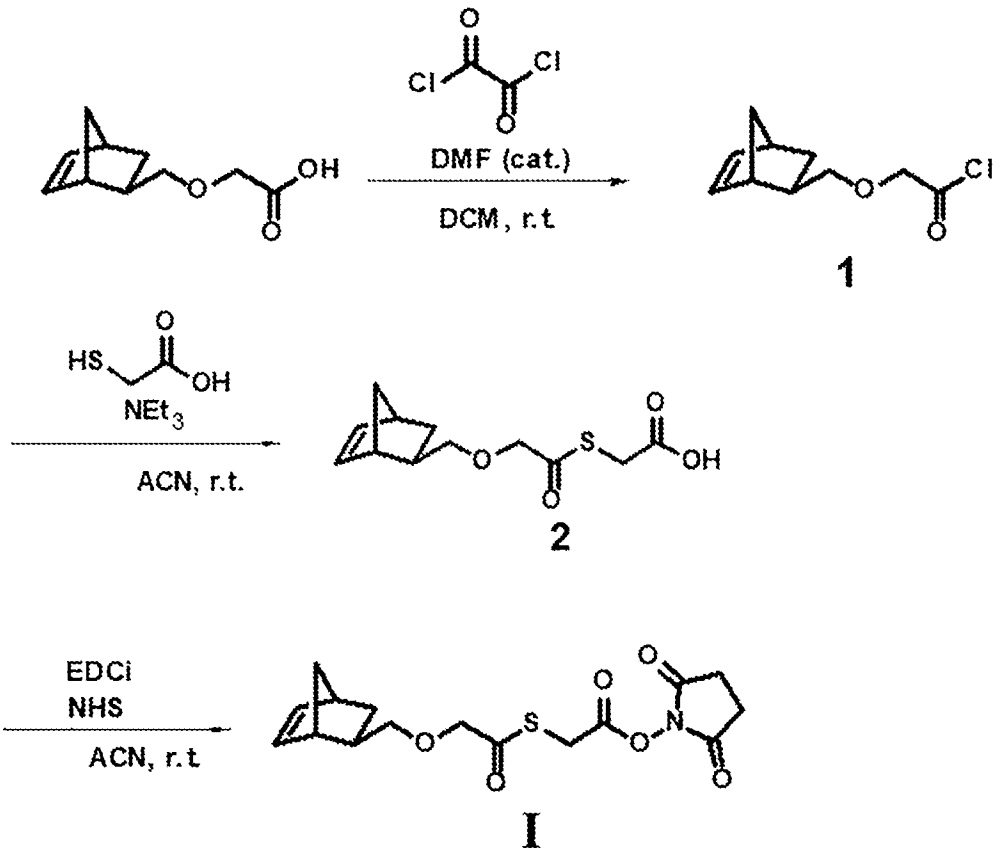


FIG. 15

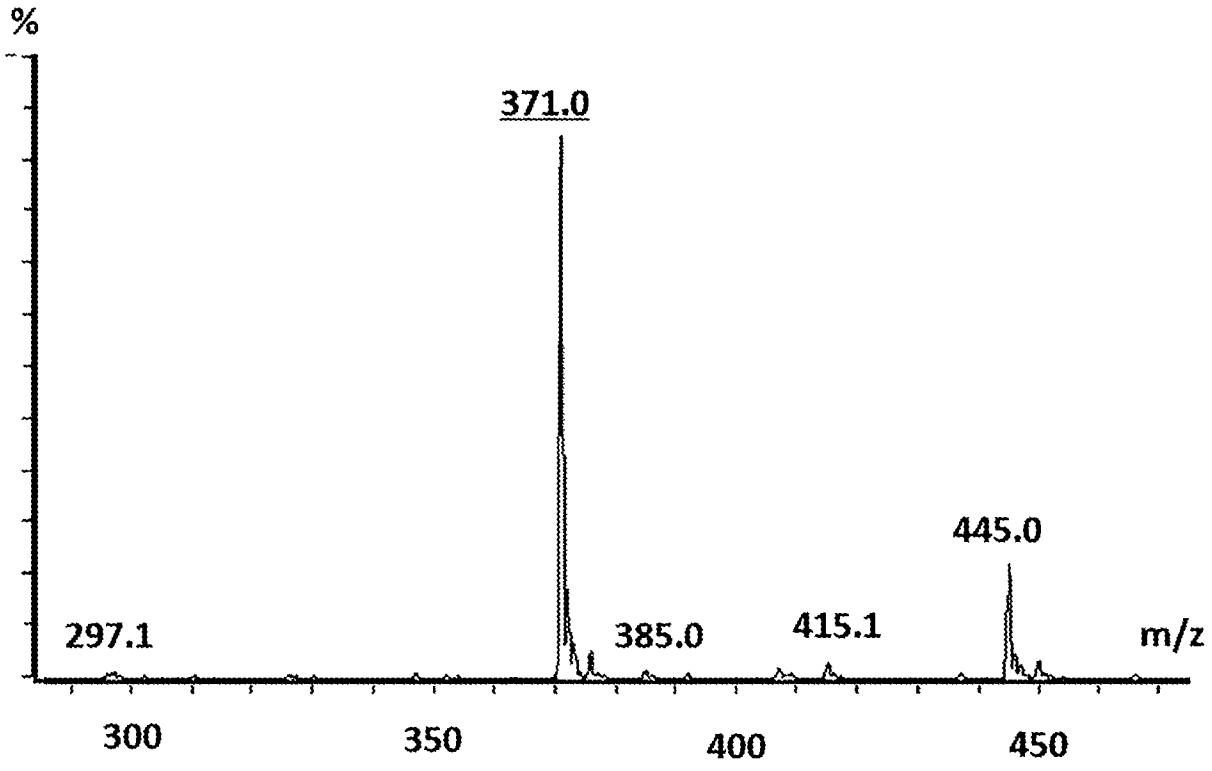


FIG. 16

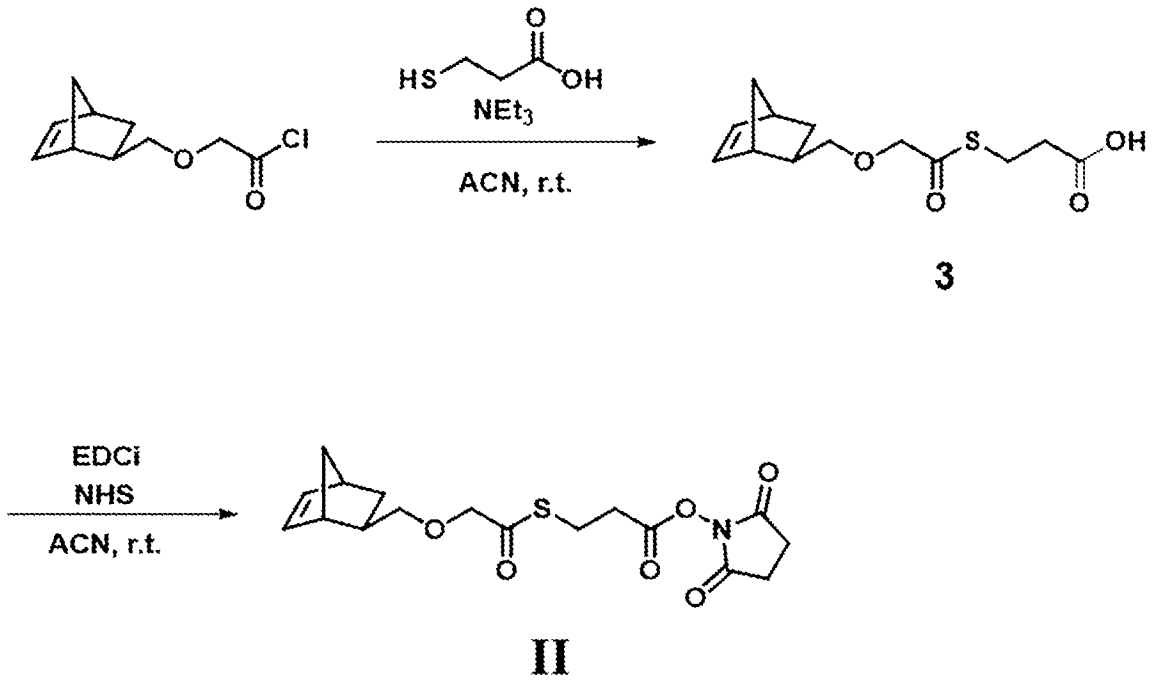


FIG. 17

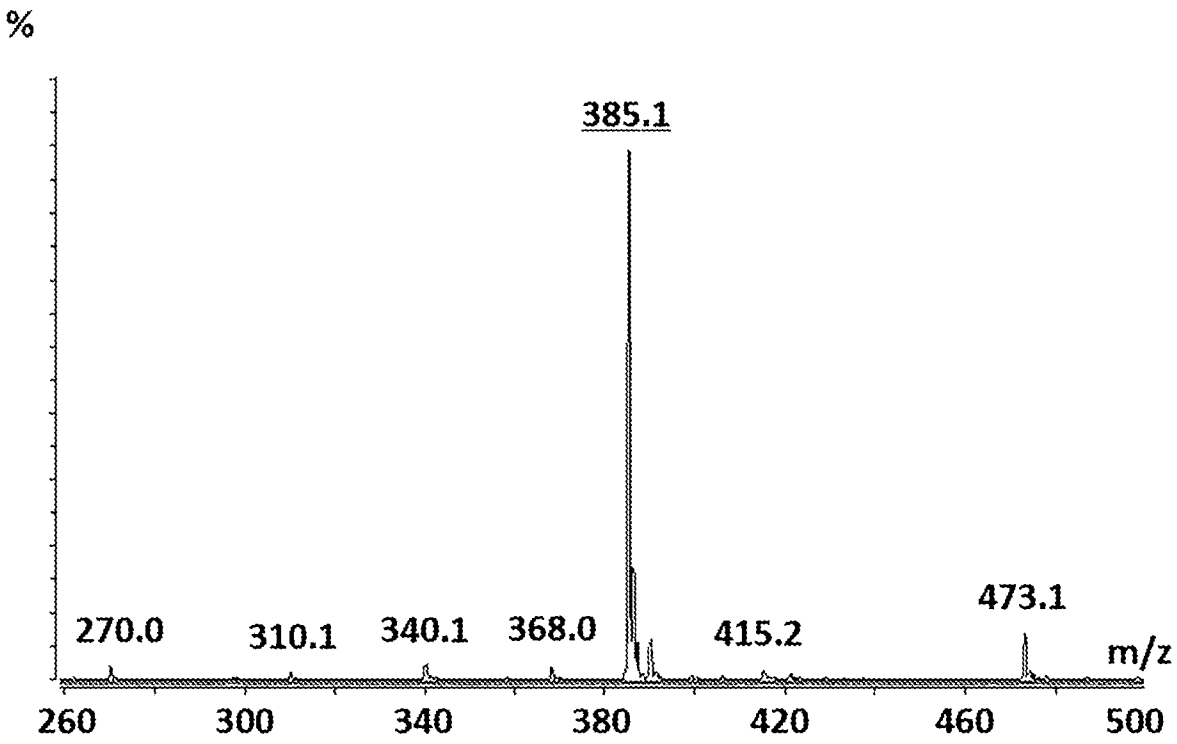

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FIG. 18

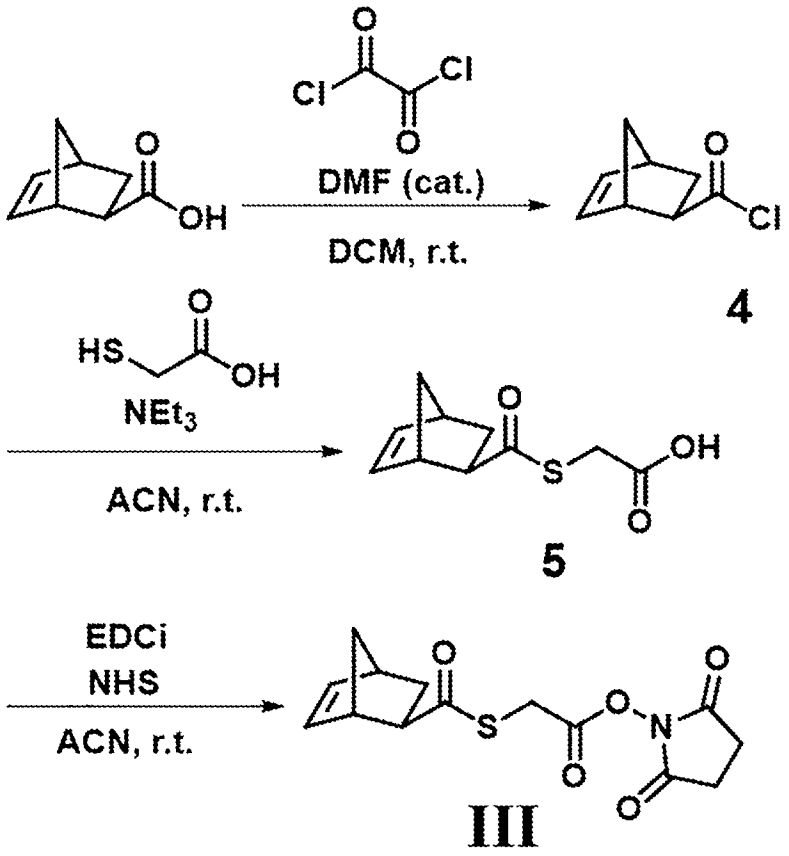


FIG. 19

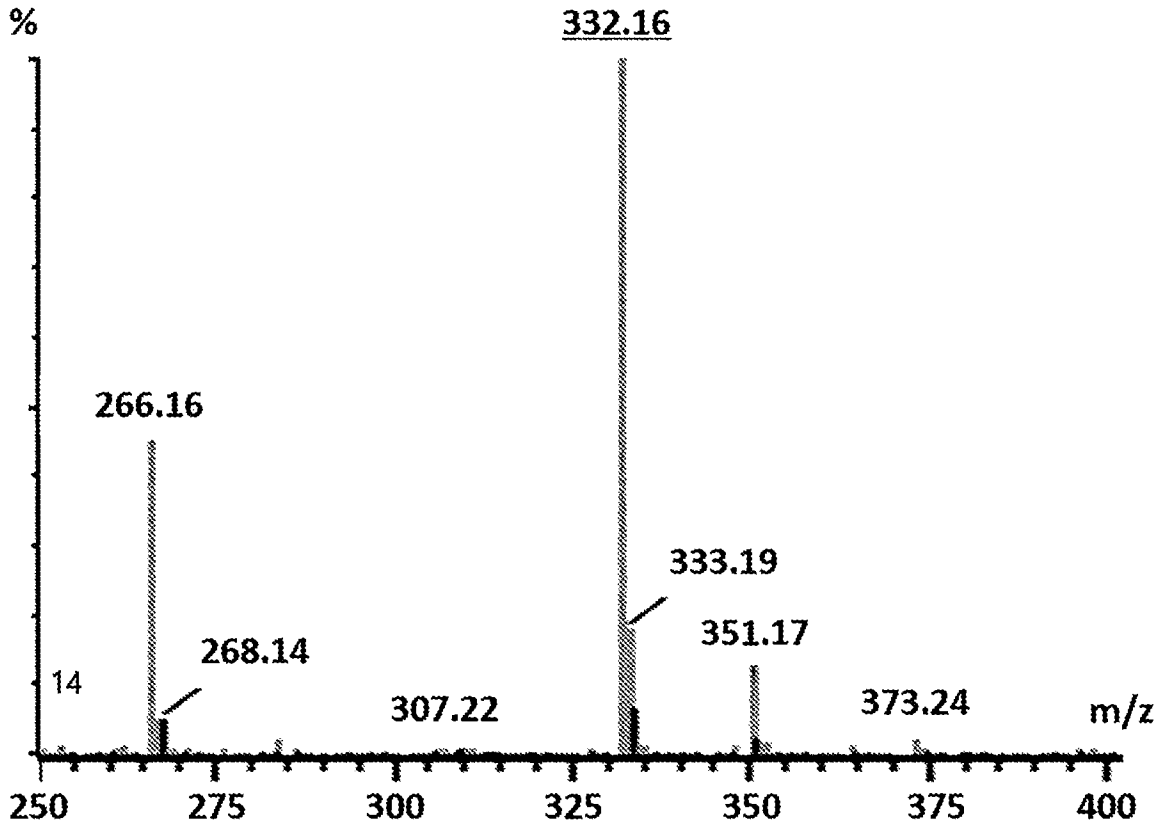

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FIG. 20

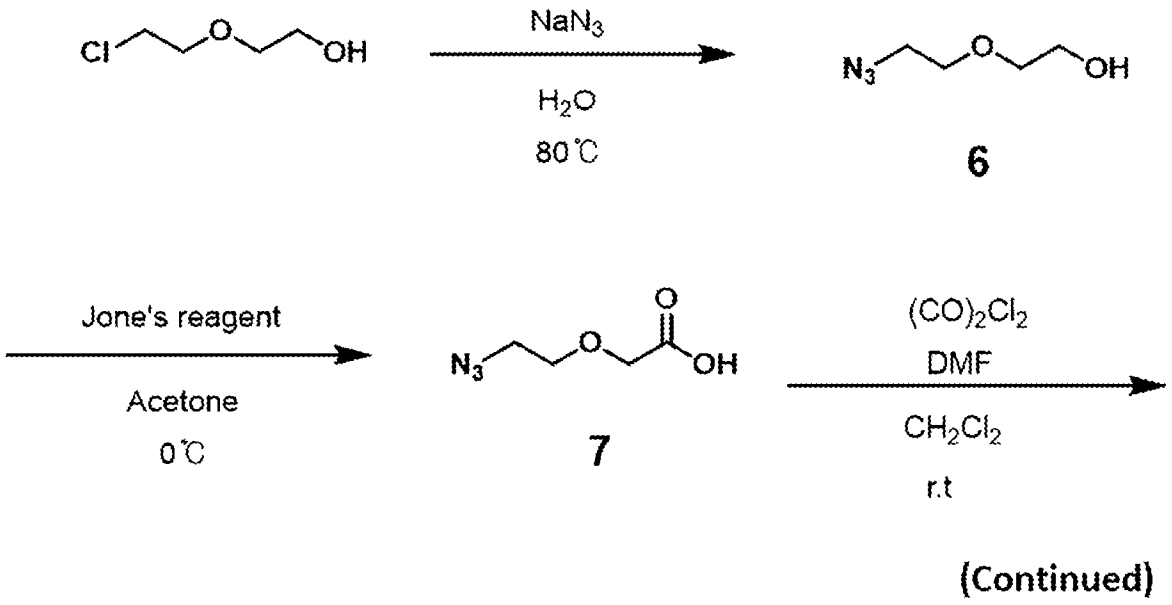

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N3
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CH2Cl2
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FIG. 21

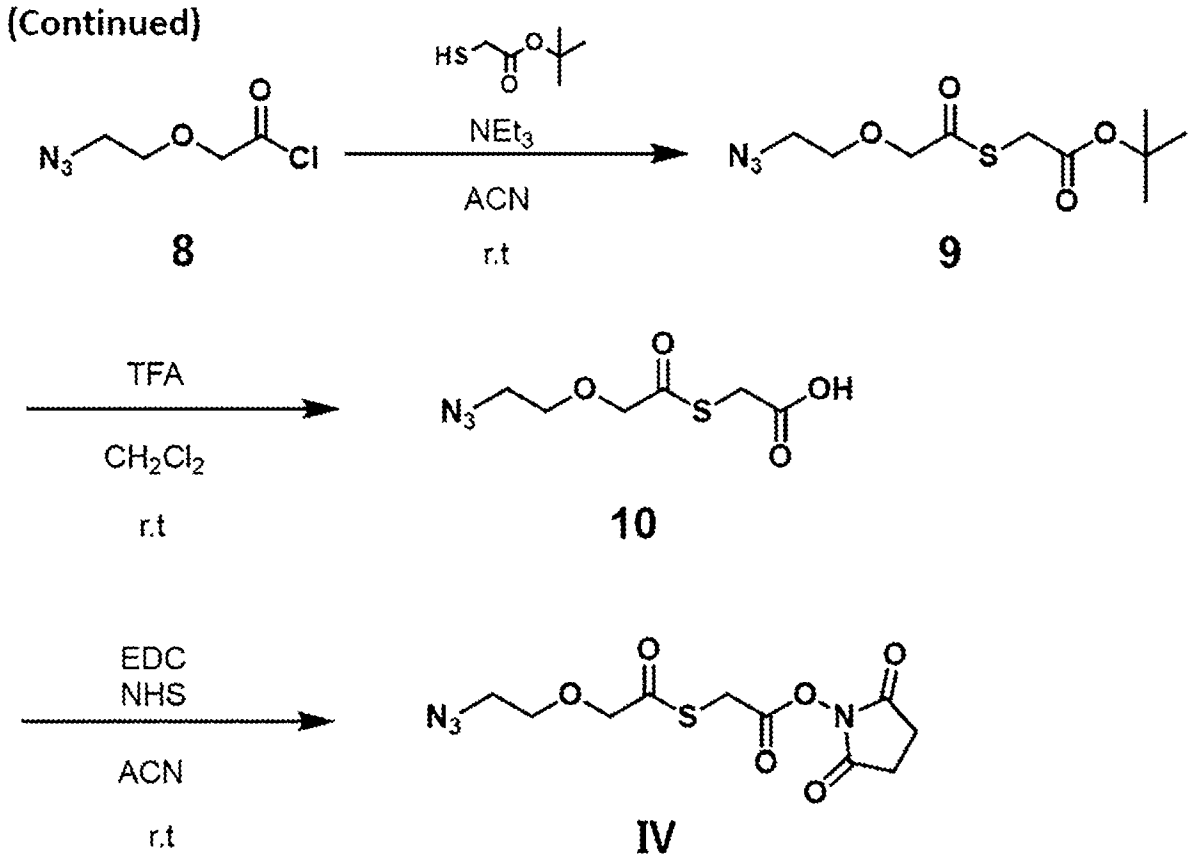

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OH
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IV

FIG. 22

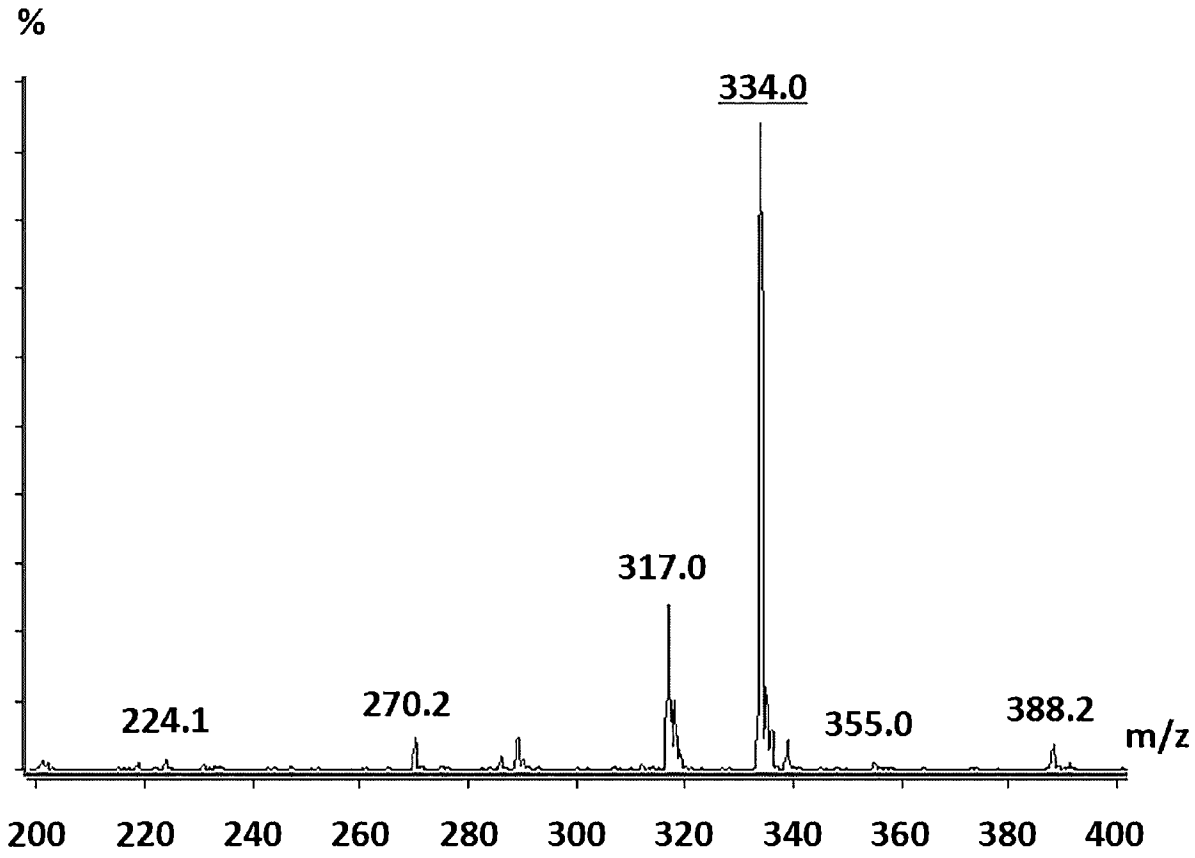

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FIG. 23

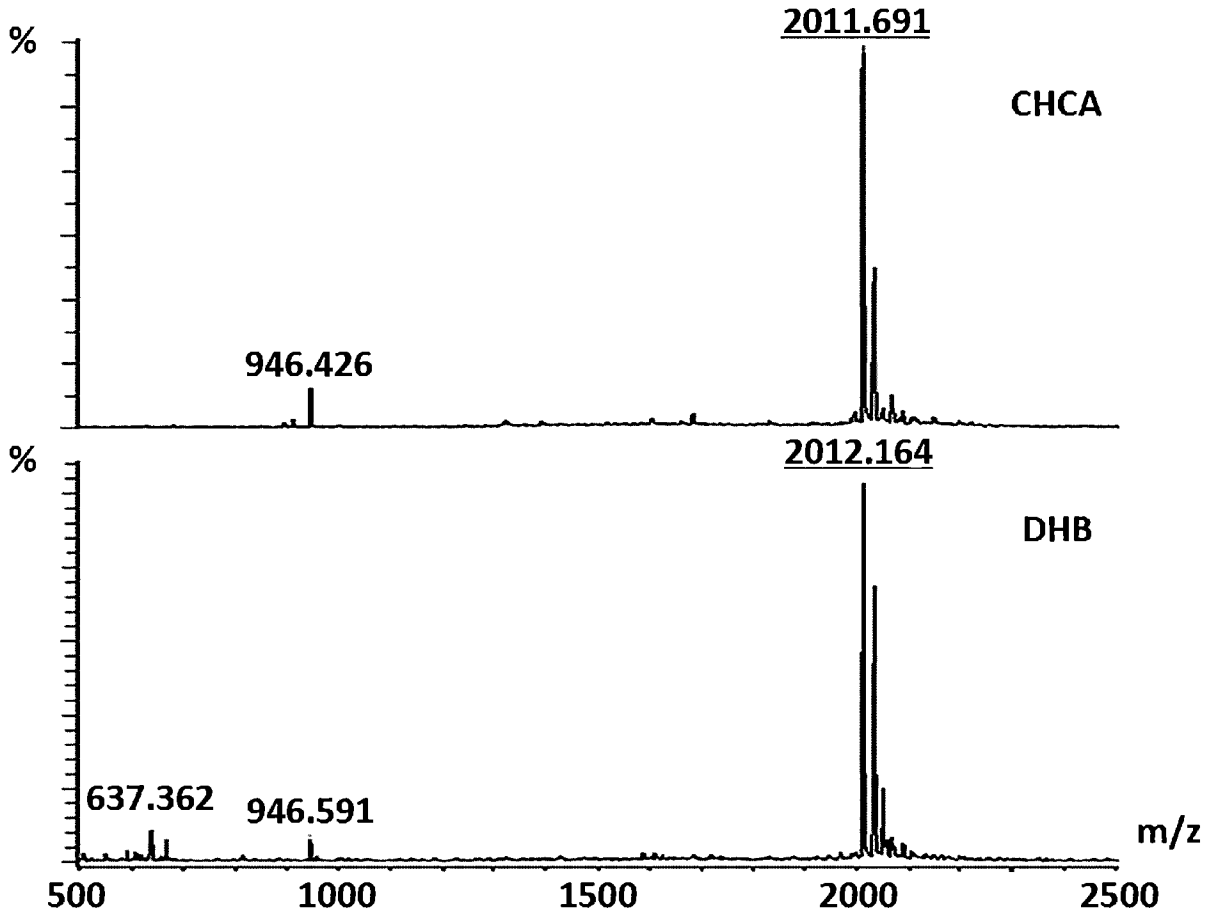

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FIG. 24

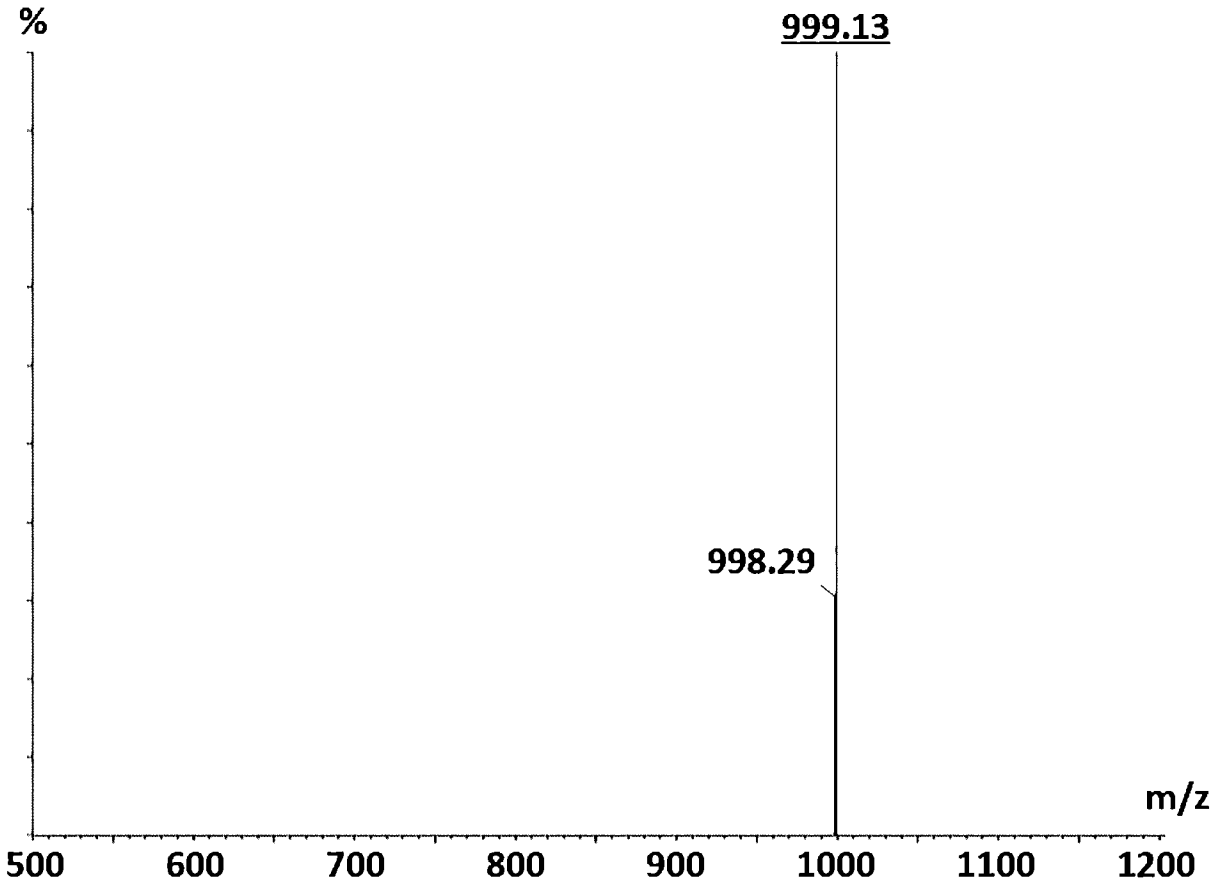


FIG. 25

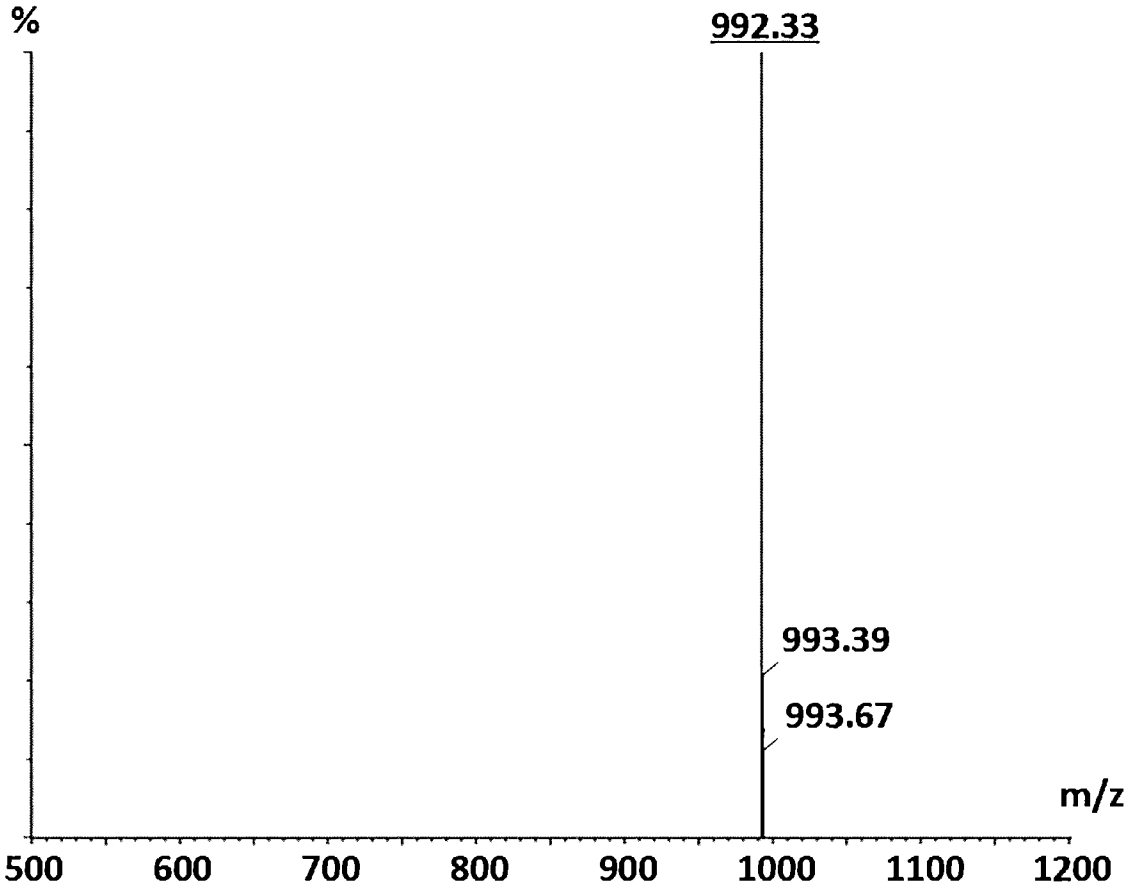


FIG. 26

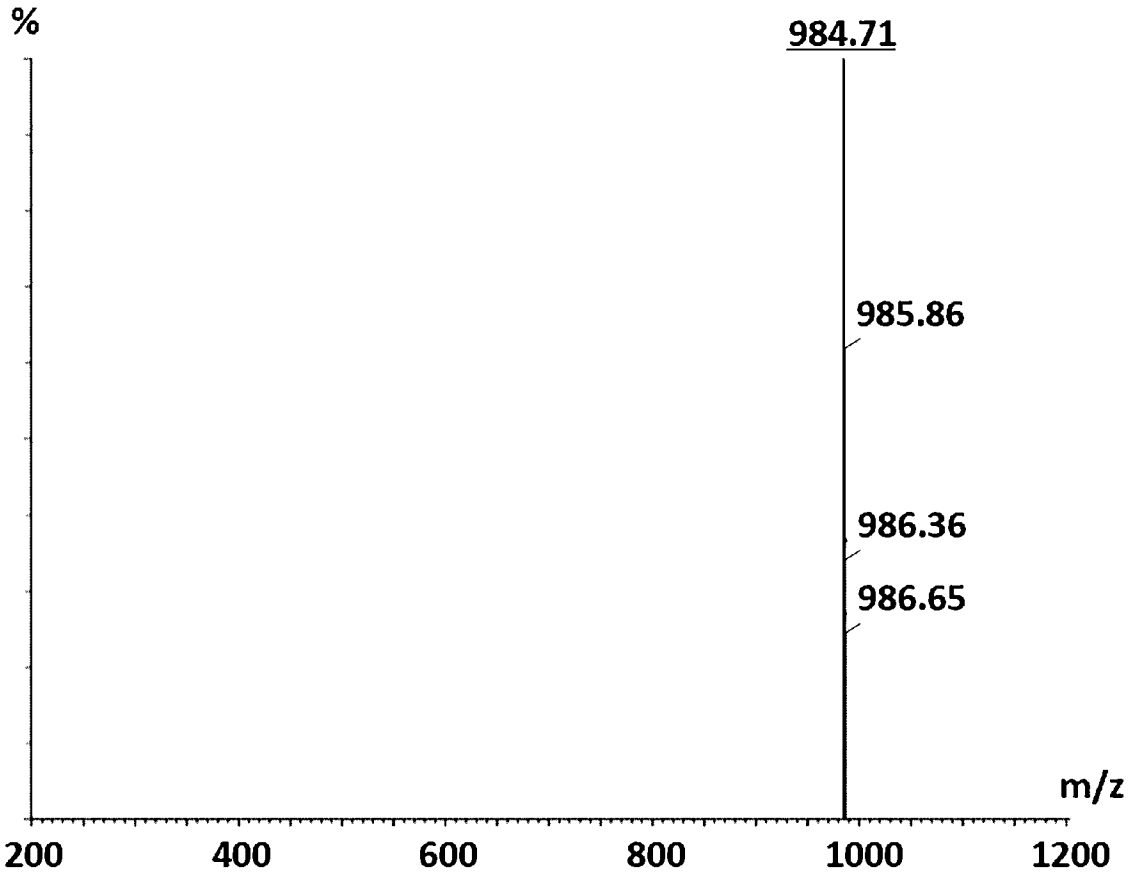


FIG. 27

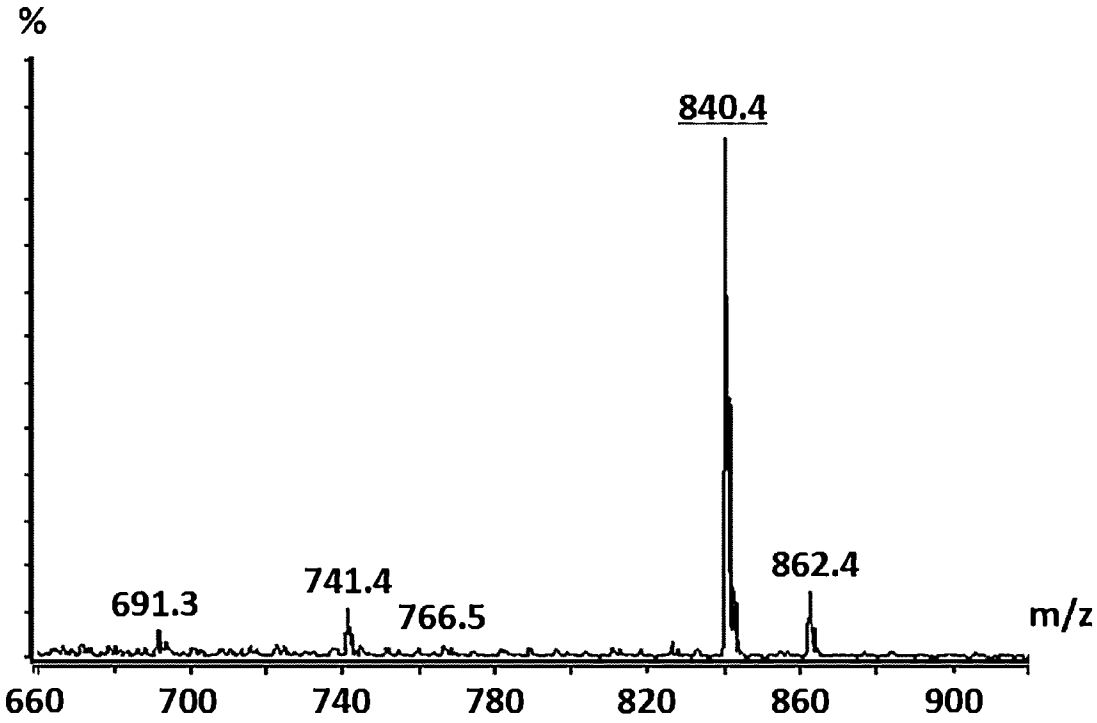


FIG. 28

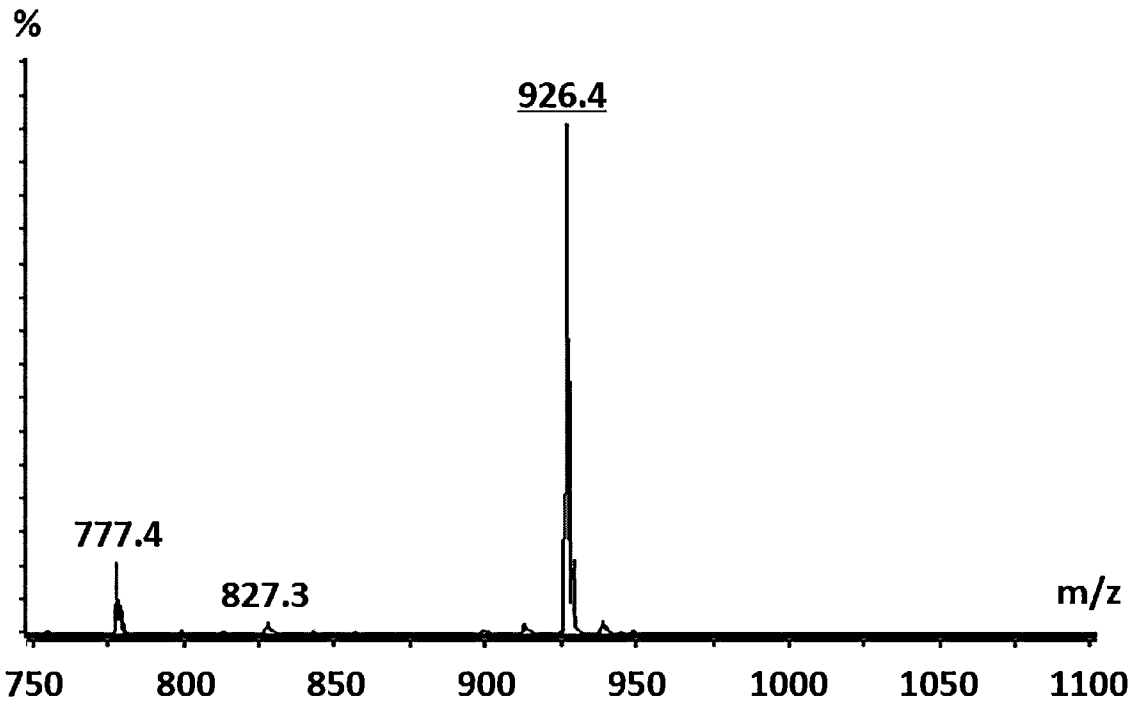


FIG. 29

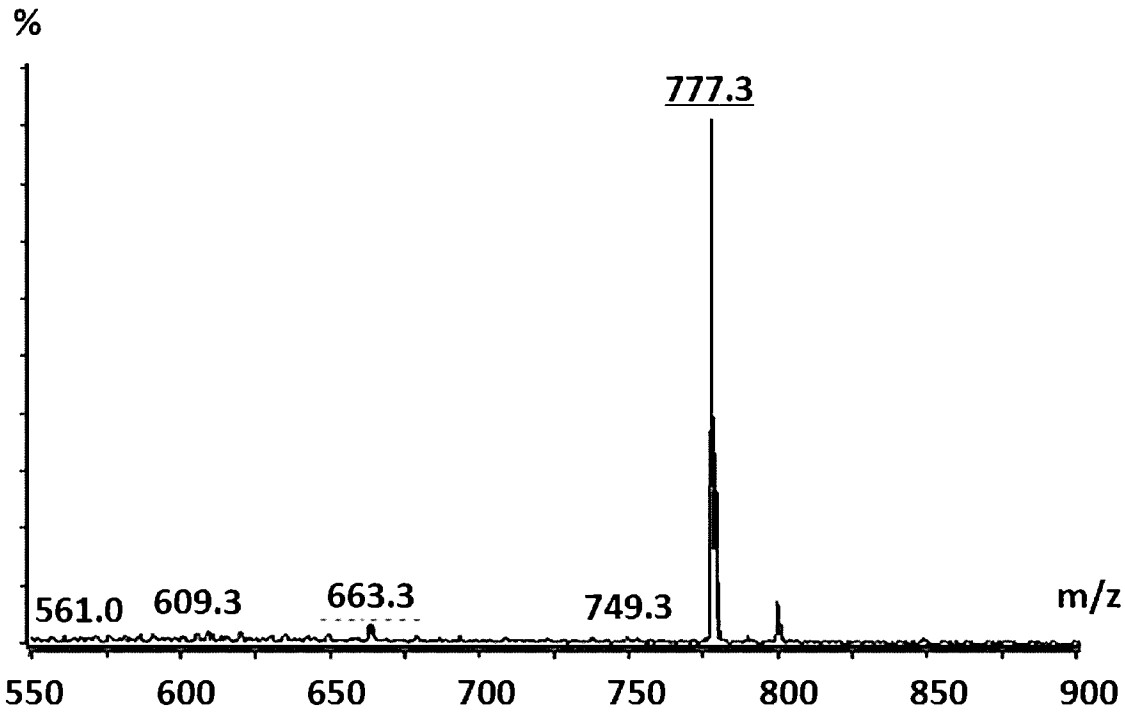


FIG. 30

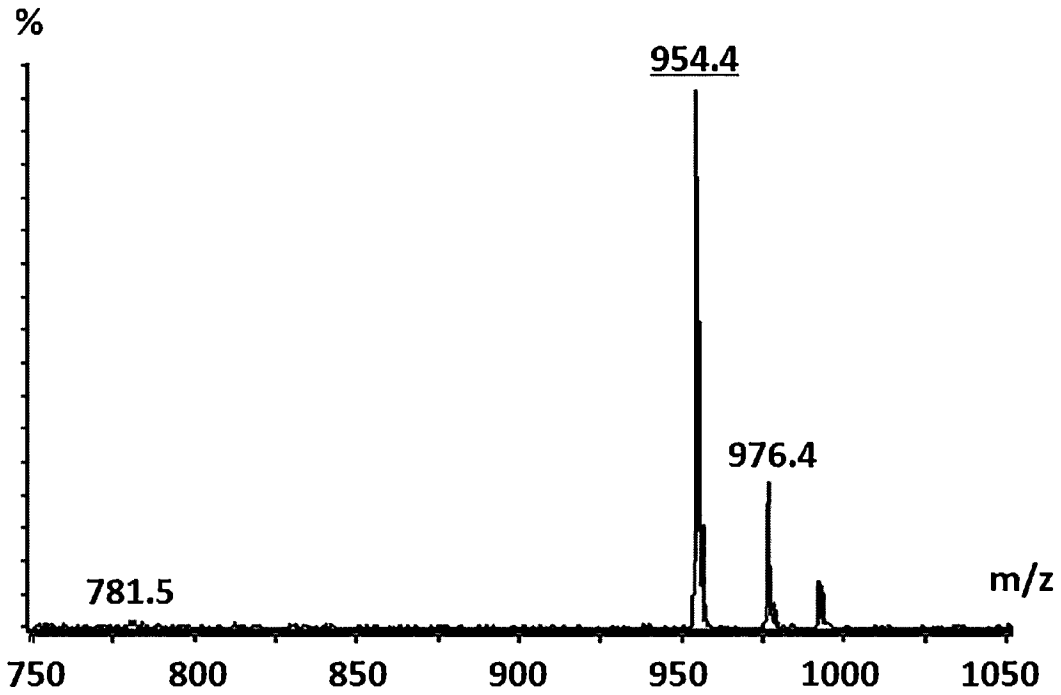


FIG. 31

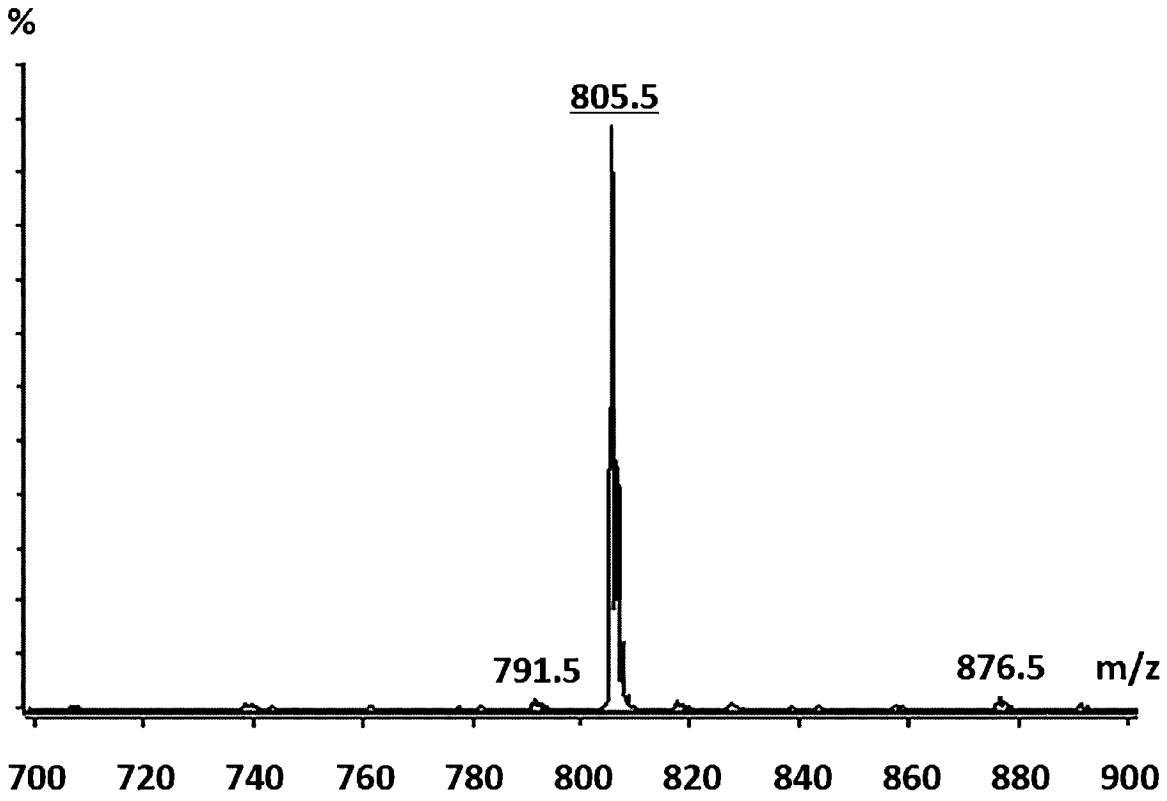


FIG. 32

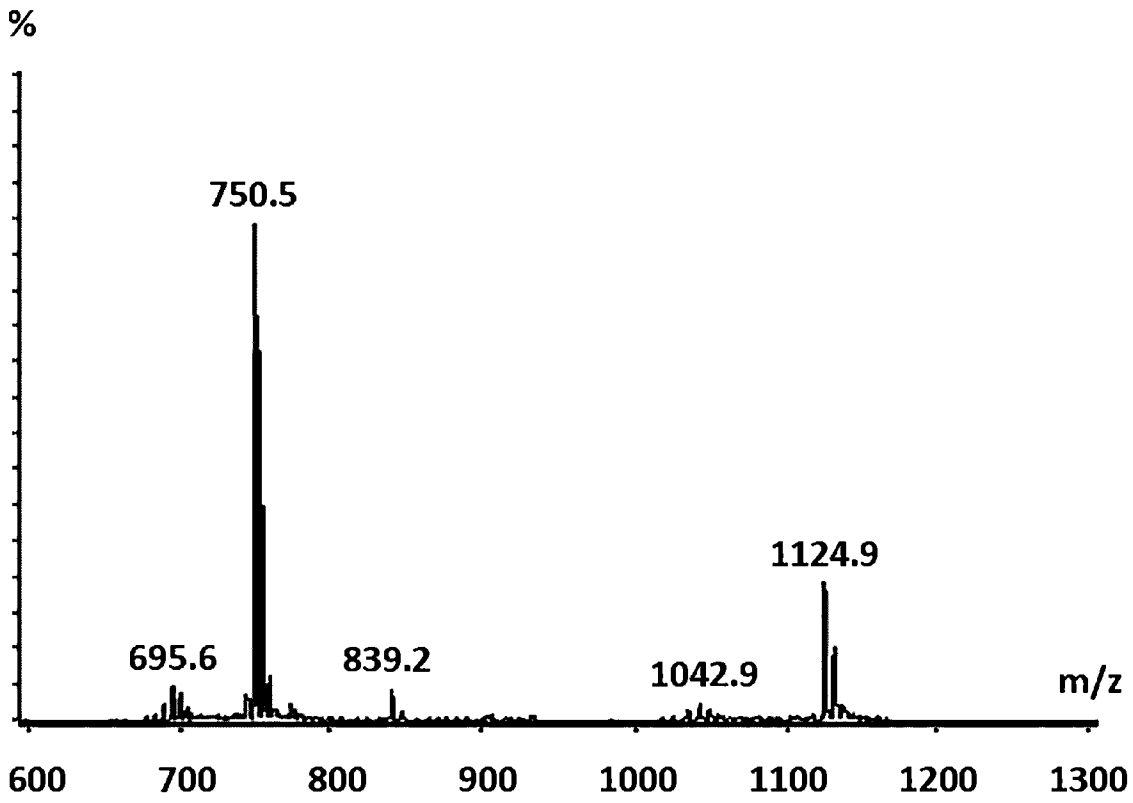

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1000
1100
1200
1300

FIG. 33

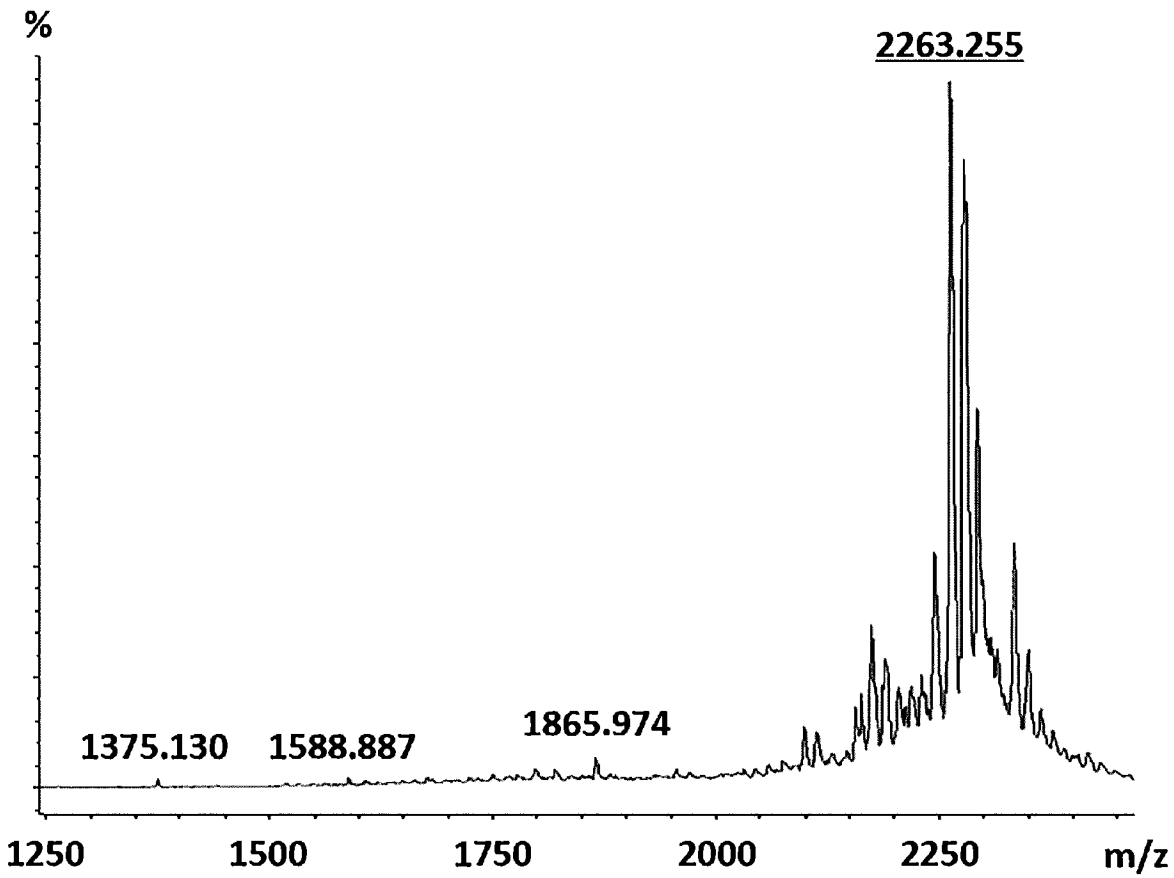

%
2263.255
1865.974
1375.130
1588.887
1250
1500
1750
2000
2250
m/z

FIG. 34

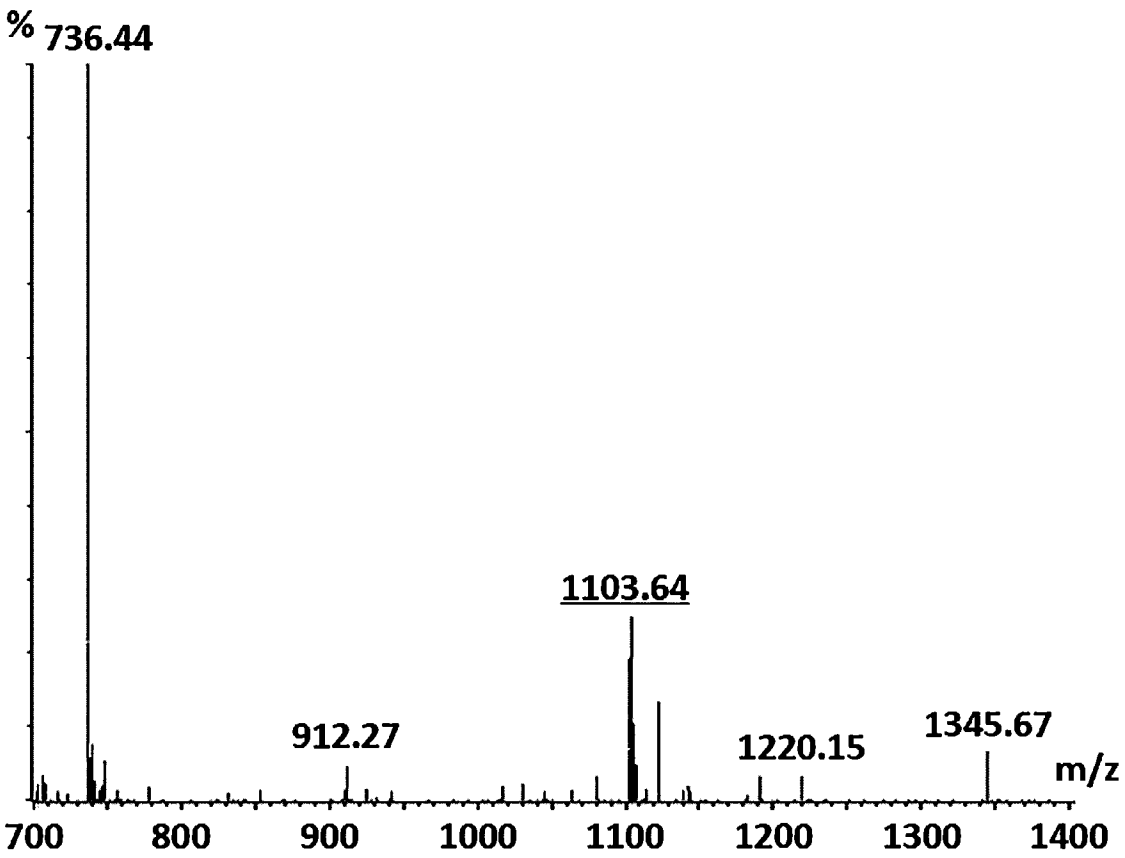

%
736.44
1103.64
912.27
1220.15
1345.67
m/z
700
800
900
1000
1100
1200
1300
1400

FIG. 35

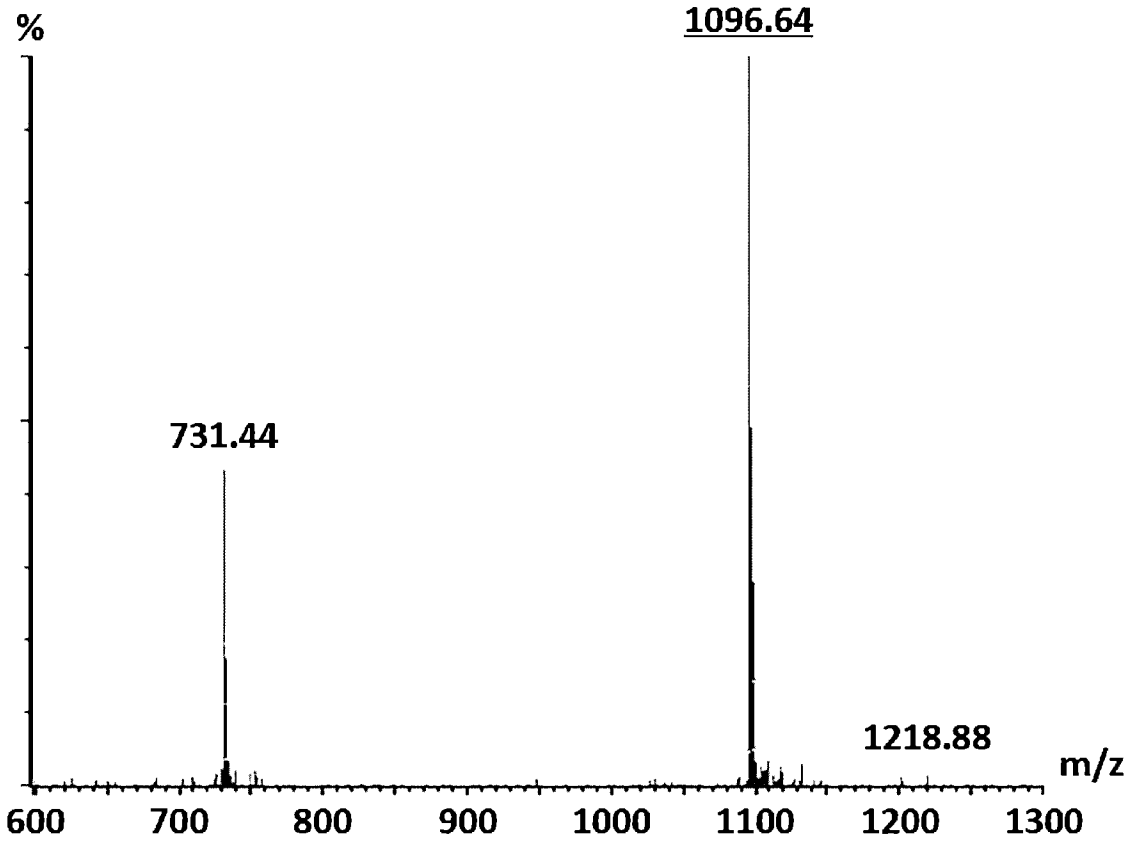


FIG. 36

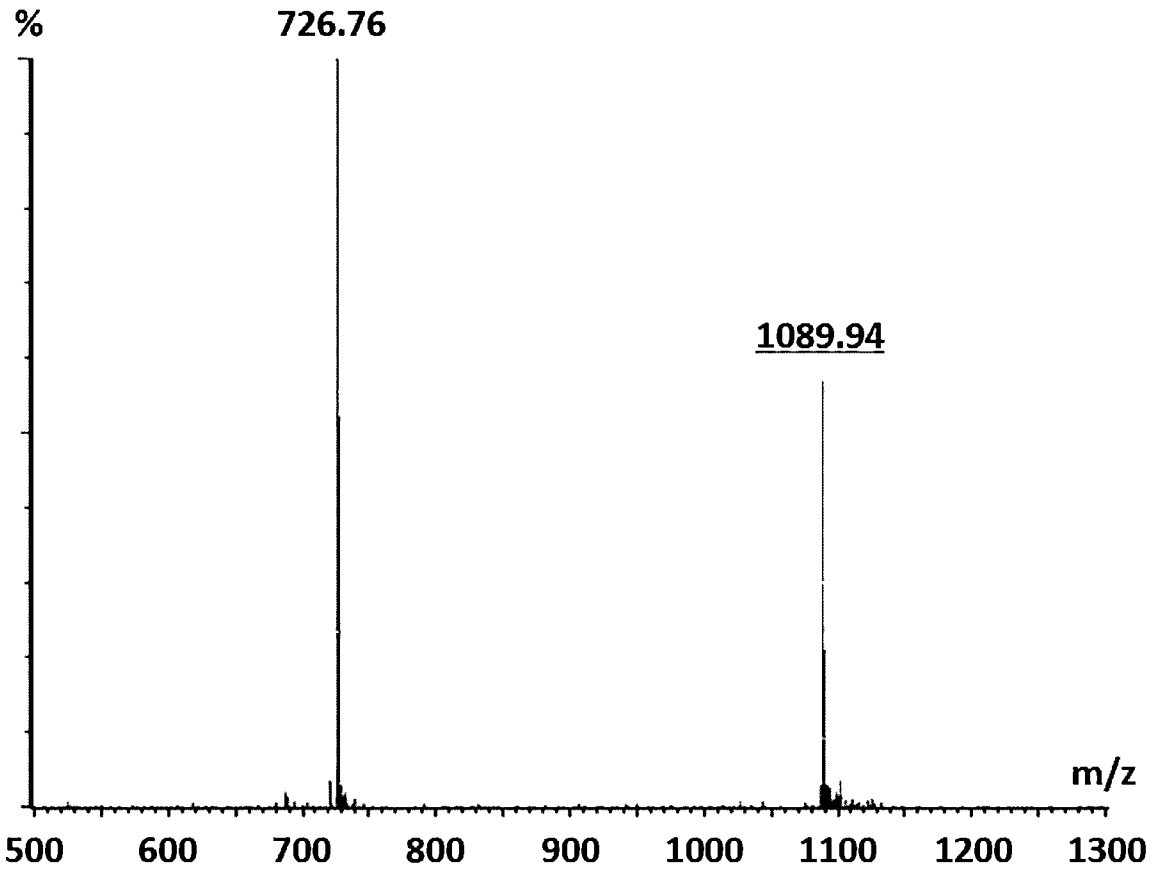


FIG. 37

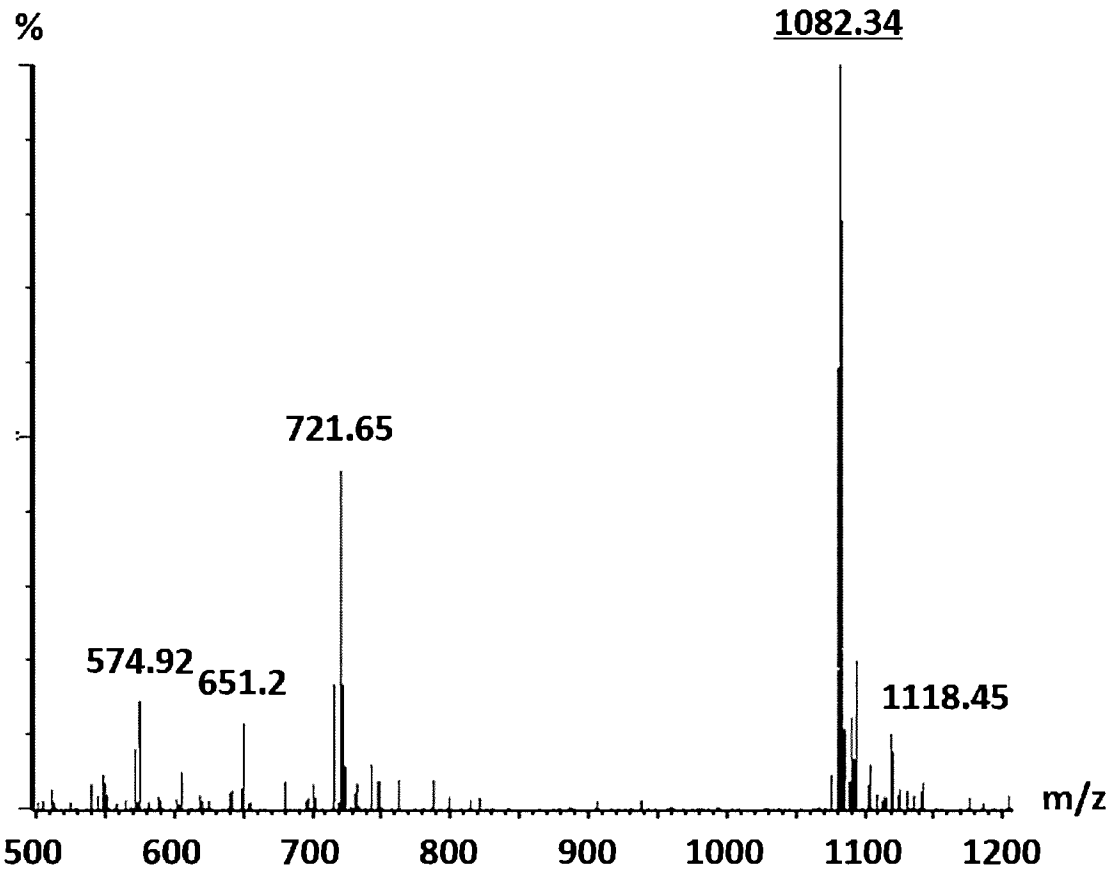

%
1082.34
721.65
574.92
651.2
1118.45
m/z
500
600
700
800
900
1000
1100
1200

FIG. 38

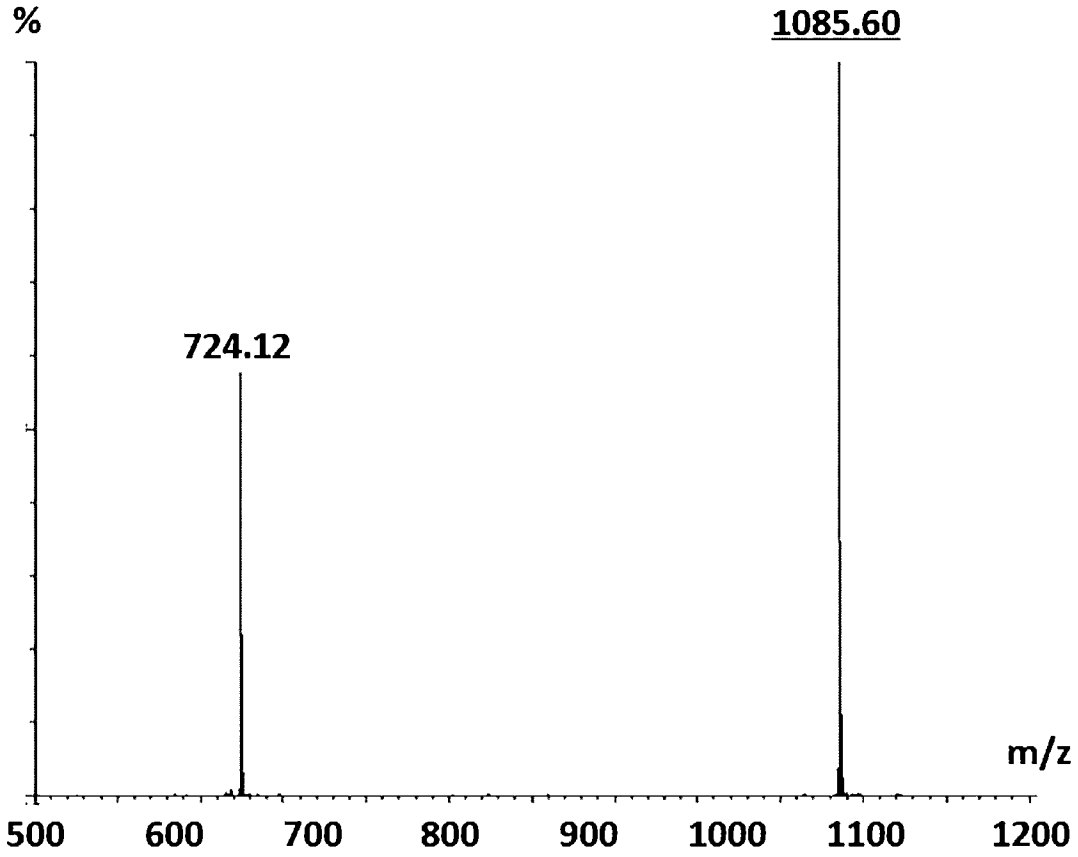


FIG. 39

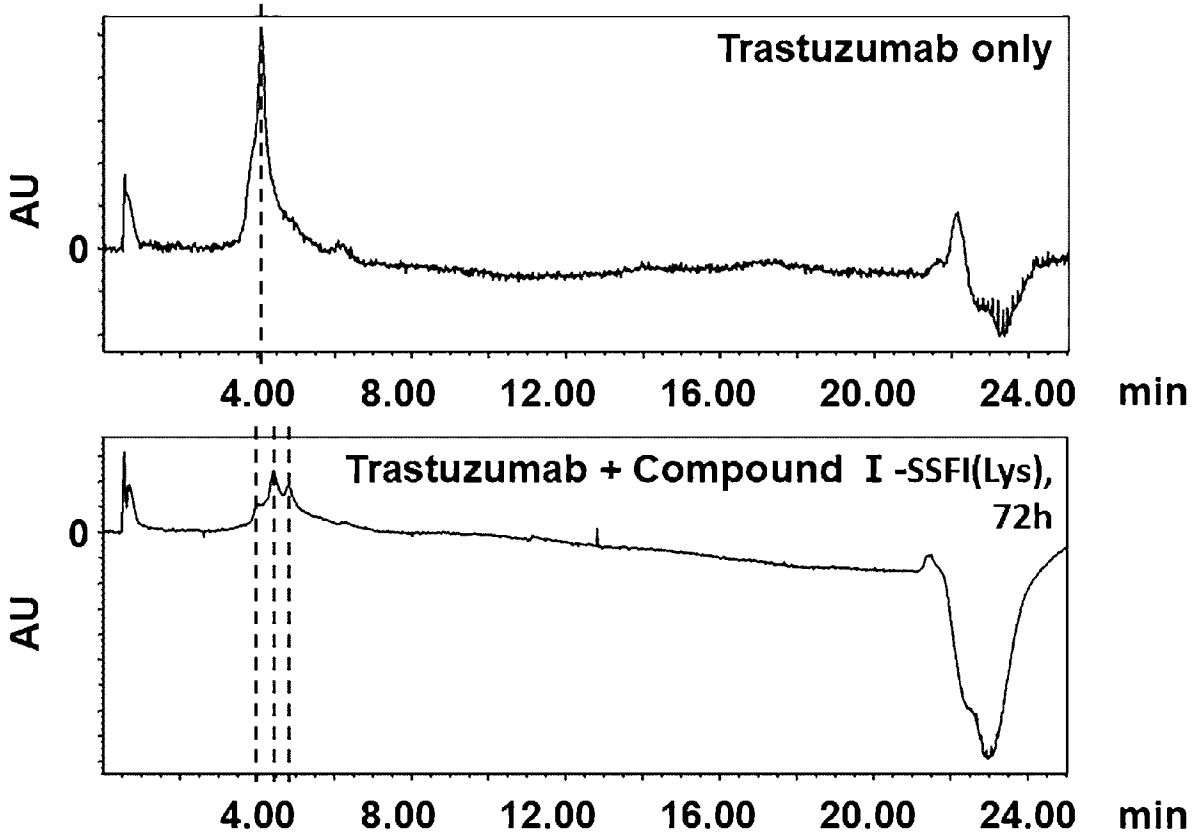

Trastuzumab only
AU
0
4.00
8.00
12.00
16.00
20.00
24.00
min
Trastuzumab + Compound I -SSFI(Lys), 72h
AU
0
4.00
8.00
12.00
16.00
20.00
24.00
min

FIG. 40

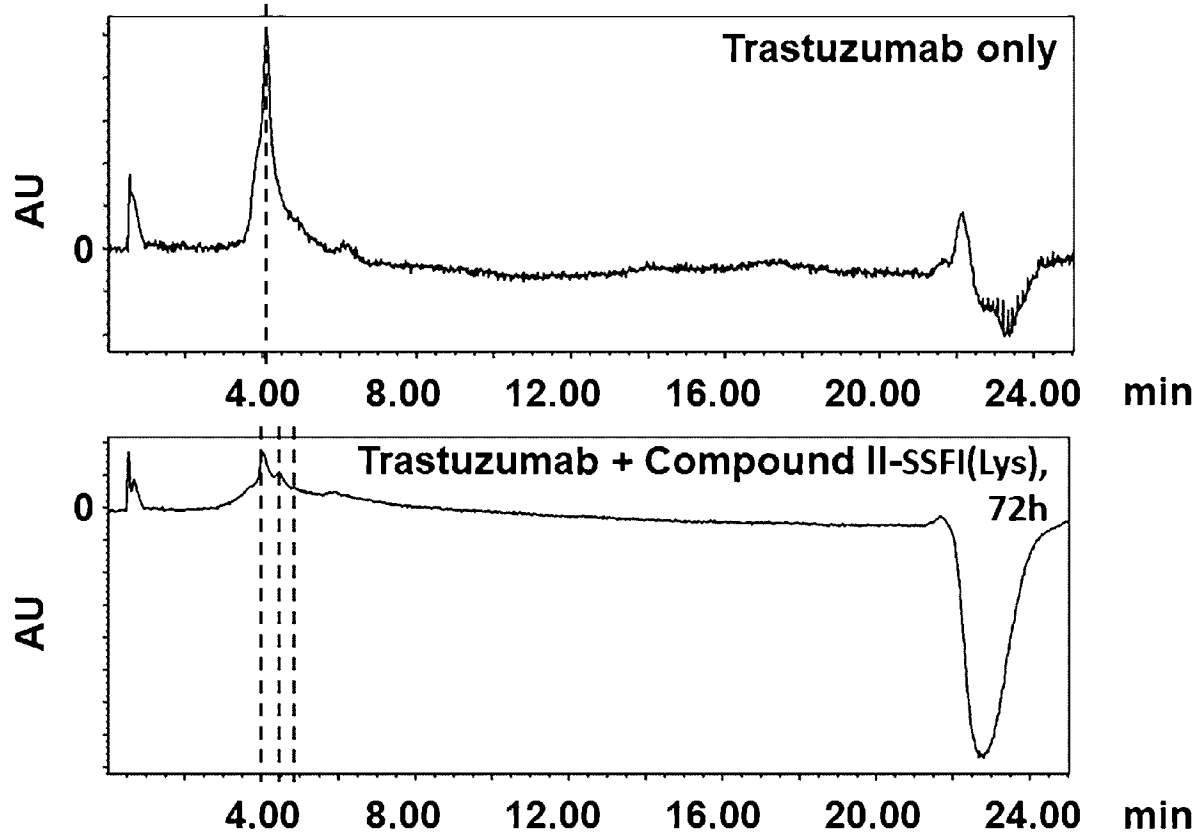
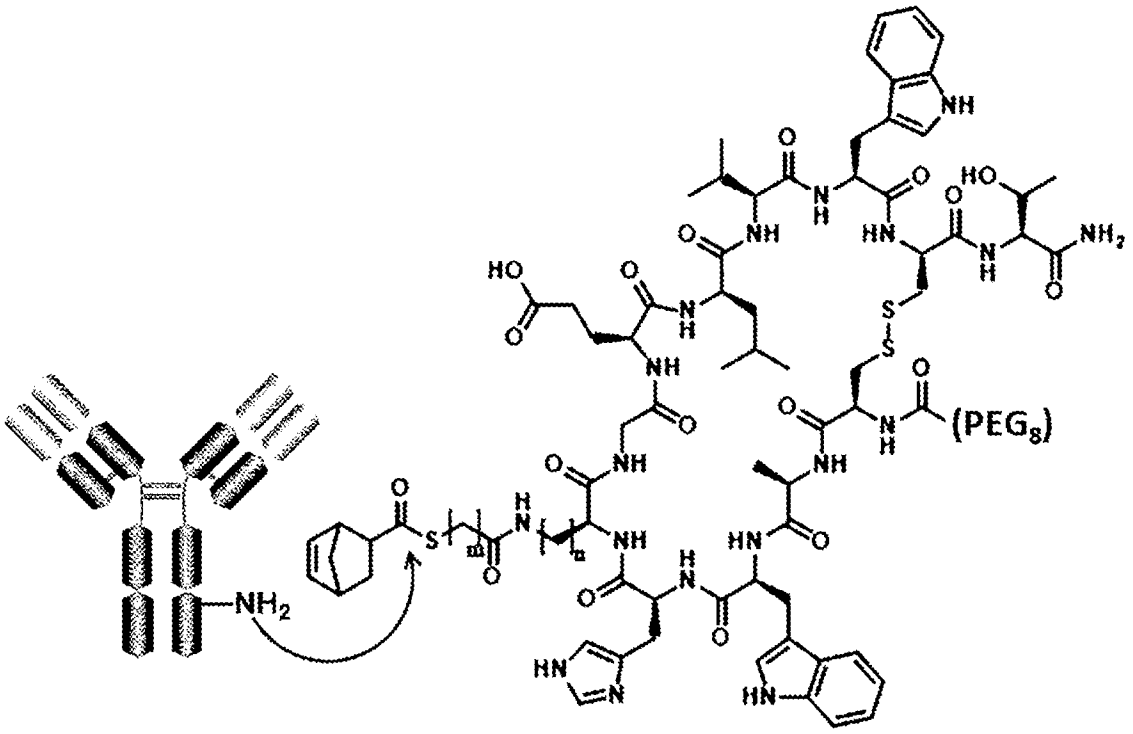

Trastuzumab only
AU
0
4.00
8.00
12.00
16.00
20.00
24.00
min
Trastuzumab + Compound II-SSFI(Lys), 72h
AU
0
4.00
8.00
12.00
16.00
20.00
24.00
min

FIG. 41



(chemical structure of formula 16 pegylated)

FIG. 42

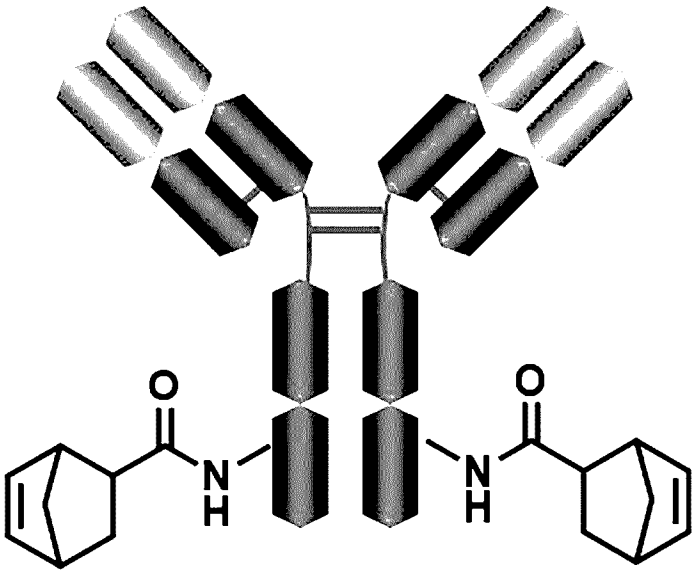


FIG. 43

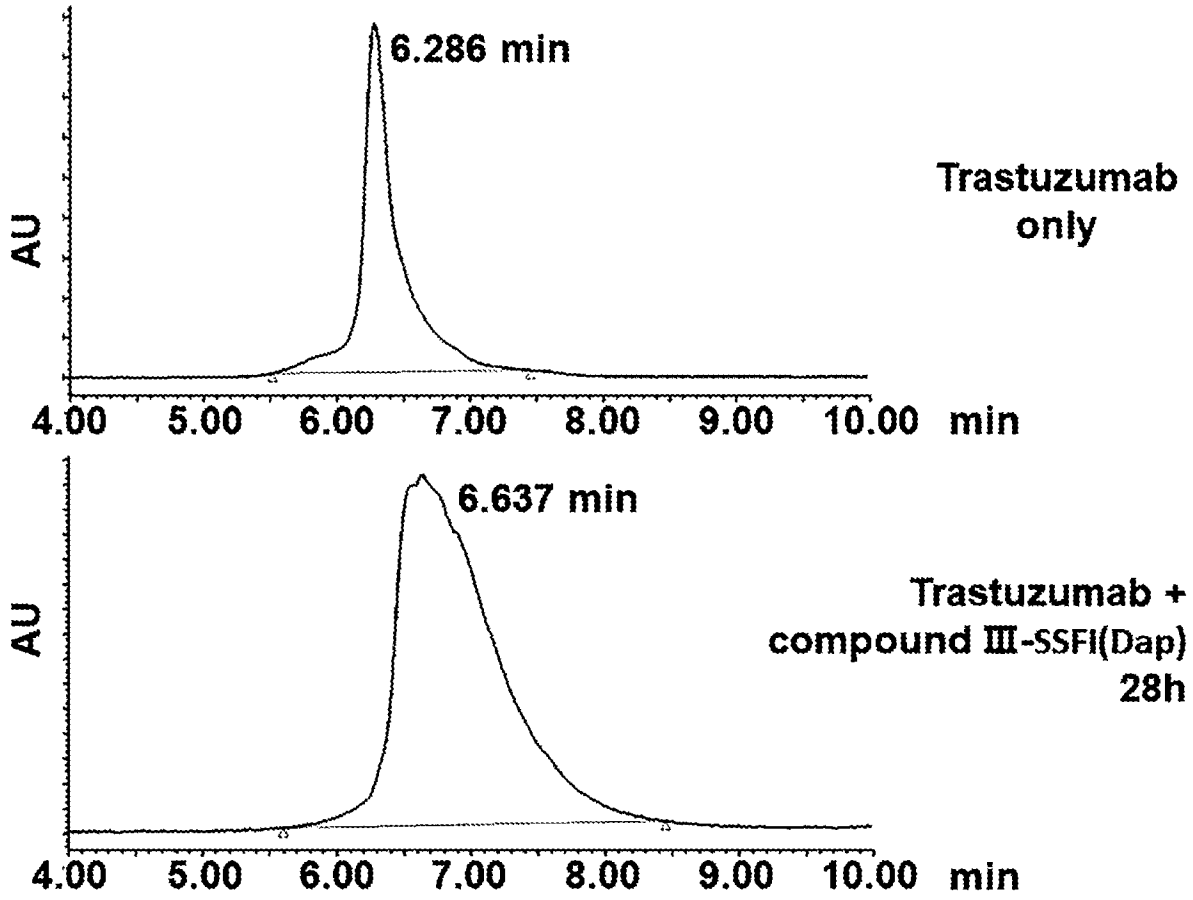

6.286 min
AU
Trastuzumab only
4.00
5.00
6.00
7.00
8.00
9.00
10.00
min
6.637 min
AU
Trastuzumab + compound Ⅲ-SSFI(Dap) 28h
4.00
5.00
6.00
7.00
8.00
9.00
10.00
min

FIG. 44

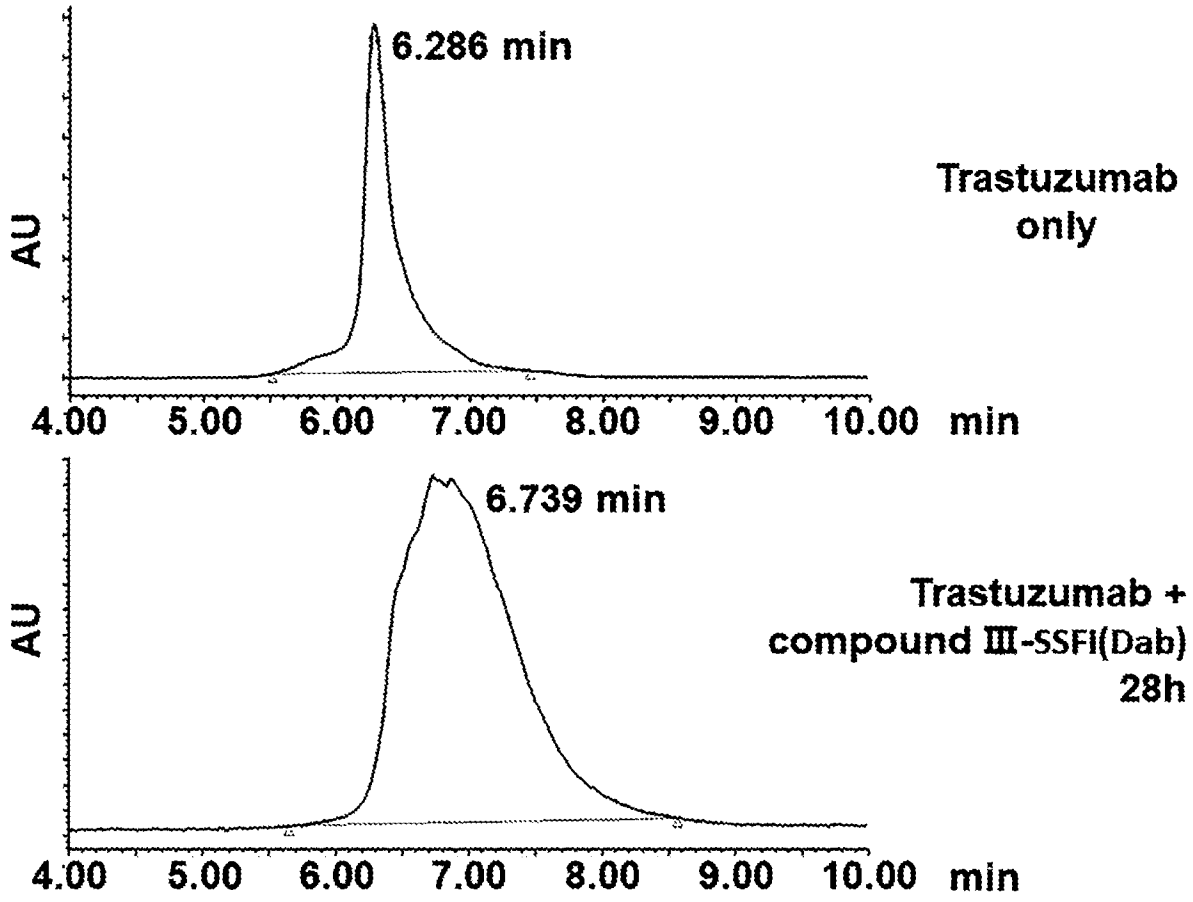

6.286 min
AU
Trastuzumab only
4.00
5.00
6.00
7.00
8.00
9.00
10.00
min
6.739 min
AU
Trastuzumab + compound Ⅲ-SSFI(Dab) 28h
4.00
5.00
6.00
7.00
8.00
9.00
10.00
min

FIG. 45

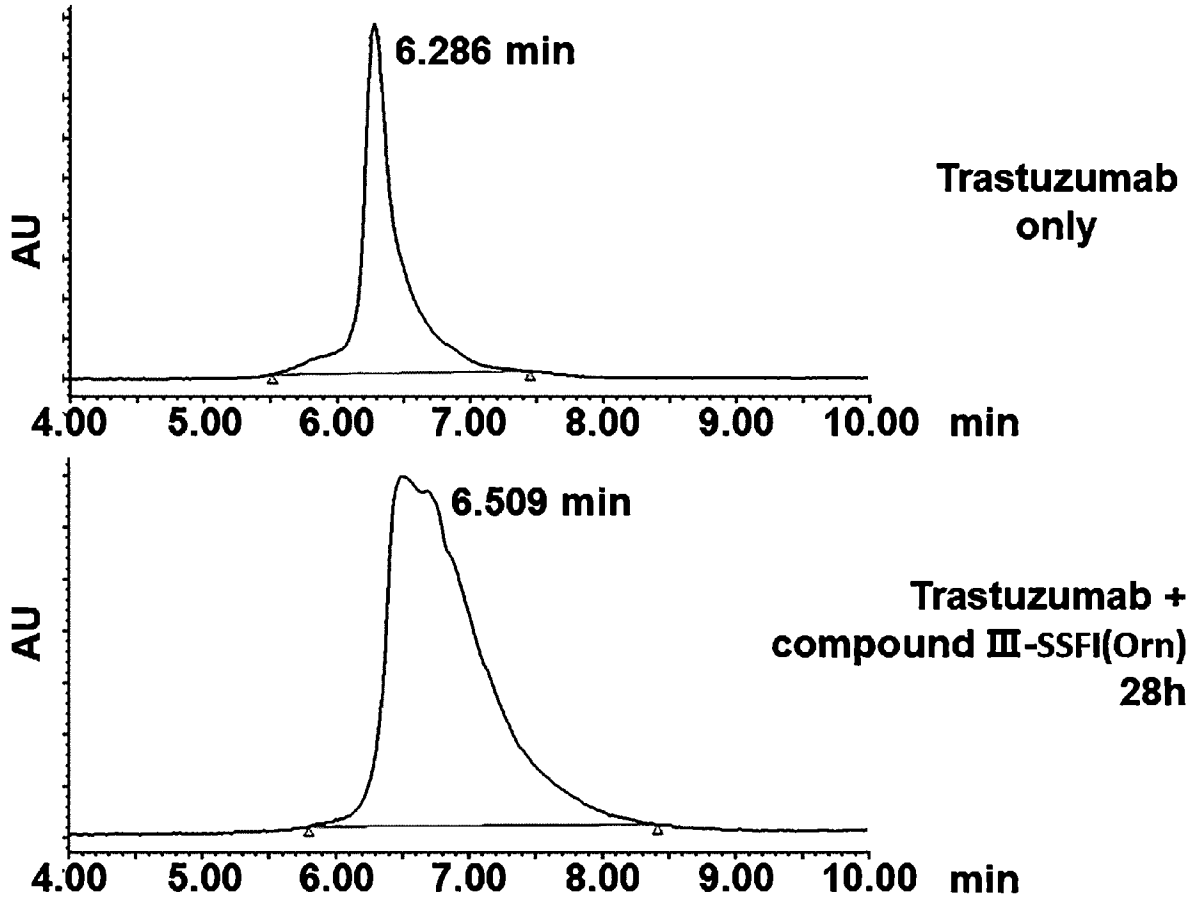

6.286 min
AU
Trastuzumab only
4.00
5.00
6.00
7.00
8.00
9.00
10.00
min
6.509 min
AU
Trastuzumab + compound Ⅲ-SSFI(Orn) 28h
4.00
5.00
6.00
7.00
8.00
9.00
10.00
min

FIG. 46

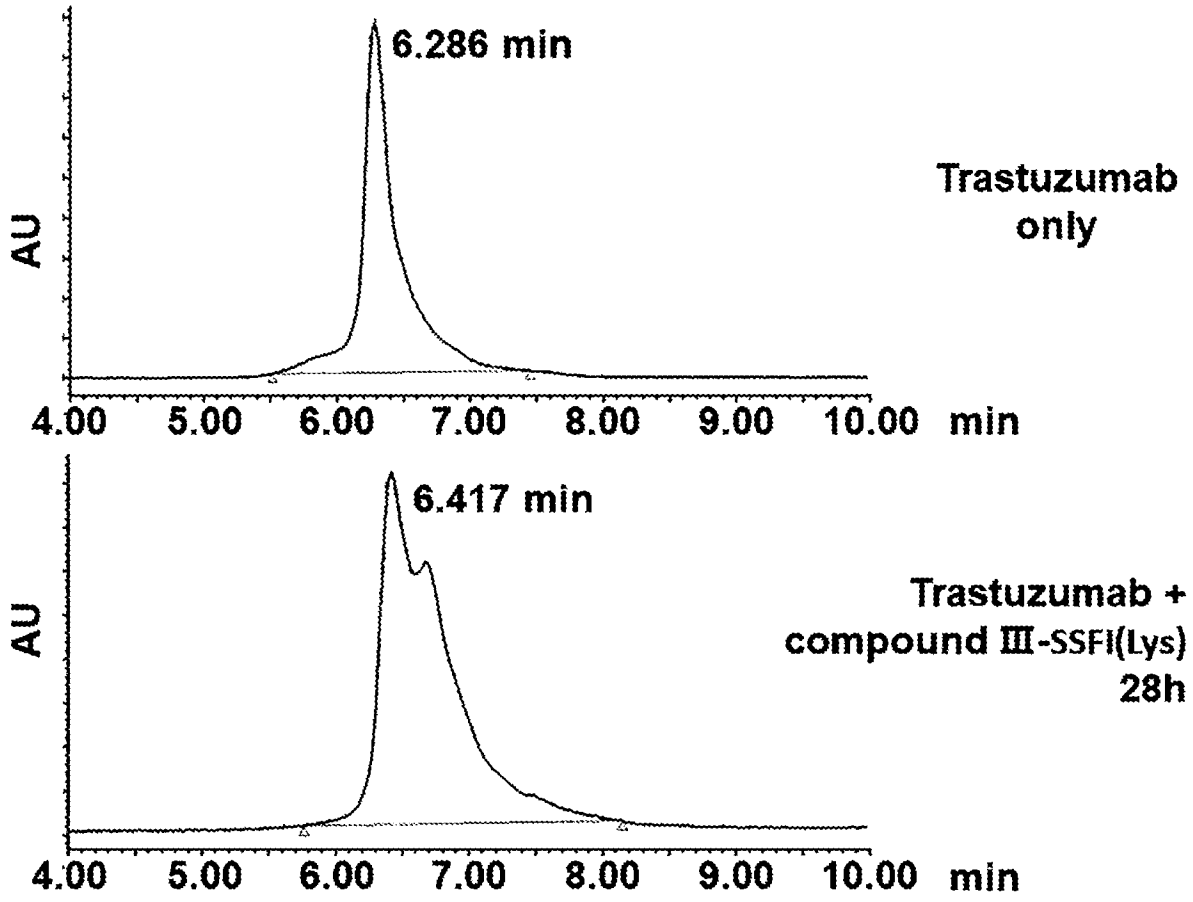

6.286 min
AU
Trastuzumab only
4.00
5.00
6.00
7.00
8.00
9.00
10.00
min
6.417 min
AU
Trastuzumab + compound Ⅲ-SSFI(Lys) 28h
4.00
5.00
6.00
7.00
8.00
9.00
10.00
min

FIG. 47

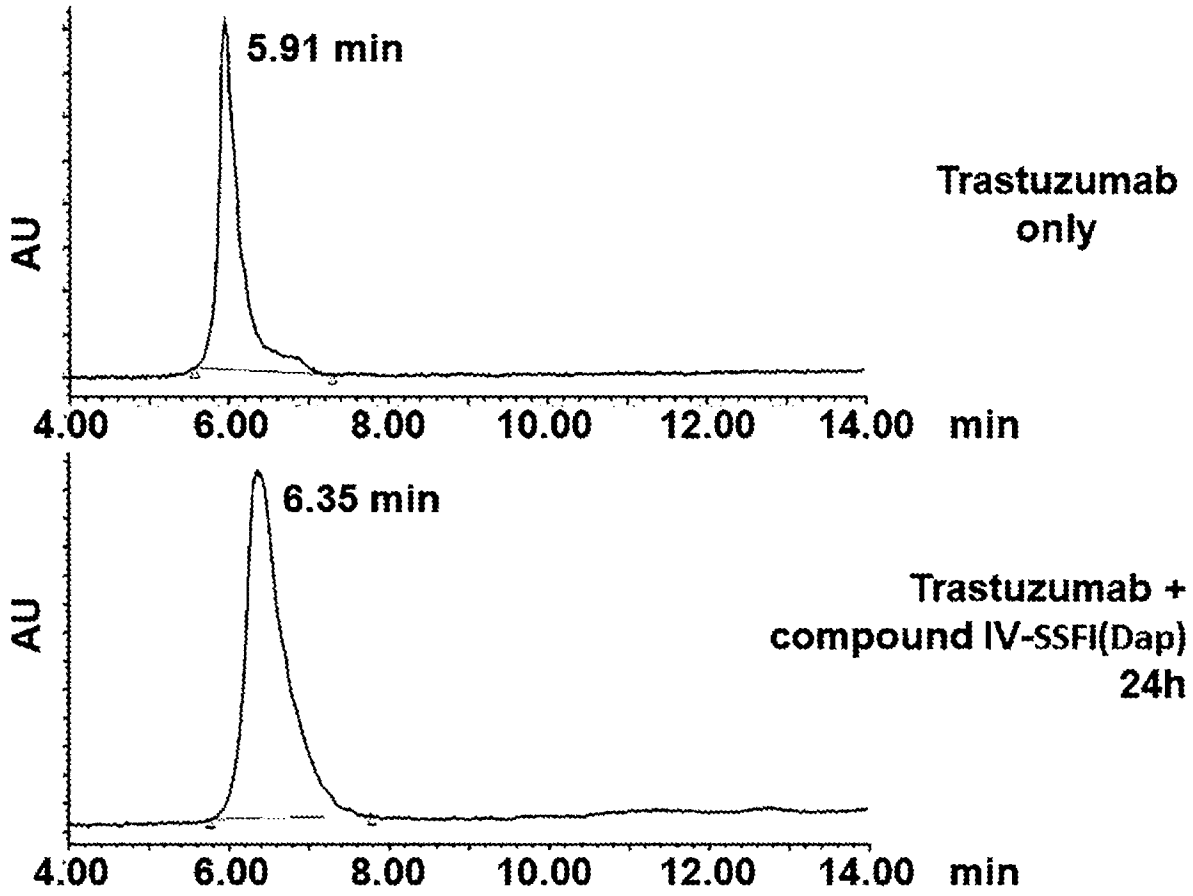

5.91 min
AU
Trastuzumab only
4.00
6.00
8.00
10.00
12.00
14.00
min
6.35 min
AU
Trastuzumab + compound IV-SSFI(Dap) 24h
4.00
6.00
8.00
10.00
12.00
14.00
min

FIG. 48

Trastuzumab only (Fc/2)

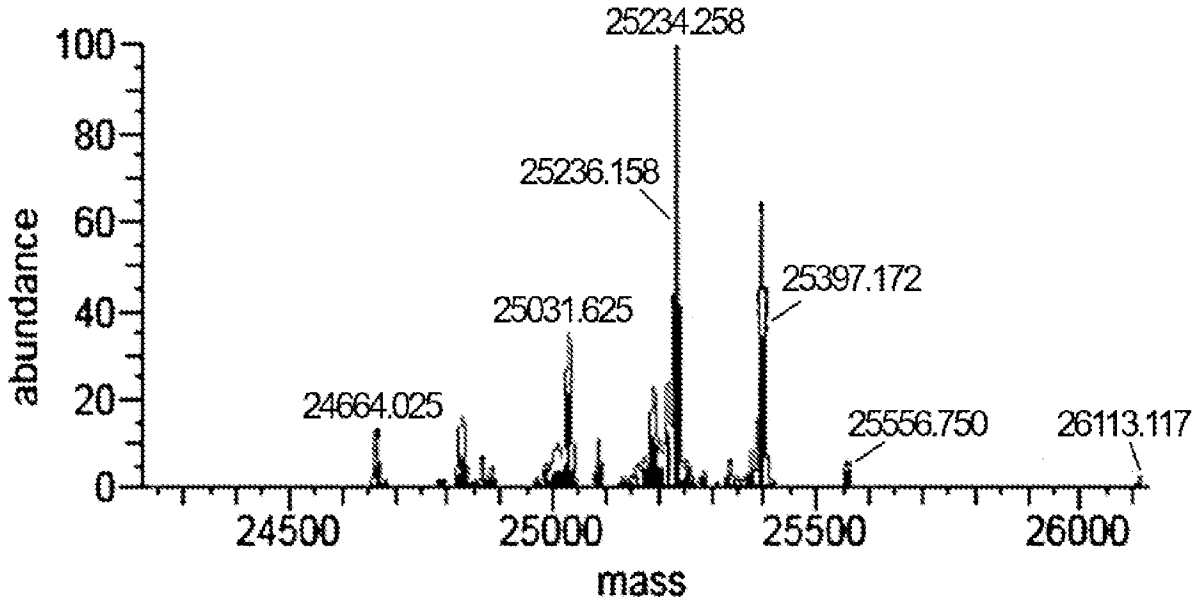


FIG. 49

Trastuzumab-norbornene conjugate (Fc/2)

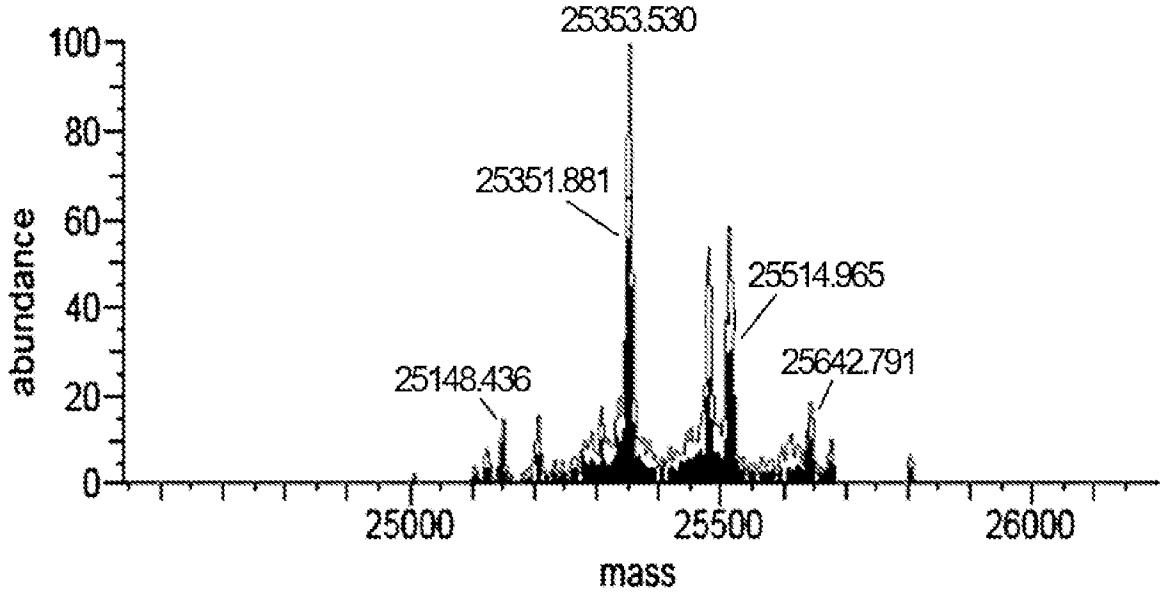


FIG. 50

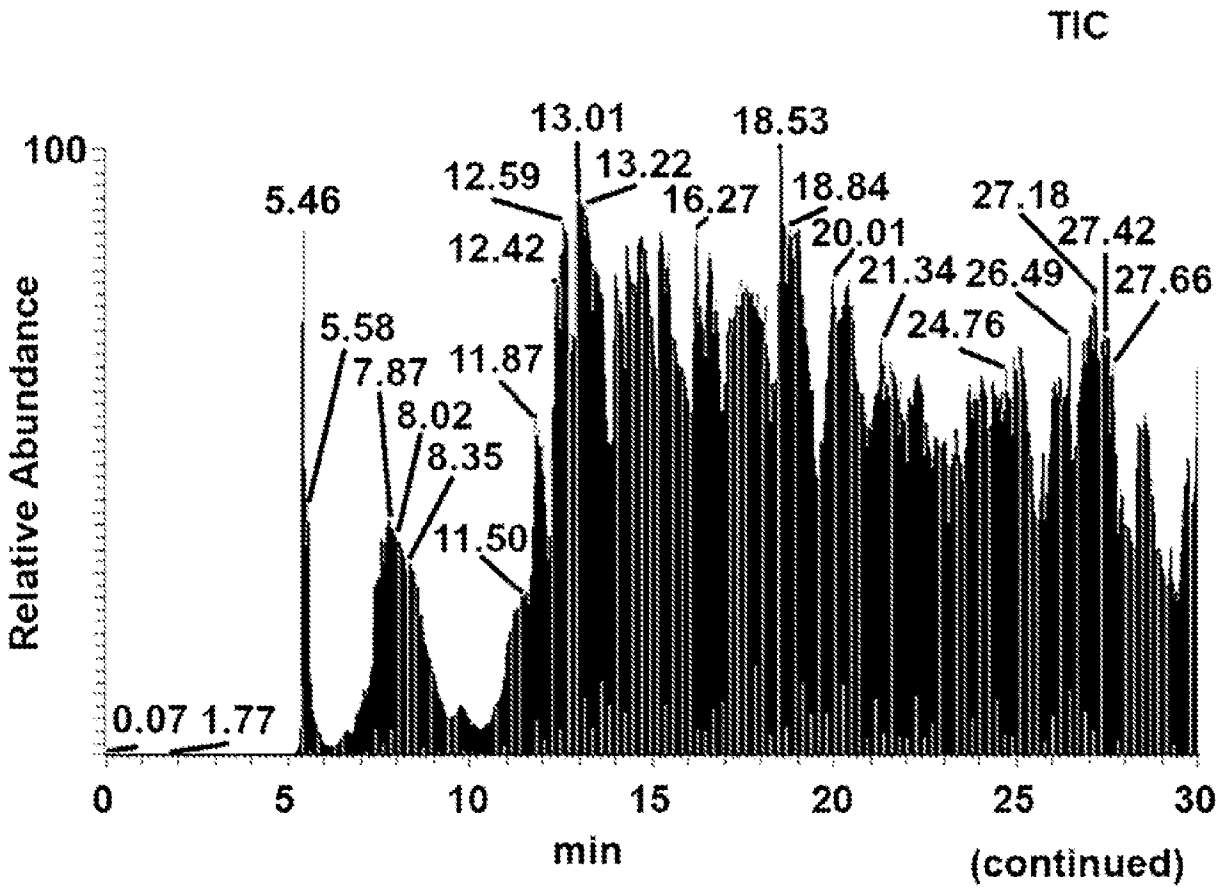

TIC
100
Relative Abundance
13.01
18.53
12.59
13.22
5.46
16.27
18.84
27.18
12.42
20.01
27.42
21.34
26.49
27.66
5.58
24.76
7.87
11.87
8.02
8.35
11.50
0.07
1.77
0
5
10
15
20
25
30
min
(continued)

FIG. 51

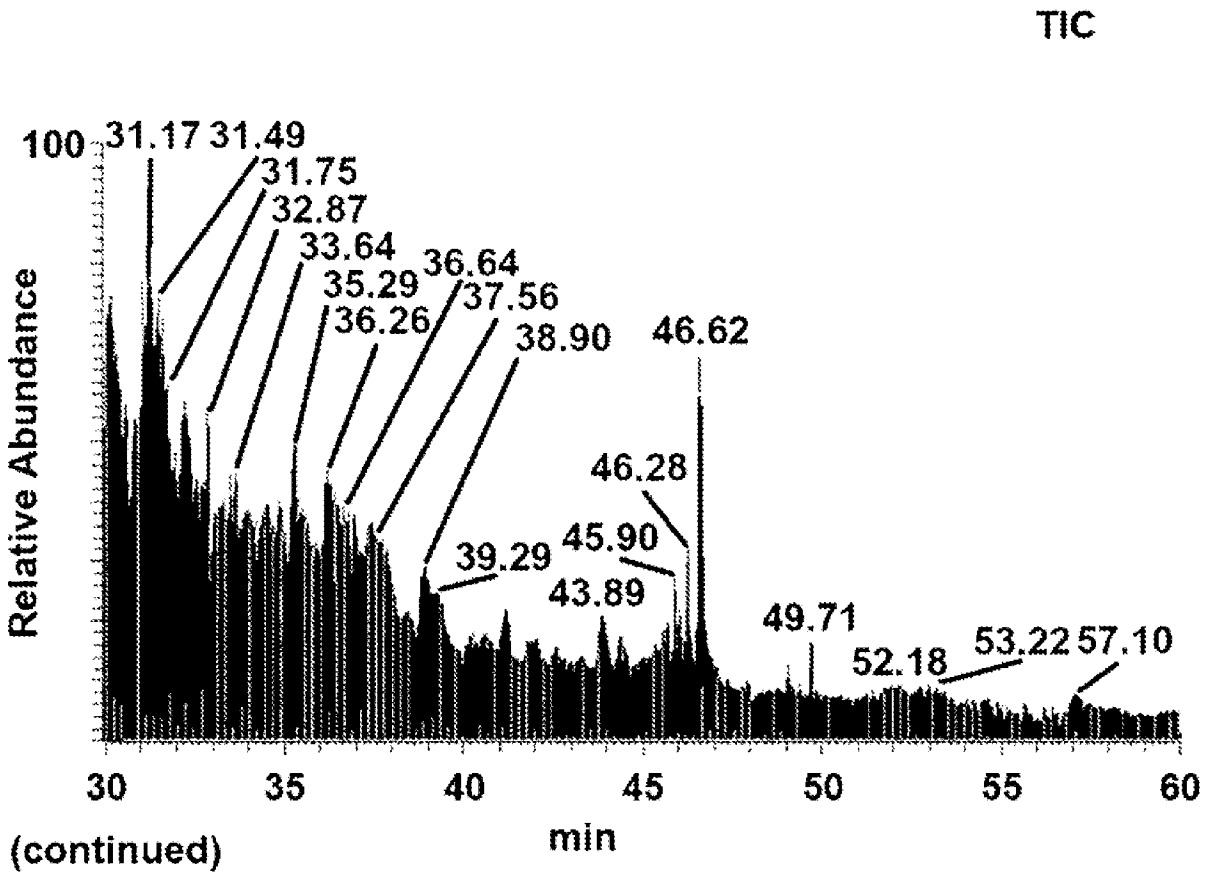

TIC
100
31.17
31.49
31.75
32.87
33.64
35.29
36.26
36.64
37.56
38.90
46.62
46.28
45.90
39.29
43.89
49.71
52.18
53.22
57.10
Relative Abundance
30
35
40
45
50
55
60
min
(continued)

FIG. 52

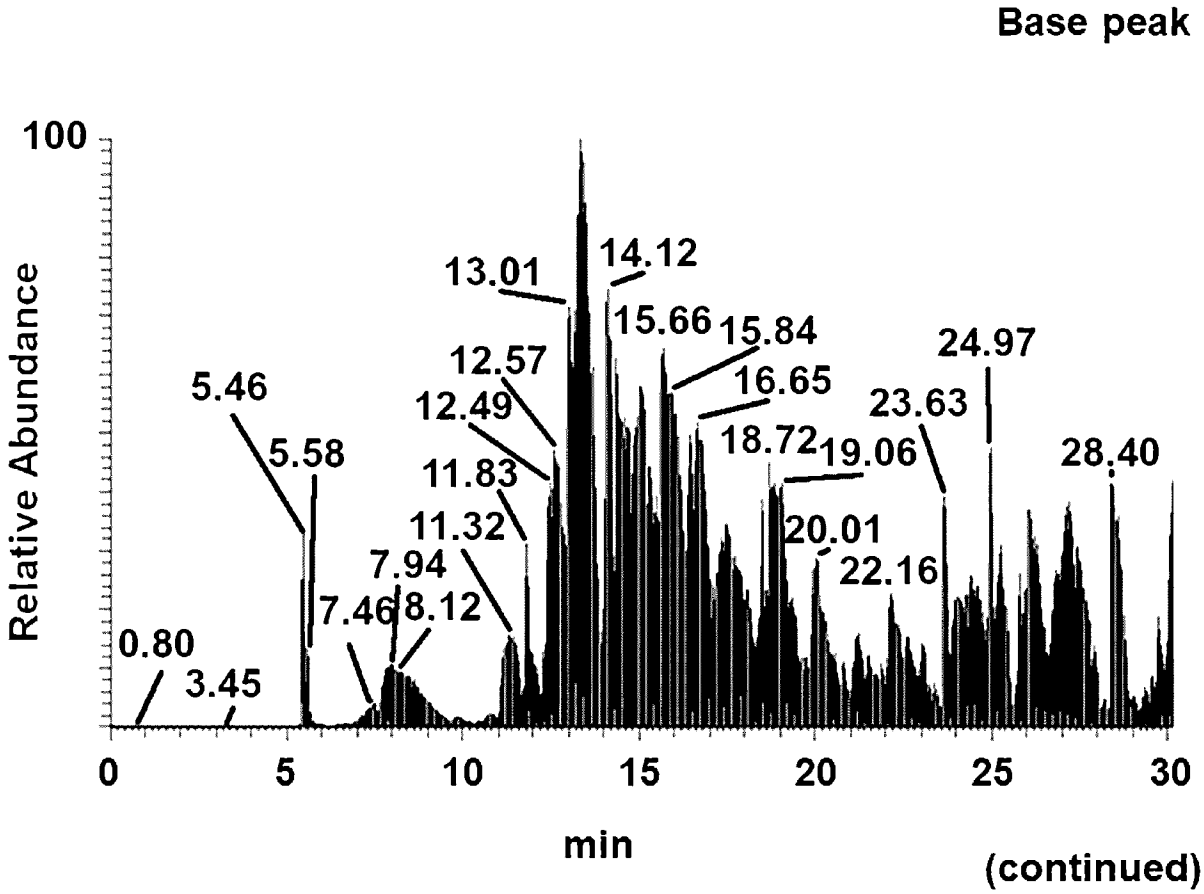

Base peak
100
Relative Abundance
13.01
14.12
15.66
15.84
24.97
12.57
5.46
16.65
12.49
23.63
5.58
18.72
19.06
28.40
11.83
11.32
20.01
7.94
22.16
7.46
8.12
0.80
3.45
0
5
10
15
20
25
30
min
(continued)

FIG. 53

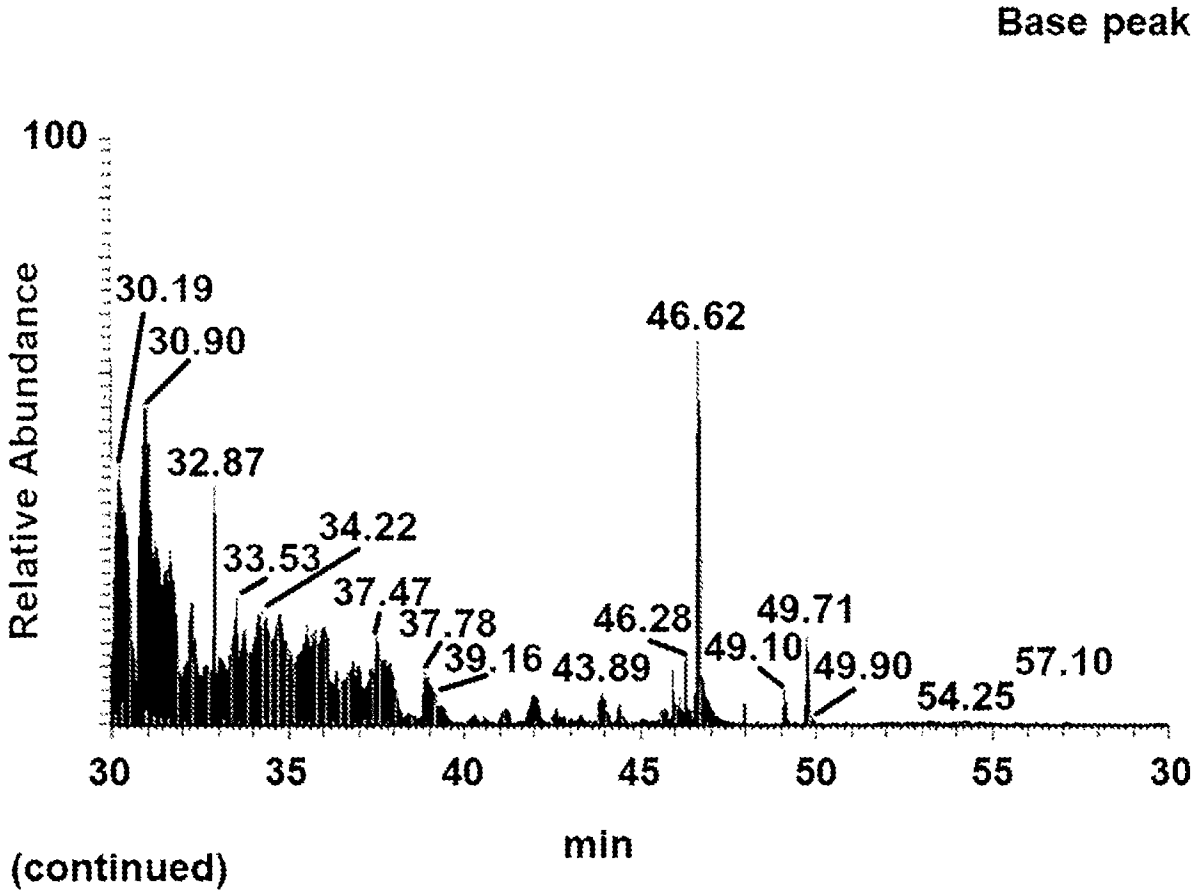

Base peak
100
Relative Abundance
30.19
30.90
32.87
33.53
34.22
37.47
37.78
39.16
43.89
46.28
46.62
49.10
49.71
49.90
54.25
57.10
30
35
40
45
50
55
30
min
(continued)

FIG. 54

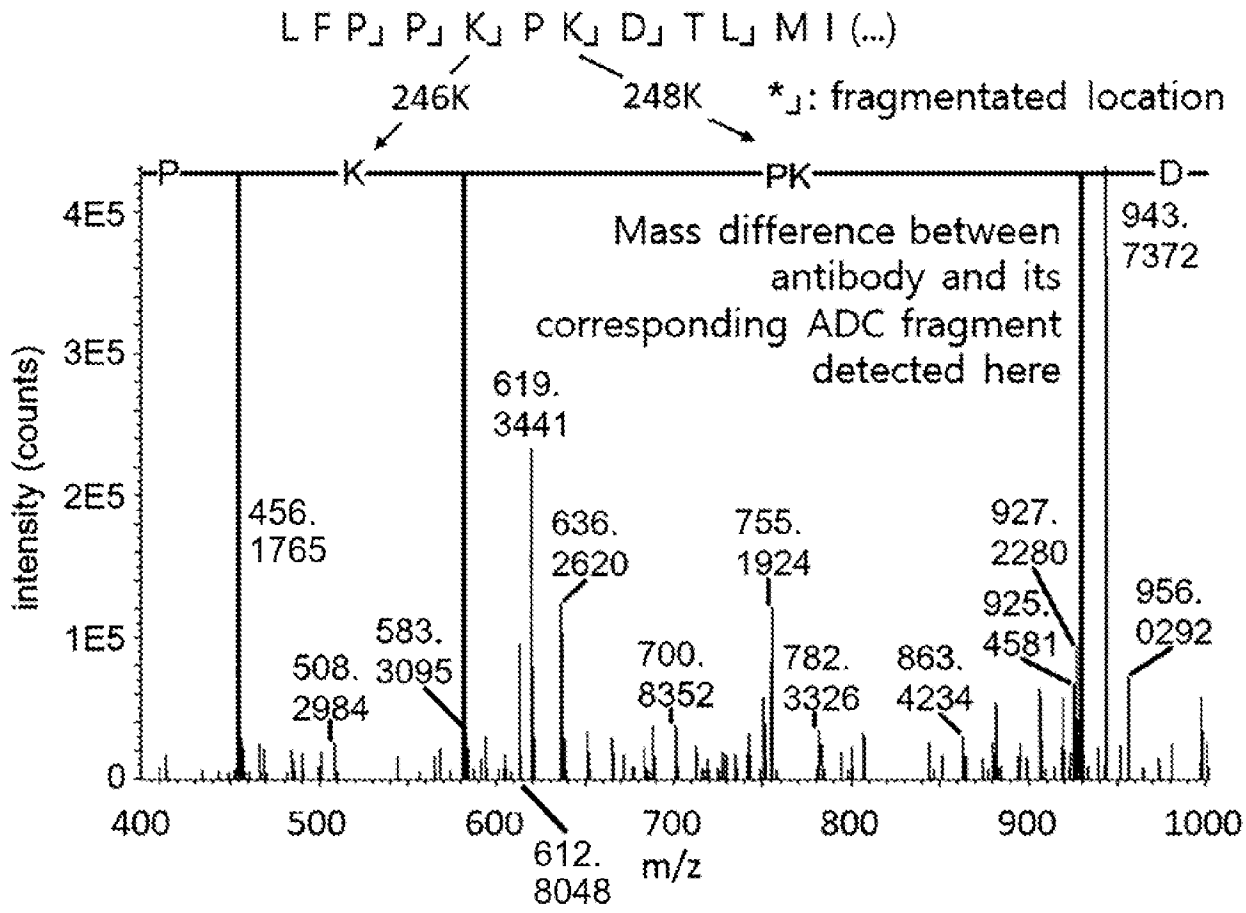

L F P」 P」 K」 P K」 D」 T L」 M I (...)
246K
248K
*」: fragmentated location
P
K
PK
D
Mass difference between antibody and its corresponding ADC fragment detected here
943. 7372
619. 3441
456. 1765
636. 2620
755. 1924
927. 2280
925. 4581
956. 0292
583. 3095
508. 2984
700. 8352
782. 3326
863. 4234
612. 8048
intensity (counts)
4E5
3E5
2E5
1E5
0
400
500
600
700
800
900
1000
m/z

FIG. 55

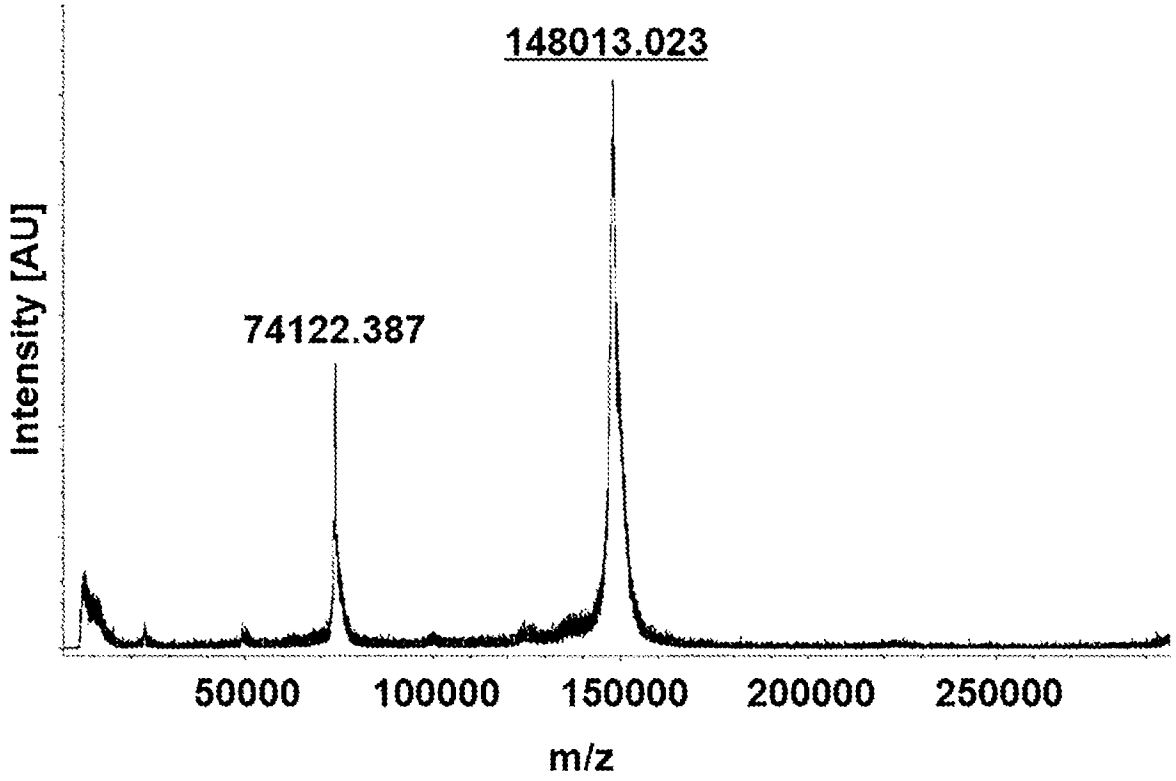

148013.023
74122.387
Intensity [AU]
50000
100000
150000
200000
250000
m/z

FIG. 56

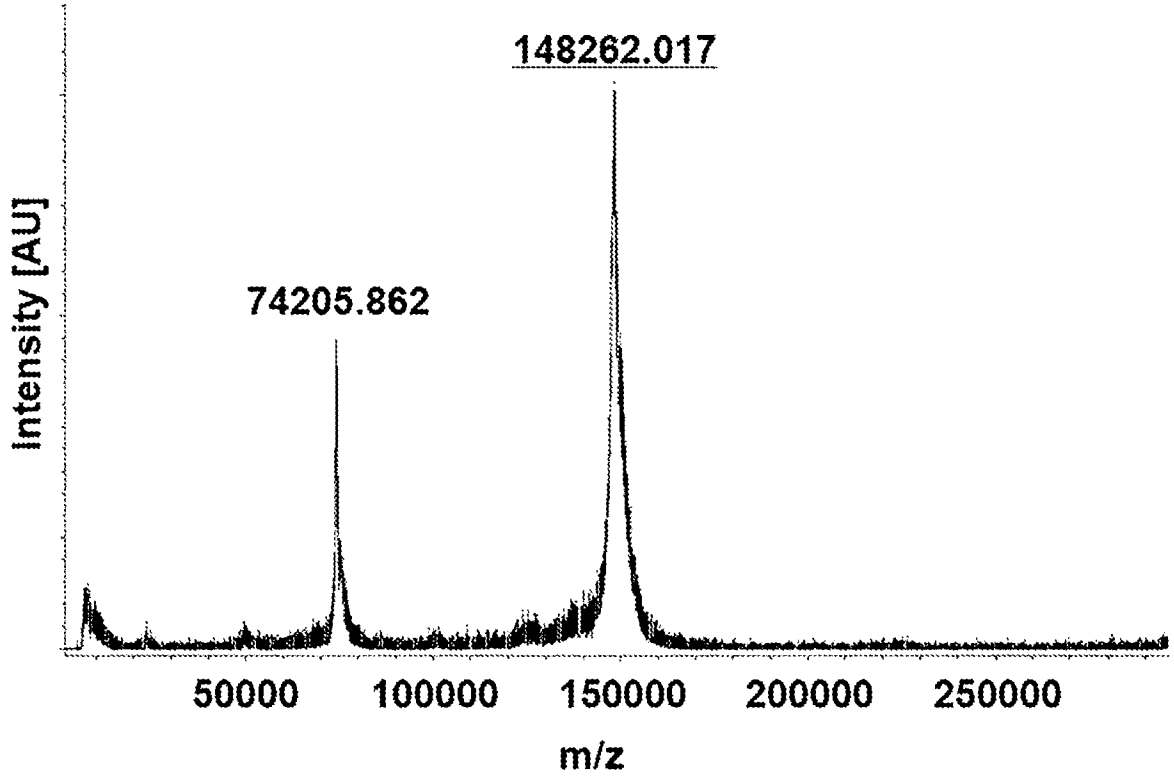

148262.017
74205.862
Intensity [AU]
50000
100000
150000
200000
250000
m/z

FIG. 57

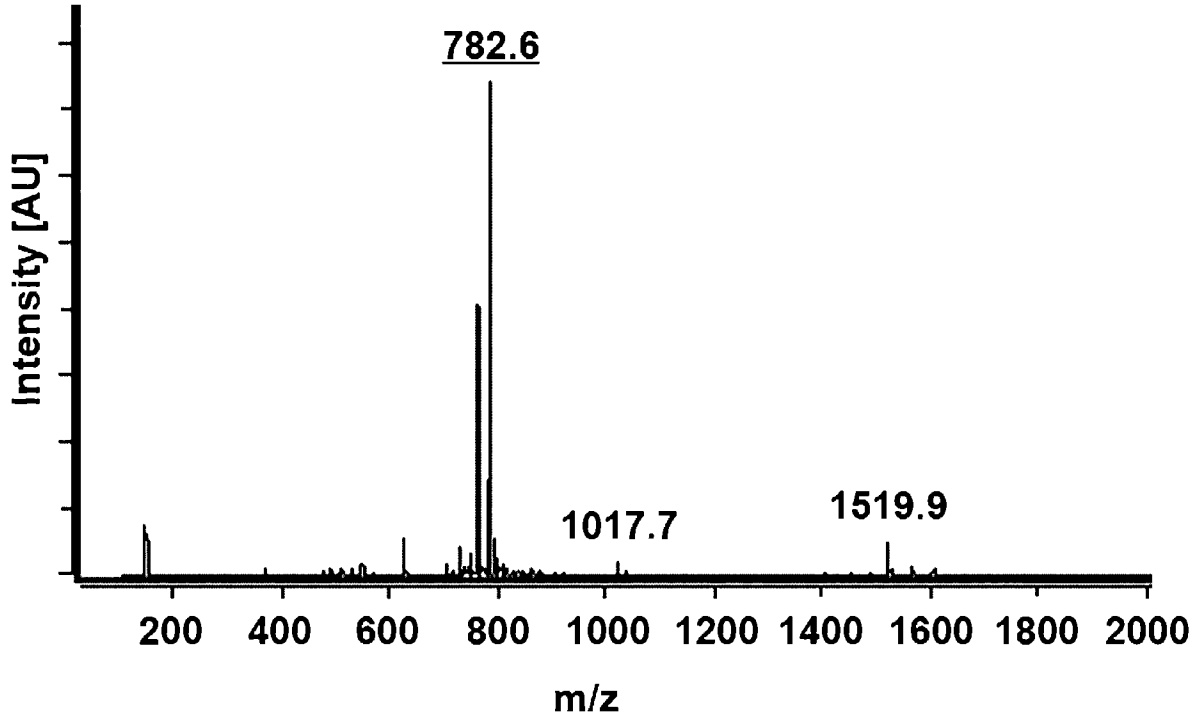

782.6
1017.7
1519.9
Intensity [AU]
200
400
600
800
1000
1200
1400
1600
1800
2000
m/z

FIG. 58

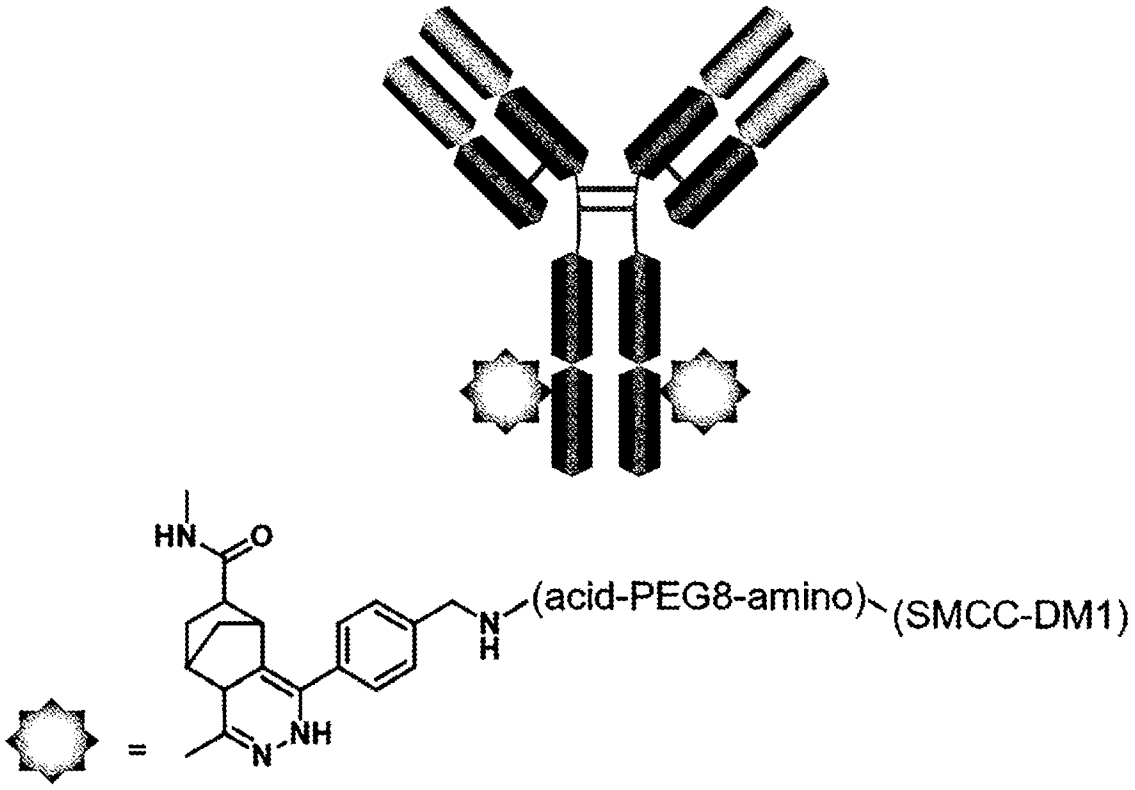


FIG. 59

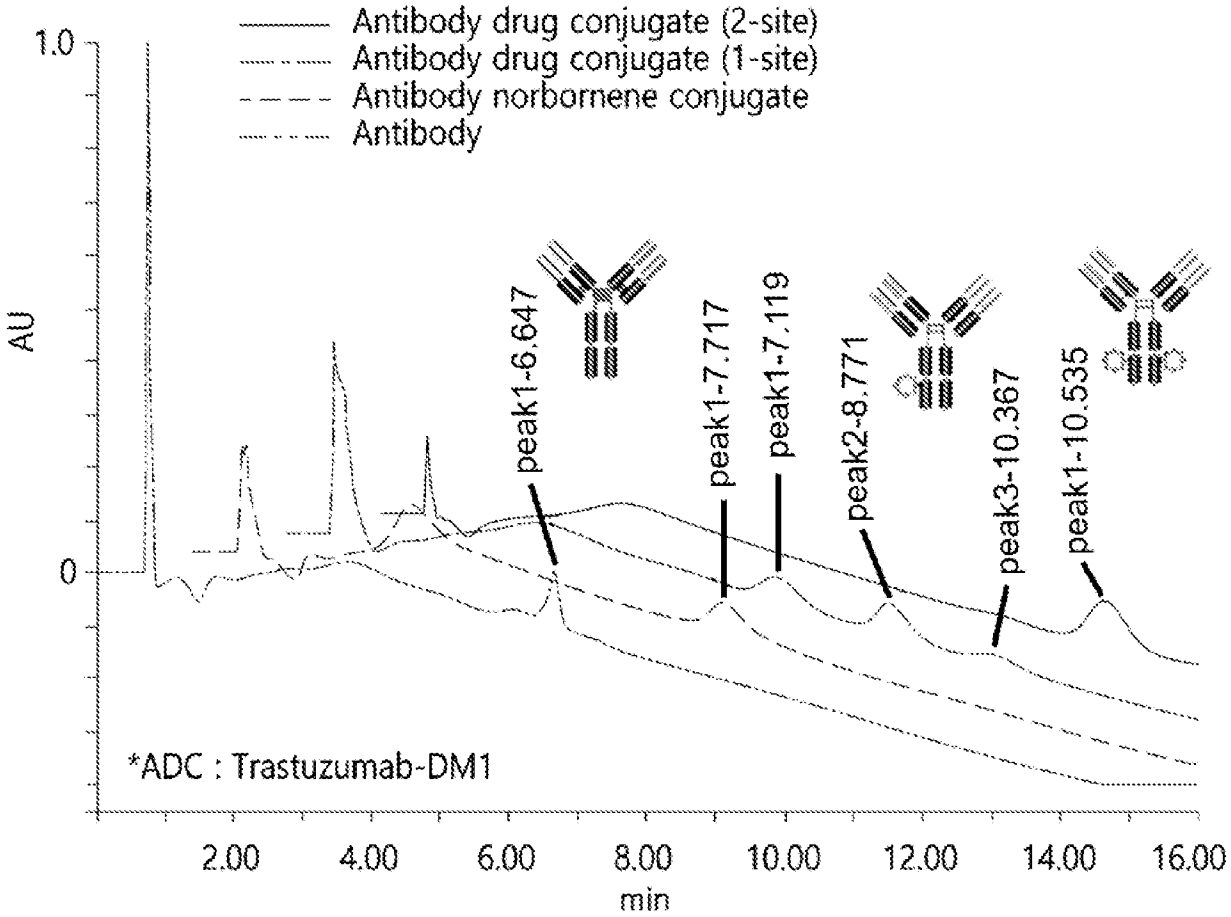

Antibody drug conjugate (2-site)
Antibody drug conjugate (1-site)
Antibody norbornene conjugate
Antibody
1.0
AU
0
peak1-6.647
peak1-7.717
peak1-7.119
peak2-8.771
peak3-10.367
peak1-10.535
*ADC : Trastuzumab-DM1
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
min

FIG. 60

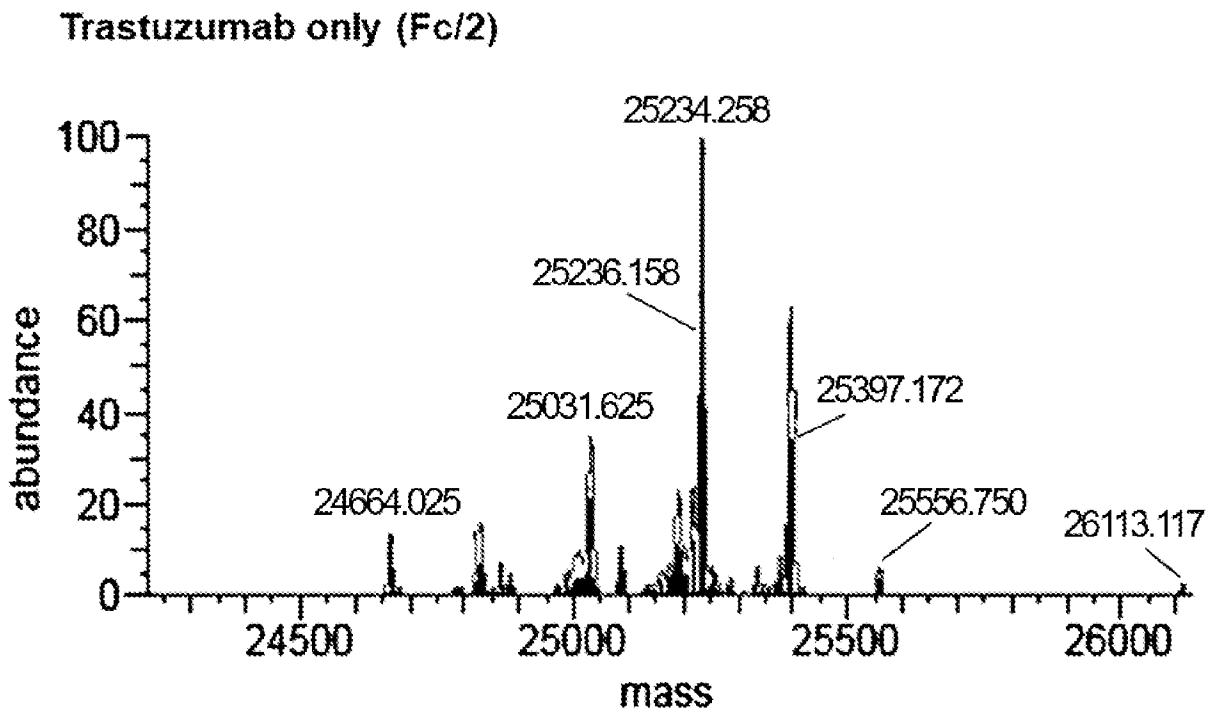

Trastuzumab only (Fc/2)
25234.258
25236.158
25397.172
25031.625
24664.025
25556.750
26113.117
100
80
60
40
20
0
abundance
24500
25000
25500
26000
mass

FIG. 61

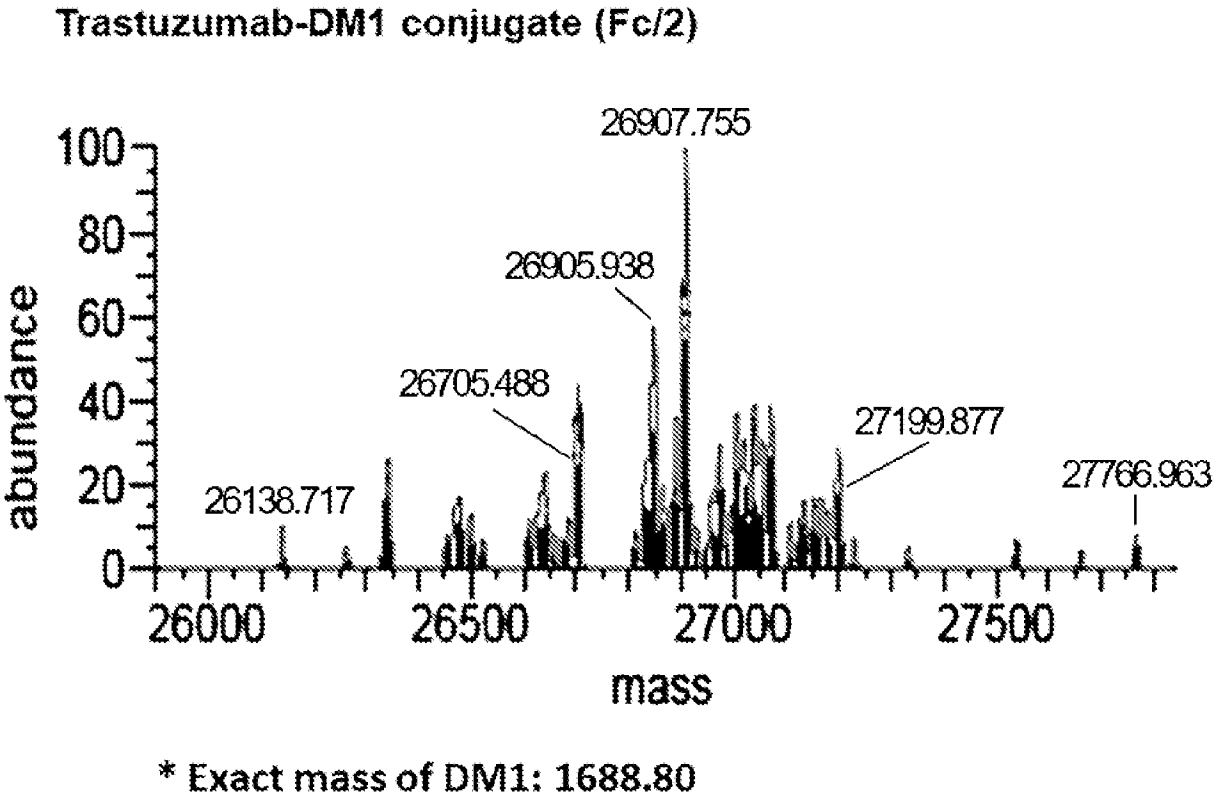
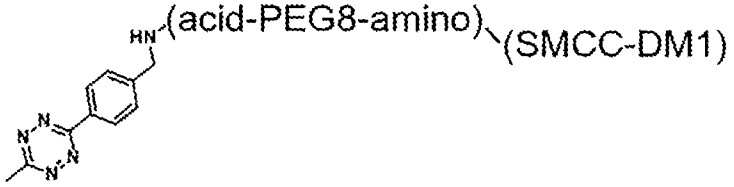
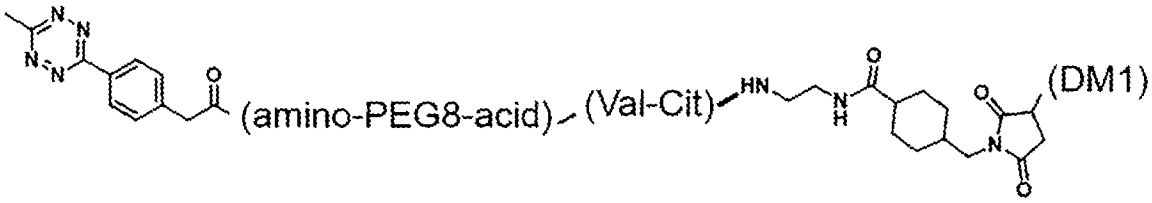

Trastuzumab-DM1 conjugate (Fc/2)
26907.755
26905.938
26705.488
27199.877
27766.963
26138.717
100
80
60
40
20
0
abundance
26000
26500
27000
27500
mass
* Exact mass of DM1: 1688.80

FIG. 62

1. NC-DM1 : non-cleavable DM1 (tetrazine-PEG8-DM1)



2. VC-DM1 : cleavable DM1 (tetrazine-PEG8-valine-citrulline-DM1)



3. VC-MMAE : cleavable DM1 (tetrazine-PEG8-valine-citrulline-PAB-MMAE)

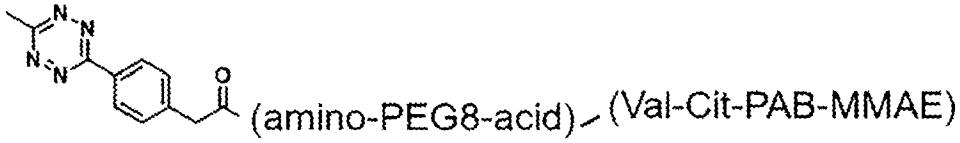


FIG. 63

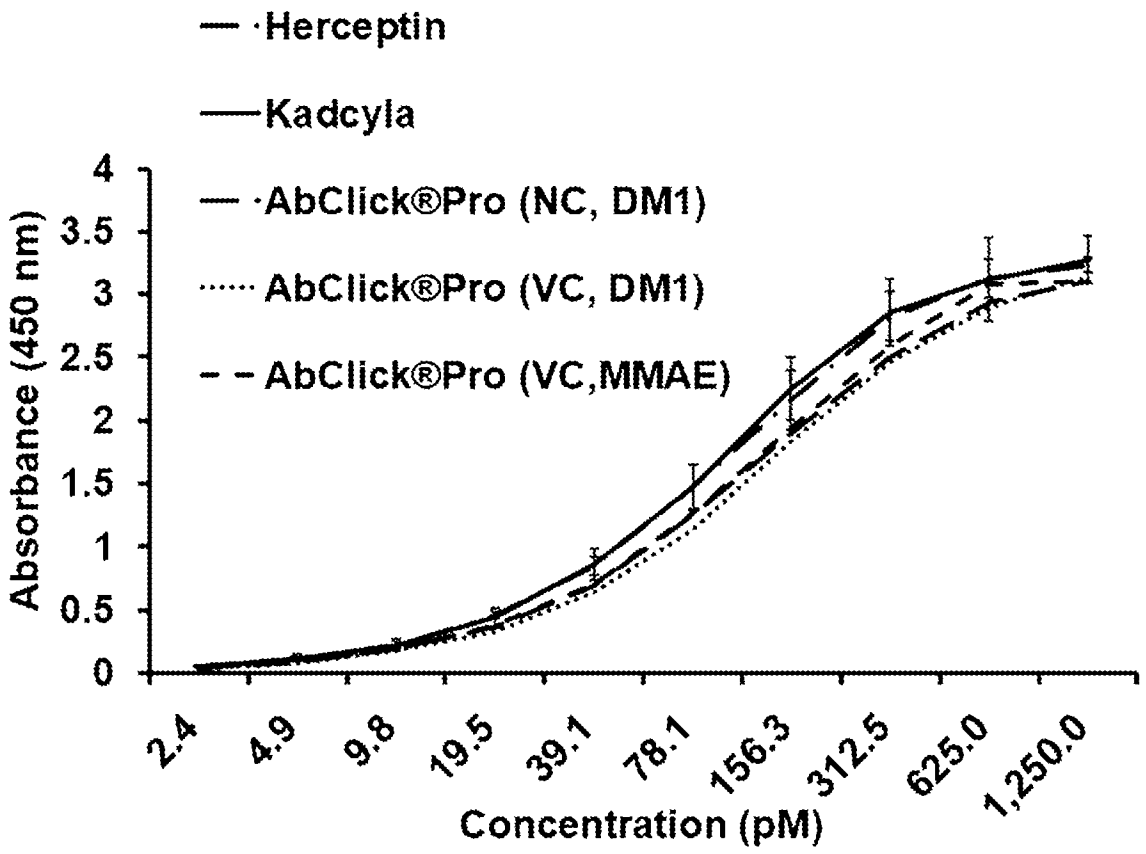

Herceptin
Kadcyla
AbClick®Pro (NC, DM1)
AbClick®Pro (VC, DM1)
AbClick®Pro (VC,MMAE)
Absorbance (450 nm)
0
0.5
1
1.5
2
2.5
3
3.5
4
2.4
4.9
9.8
19.5
39.1
78.1
156.3
312.5
625.0
1,250.0
Concentration (pM)

FIG. 64

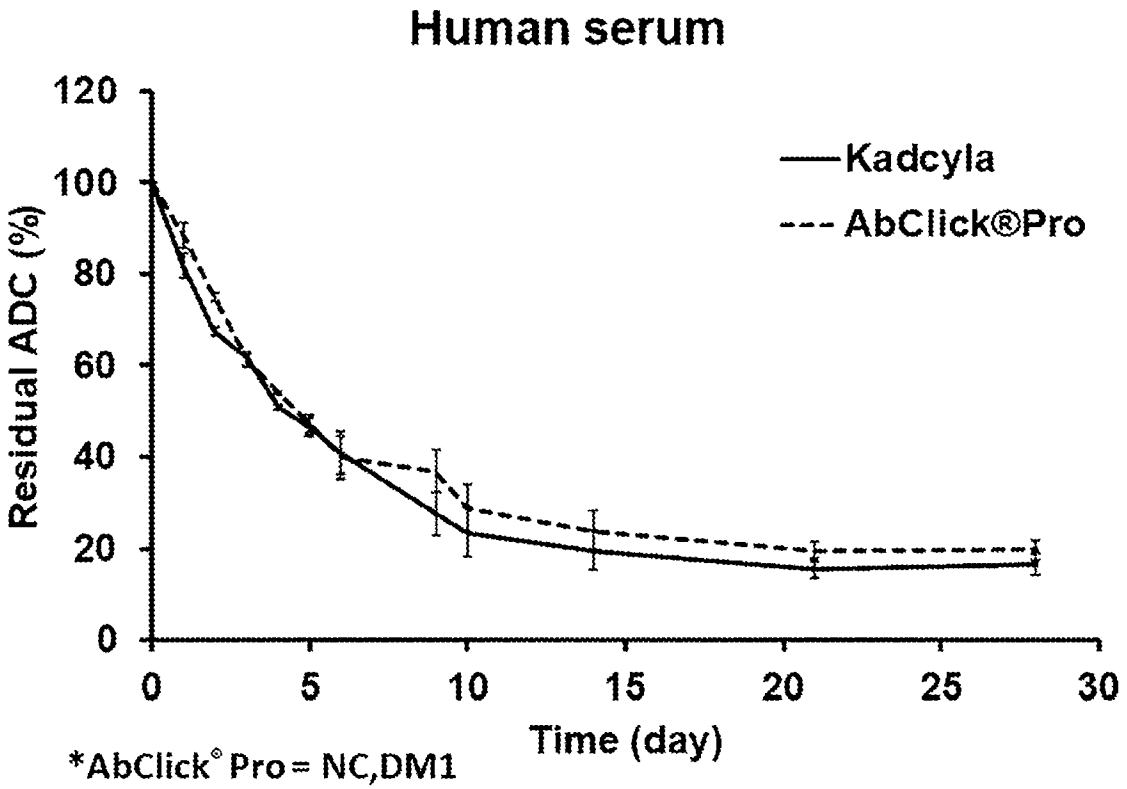

Human serum
Residual ADC (%)
120
100
80
60
40
20
0
0
5
10
15
20
25
30
Time (day)
Kadcyla
AbClick®Pro
*AbClick® Pro = NC,DM1

FIG. 65

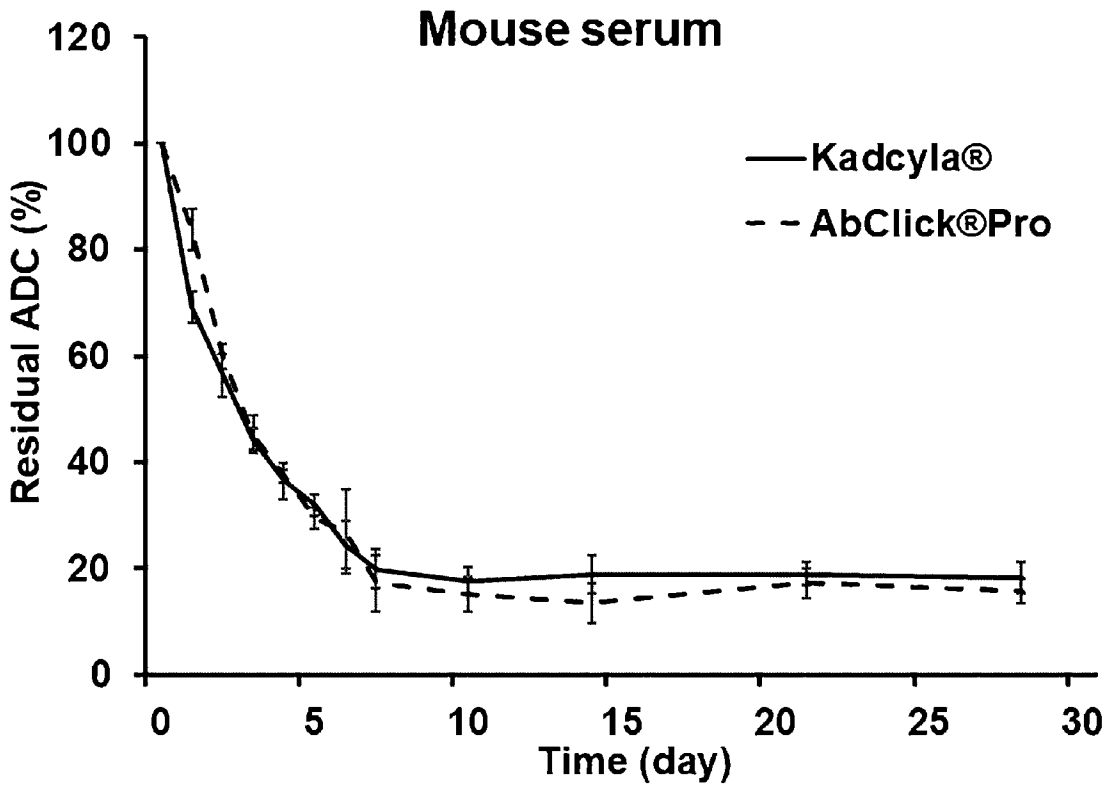

Mouse serum
Kadcyla®
AbClick®Pro
Residual ADC (%)
120
100
80
60
40
20
0
0
5
10
15
20
25
30
Time (day)

FIG. 66

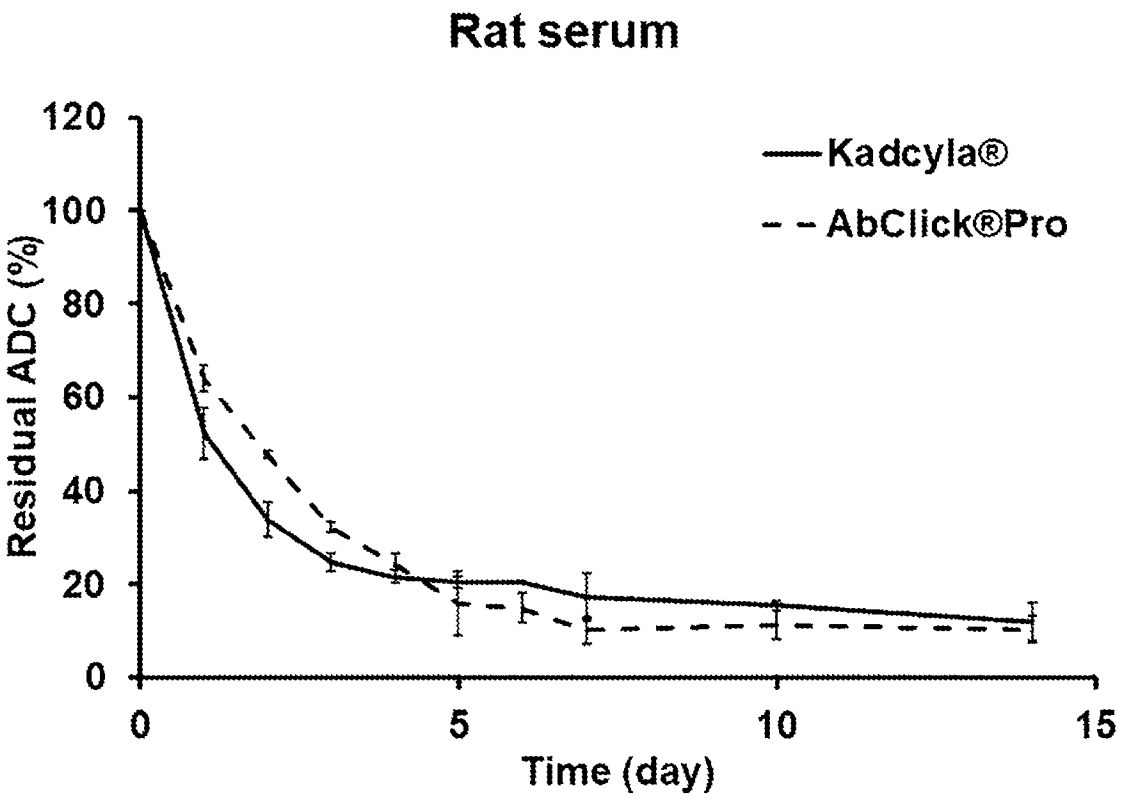

Rat serum
Kadcyla®
AbClick®Pro
Residual ADC (%)
120
100
80
60
40
20
0
0
5
10
15
Time (day)

FIG. 67

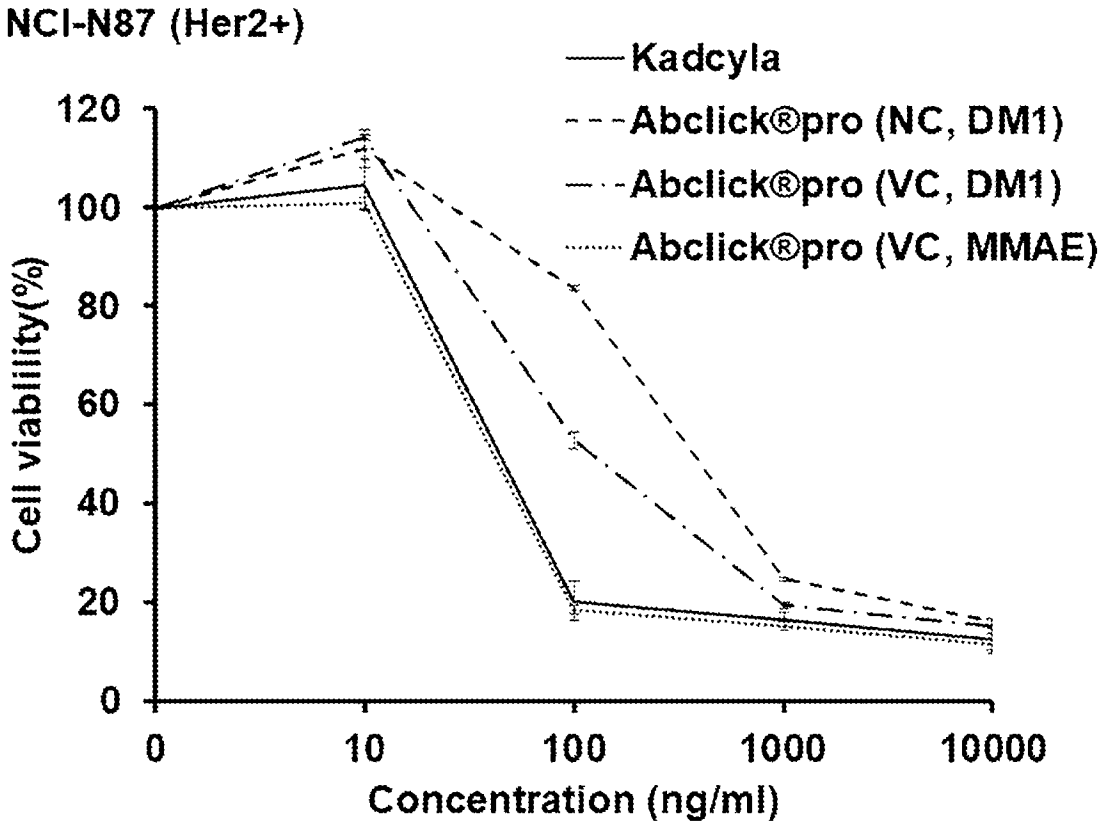

NCI-N87 (Her2+)
Kadcyla
Abclick®pro (NC, DM1)
Abclick®pro (VC, DM1)
Abclick®pro (VC, MMAE)
Cell viability(%)
120
100
80
60
40
20
0
0
10
100
1000
10000
Concentration (ng/ml)

FIG. 68

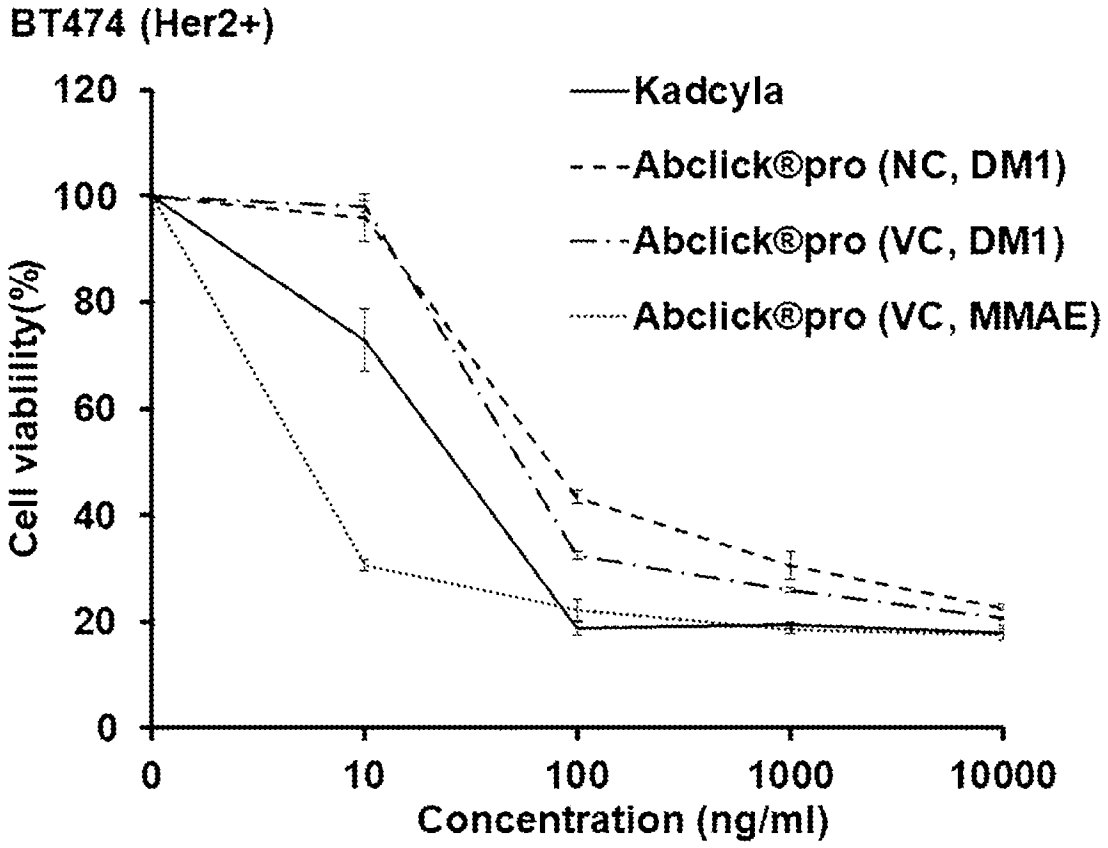

BT474 (Her2+)
Kadcyla
Abclick®pro (NC, DM1)
Abclick®pro (VC, DM1)
Abclick®pro (VC, MMAE)
Cell viability(%)
120
100
80
60
40
20
0
0
10
100
1000
10000
Concentration (ng/ml)

FIG. 69

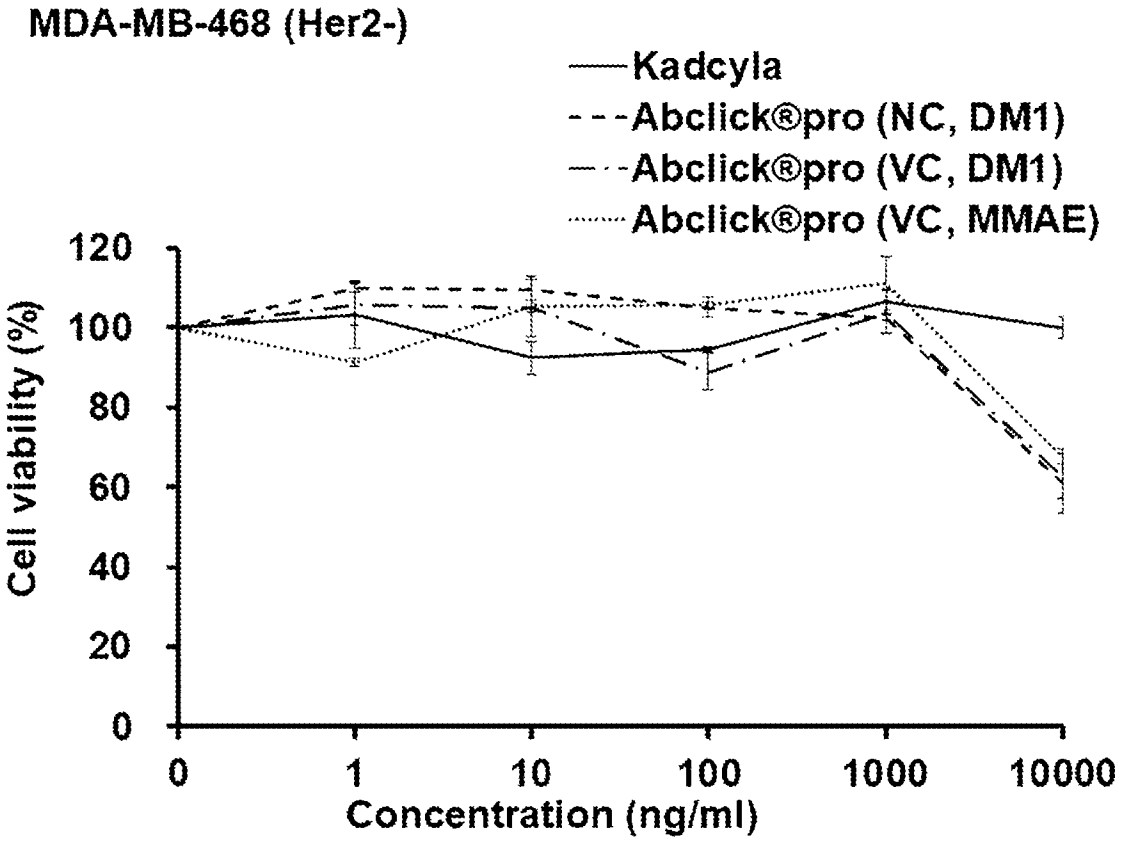

MDA-MB-468 (Her2-)
Kadcyla
Abclick®pro (NC, DM1)
Abclick®pro (VC, DM1)
Abclick®pro (VC, MMAE)
Cell viability (%)
120
100
80
60
40
20
0
0
1
10
100
1000
10000
Concentration (ng/ml)

FIG. 70

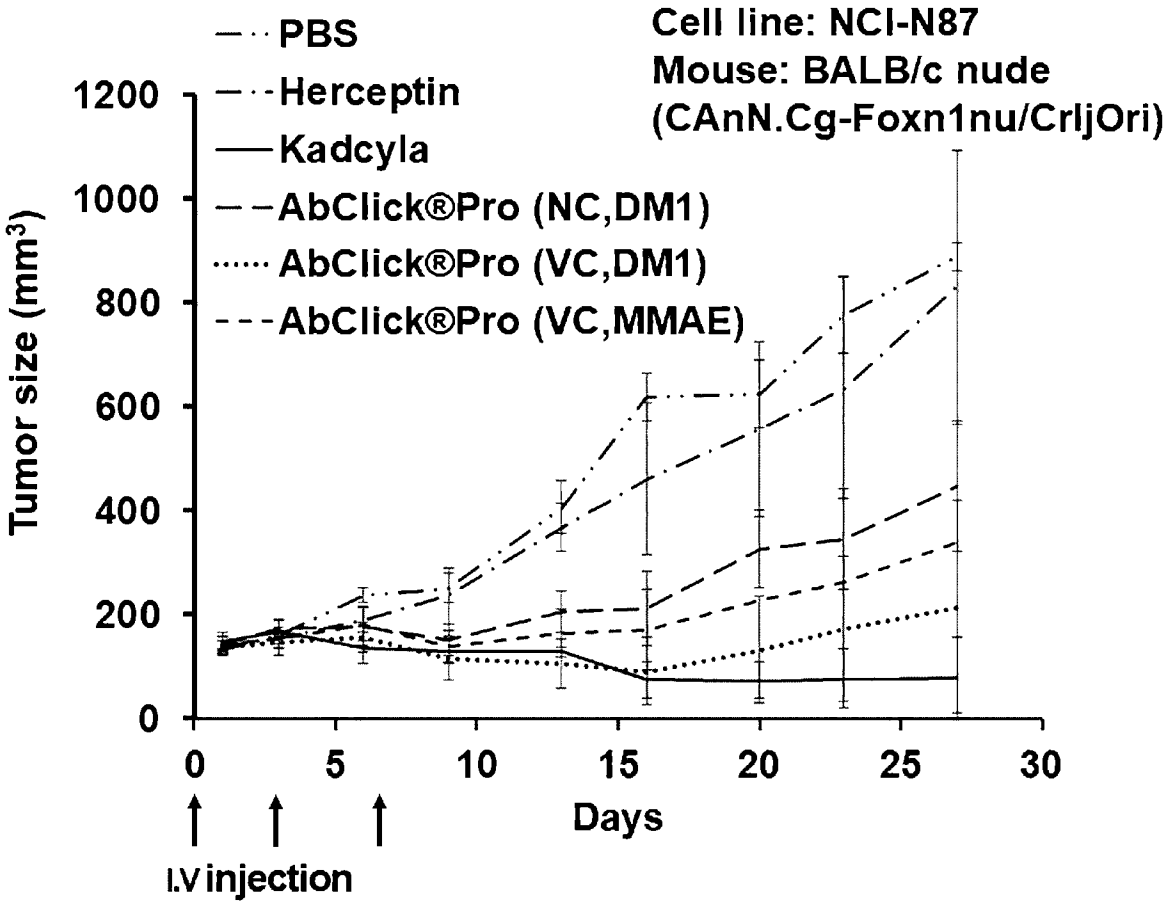

PBS
Herceptin
Kadcyla
AbClick®Pro (NC,DM1)
AbClick®Pro (VC,DM1)
AbClick®Pro (VC,MMAE)
Cell line: NCI-N87
Mouse: BALB/c nude
(CAnN.Cg-Foxn1nu/CrljOri)
Tumor size (mm3)
1200
1000
800
600
400
200
0
0
5
10
15
20
25
30
Days
I.V injection

FIG. 71

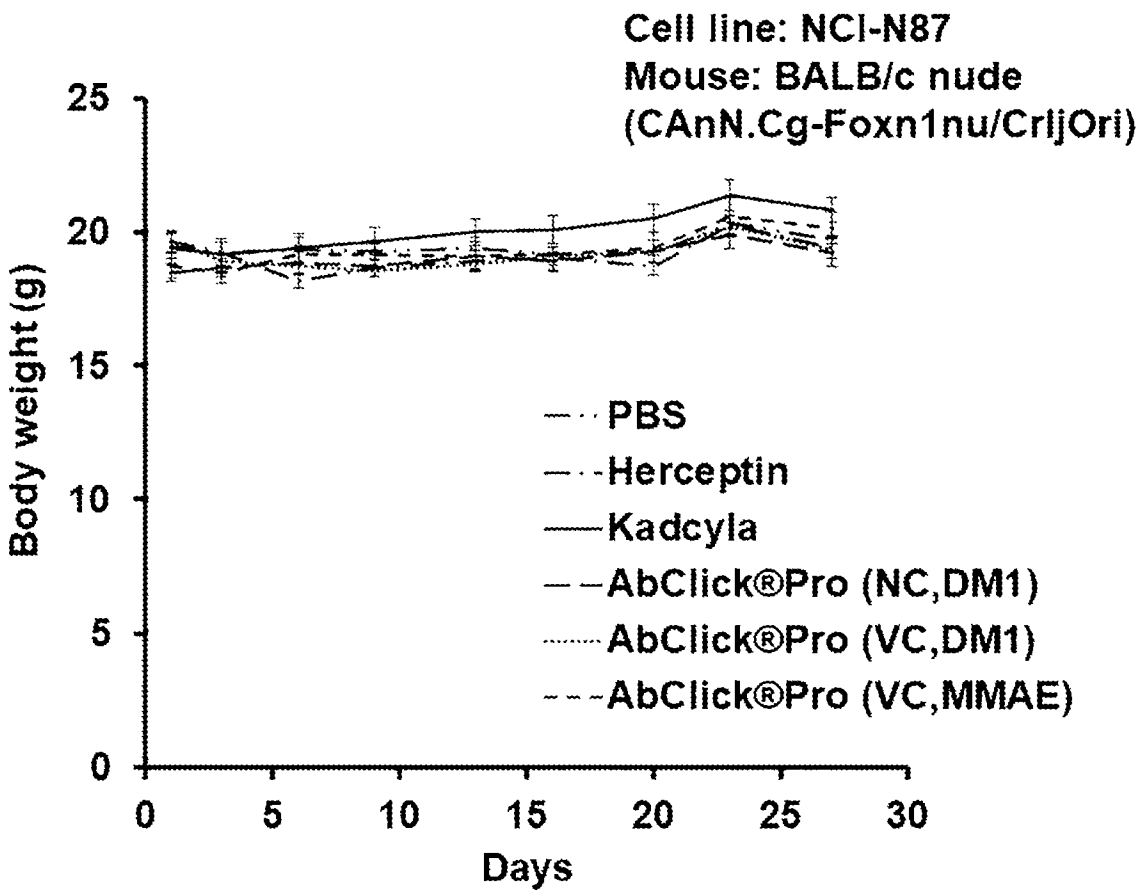

Cell line: NCI-N87
Mouse: BALB/c nude
(CAnN.Cg-Foxn1nu/CrljOri)
Body weight (g)
25
20
15
10
5
0
0
5
10
15
20
25
30
Days
PBS
Herceptin
Kadcyla
AbClick®Pro (NC,DM1)
AbClick®Pro (VC,DM1)
AbClick®Pro (VC,MMAE)

FIG. 72

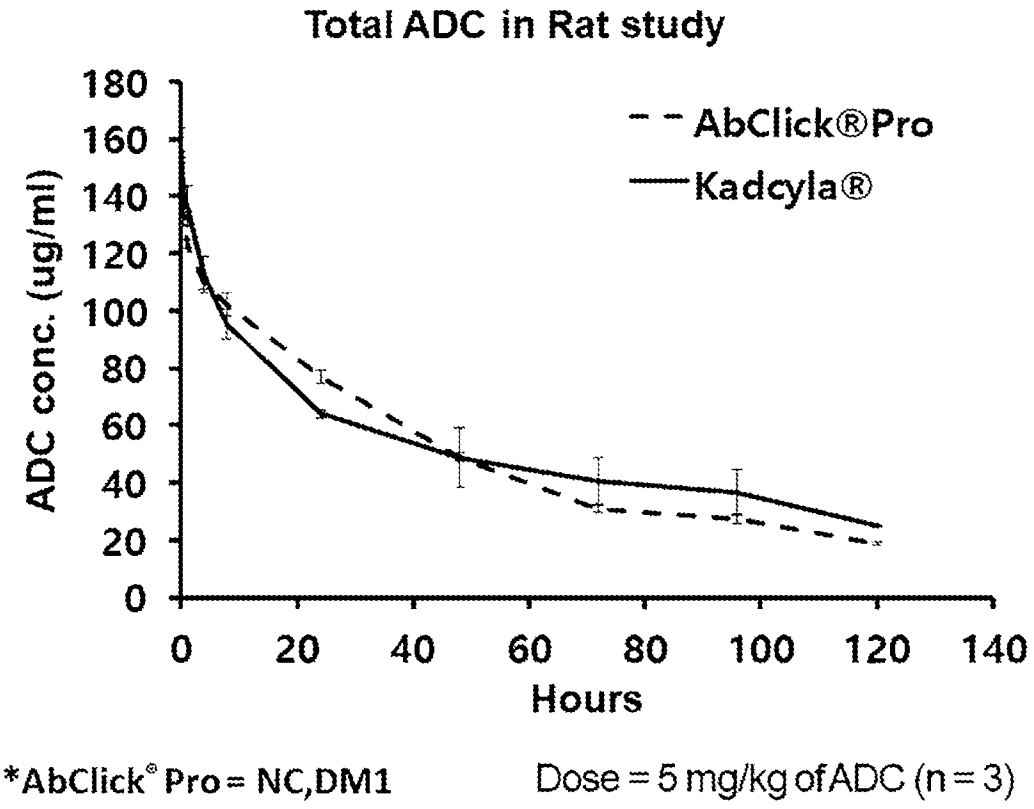

Total ADC in Rat study
ADC conc. (ug/ml)
180
160
140
120
100
80
60
40
20
0
0
20
40
60
80
100
120
140
Hours
AbClick®Pro
Kadcyla®
*AbClick® Pro = NC,DM1
Dose = 5 mg/kg of ADC (n = 3)

FIG. 73

AMENDMENTS TO THE DRAWINGS

The attached "Replacement Sheets" of drawings include changes to Figures 51 and 52. The attached "Replacement Sheets" which include Figures 1-73, replace the original sheets including Figures 1-73.

Attachment: Replacement Sheets

REMARKS

Applicant has amended the drawings (Figs. 51 and 52) wherein reference numbers were truncated and are now removed entirely as required by the Notice to File Corrected Application Papers received.

Although Applicant believes no fee is due in connection with this filing, the Patent Office is hereby authorized to charge any required fee or credit any overpayment to Deposit Account No. **08-0750**.

If the Examiner believes that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at (314) 446-7670.

Respectfully submitted,

Dated: 10 January 2023

By: / Kisuk Lee /
Kisuk Lee, Reg. No. 66,861

HARNESS, DICKEY & PIERCE, P.L.C.
7700 Bonhomme, Suite 400
St. Louis, MO 63105
314-446-7670 (telephone)
314-726-7501 (facsimile)

SCORE Placeholder Sheet for IFW Content

Application Number: 18154998 Document Date: 01/10/2024

The presence of this form in the IFW record indicates that the following document type was received in electronic format on the date identified above. This content is stored in the SCORE database.

Since this was an electronic submission, there is no physical artifact folder, no artifact folder is recorded in PALM, and no paper documents or physical media exist. The TIFF images in the IFW record were created from the original documents that are stored in SCORE.

- Drawing

At the time of document entry (noted above):

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- External customers may access SCORE content via PAIR using the Supplemental Content tab.

Form Revision Date: March 1, 2019

PART B–FEE(S) TRANSMITTAL

Complete and send this form, together with the applicable fee(s), by mail or fax, or via the USPTO patent electronic filing system.

By mail, send to: Mail Stop ISSUE FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

By fax, send to: (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 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. **Because electronic patent issuance may occur shortly after issue fee payment, any desired continuing application should preferably be filed prior to payment of this issue fee in order not to jeopardize copendency.**

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

HARNESS, DICKEY & PIERCE, P.L.C.
7700 Bonhomme
Suite 400
Clayton, Missouri 63105

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 transmitted to the USPTO via the USPTO patent electronic filing system or by facsimile to (571) 273-2885, on the date below.

Kisuk Lee	(Typed or printed name)
/Kisuk Lee/	(Signature)
8 December 2023	(Date)

APPLICATION NO	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO	CONFIRMATION NO
18/154,998	01/16/2023	Sang Jeon CHUNG	18896-000072-US-COA	5143

TITLE OF INVENTION: SITE-SPECIFIC ANTIBODY CONJUGATION AND ANTIBODY-DRUG CONJUGATE AS SPECIFIC EMBODIMENT THEREOF

APPLN: TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	SMALL	$480.00	$0	$0	$480.00	03/07/2024

EXAMINER	ART UNIT	CLASS-SUBCLASS
Y. Xiao	1642	424/133.100

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/AIA/47 or PTO/SB/47; Rev 03-09 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. Harness, Dickey & Pierce, P.L.C.
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 must have been previously recorded, or filed for recordation, as set forth in 37 CFR 3.11 and 37 CFR 3.81(a). Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE: AbTis Co., Ltd.

(B) RESIDENCE: (CITY and STATE or COUNTRY): Gyeonggi-do, Republic of Korea

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

4a. Fees Submitted: ☒ Issue Fee ☐ Publication Fee (if required)

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

☒ Electronic Payment via the USPTO patent electronic filing system ☐ Enclosed check ☐ Non-electronic payment by credit card (Attach form PTO-2038)

☒ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment to Deposit Account No. 08-0750

5. Change of Entity Status (from status indicated above)

☐ Applicant certifying micro entity status. See 37 CFR 1.29. NOTE: Absent a valid Certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.

☐ Applicant asserting small entity status. See 37 CFR 1.27. NOTE: If the application was previously under micro entity status, checking this box will be taken as a notification of loss of entitlement to micro entity status.

☐ Applicant changing to regular undiscounted fee status. NOTE: Checking this box will be taken as a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature: /Kisuk Lee/ Date: December 8, 2023

Typed or printed name: Kisuk Lee Registration No.: 66,861

UNITED STATES
PATENT AND TRADEMARK OFFICE

P.O. Box 1450
Alexandria, VA 22313 - 1450
www.uspto.gov

ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
18/154,998	**12/08/2023 12:44:04 PM Z ET**	**18896-000072-US-COA**

Title of Invention

SITE-SPECIFIC ANTIBODY CONJUGATION AND ANTIBODY-DRUG CONJUGATE AS SPECIFIC EMBODIMENT THEREOF

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	5143	FILED BY	Erica Imergoot
PATENT CENTER #	63543182	FILING DATE	01/16/2023
CUSTOMER #	28997	FIRST NAMED INVENTOR	Sang Jeon CHUNG
CORRESPONDENCE ADDRESS	-	AUTHORIZED BY	Kisuk Lee

Documents

TOTAL DOCUMENTS: 1

DOCUMENT	PAGES	DESCRIPTION	SIZE (KB)
000072uscoa_IssueFeeTransmittal.pdf	1	Issue Fee Payment (PTO-85B)	36 KB

Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)
000072uscoa_IssueFeeTransmittal.pdf	ECB947AA0D8E7C1F3F73D74DF7C6D412915BDD9A72CE5BDF09A869C972A878D1960DF1B45F888B91E778758F8DA115ECF17D5F698F9B2F7DBF384BD878592939

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 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.

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PATENT AND TRADEMARK OFFICE

P.O. Box 1450
Alexandria, VA 22313 - 1450
www.uspto.gov

ELECTRONIC PAYMENT RECEIPT

APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
18/154,998	12/08/2023 12:44:04 PM Z ET	18896-000072-US-COA

Title of Invention

SITE-SPECIFIC ANTIBODY CONJUGATION AND ANTIBODY-DRUG CONJUGATE AS SPECIFIC EMBODIMENT THEREOF

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	5143	FILED BY	Erica Imergoot
PATENT CENTER #	63543182	AUTHORIZED BY	Kisuk Lee
CUSTOMER #	28997	FILING DATE	01/16/2023
CORRESPONDENCE ADDRESS	-	FIRST NAMED INVENTOR	Sang Jeon CHUNG

Payment Information

PAYMENT METHOD	PAYMENT TRANSACTION ID	PAYMENT AUTHORIZED BY
DA / 080750	E2023B8C46029778	Erica Imergoot

FEE CODE	DESCRIPTION	ITEM PRICE($)	QUANTITY	ITEM TOTAL($)
2501	UTILITY ISSUE FEE	480.00	1	480.00
			TOTAL AMOUNT:	$480.00

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.

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371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

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UNITED STATES PATENT AND TRADEMARK OFFICE

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United States Patent and Trademark Office
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P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

NOTICE OF ALLOWANCE AND FEE(S) DUE

28997 7590 12/07/2023

Harness Dickey (St. Louis)
7700 Bonhomme, Suite 400
St. Louis, MO 63105

EXAMINER
XIAO, YAN

ART UNIT	PAPER NUMBER
1642	

DATE MAILED: 12/07/2023

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
18/154,998	01/16/2023	Sang Jeon CHUNG	18896-000072-US-COA	5143

TITLE OF INVENTION: SITE-SPECIFIC ANTIBODY CONJUGATION AND ANTIBODY-DRUG CONJUGATE AS SPECIFIC EMBODIMENT THEREOF

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	SMALL	$480	$0.00	$0.00	$480	03/07/2024

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 ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

For purposes of this notice, small entity fees are 40% the amount of undiscounted fees, and micro entity fees are 20% the amount of undiscounted fees.

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. 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: Maintenance fees are due in utility patents issuing on applications filed on or after Dec. 12, 1980. It is patentee's responsibility to ensure timely payment of maintenance fees when due. More information is available at www.uspto.gov/PatentMaintenanceFees.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), by mail or fax, or via the USPTO patent electronic filing system.

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

By fax, send to: (571)-273-2885

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28997 7590 12/07/2023

Harness Dickey (St. Louis)
7700 Bonhomme, Suite 400
St. Louis, MO 63105

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(Typed or printed name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
18/154,998	01/16/2023	Sang Jeon CHUNG	18896-000072-US-COA	5143

TITLE OF INVENTION: SITE-SPECIFIC ANTIBODY CONJUGATION AND ANTIBODY-DRUG CONJUGATE AS SPECIFIC EMBODIMENT THEREOF

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	SMALL	$480	$0.00	$0.00	$480	03/07/2024

EXAMINER	ART UNIT	CLASS-SUBCLASS
XIAO, YAN	1642	424-133100

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/AIA/122 or PTO/SB/122) attached.

❑ "Fee Address" indication (or "Fee Address" Indication form PTO/AIA/47 or 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 must have been previously recorded, or filed for recordation, as set forth in 37 CFR 3.11 and 37 CFR 3.81(a). 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. Fees submitted: ❑ Issue Fee ❑ Publication Fee (if required)

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

❑ Electronic Payment via the USPTO patent electronic filing system ❑ Enclosed check ❑ Non-electronic payment by credit card (Attach form PTO-2038)

❑ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment to Deposit Account No. _____

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

❑ Applicant certifying micro entity status. See 37 CFR 1.29

NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.

❑ Applicant asserting small entity status. See 37 CFR 1.27

NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.

❑ Applicant changing to regular undiscounted fee status.

NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature _____ Date _____

Typed or printed name _____ Registration No. _____

UNITED STATES PATENT AND TRADEMARK OFFICE

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

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
18/154,998	01/16/2023	Sang Jeon CHUNG	18896-000072-US-COA	5143

28997 7590 12/07/2023

Harness Dickey (St. Louis)
7700 Bonhomme, Suite 400
St. Louis, MO 63105

EXAMINER	
XIAO, YAN	
ART UNIT	PAPER NUMBER
1642	

DATE MAILED: 12/07/2023

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

The Office has discontinued providing a Patent Term Adjustment (PTA) calculation with the Notice of Allowance.

Section 1(h)(2) of the AIA Technical Corrections Act amended 35 U.S.C. 154(b)(3)(B)(i) to eliminate the requirement that the Office provide a patent term adjustment determination with the notice of allowance. See Revisions to Patent Term Adjustment, 78 Fed. Reg. 19416, 19417 (Apr. 1, 2013). Therefore, the Office is no longer providing an initial patent term adjustment determination with the notice of allowance. The Office will continue to provide a patent term adjustment determination with the Issue Notification Letter that is mailed to applicant approximately three weeks prior to the issue date of the patent, and will include the patent term adjustment on the patent. Any request for reconsideration of the patent term adjustment determination (or reinstatement of patent term adjustment) should follow the process outlined in 37 CFR 1.705.

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.

OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B 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 30 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, 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.

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. The United States Patent and Trademark Office (USPTO) collects the information in this record under authority of 35 U.S.C. 2. The USPTO's system of records is used to manage all applicant and owner information including name, citizenship, residence, post office address, and other information with respect to inventors and their legal representatives pertaining to the applicant's/owner's activities in connection with the invention for which a patent is sought or has been granted. The applicable Privacy Act System of Records Notice for the information collected in this form is COMMERCE/PAT-TM-7 Patent Application Files, available in the Federal Register at 78 FR 19243 (March 29, 2013).
https://www.govinfo.gov/content/pkg/FR-2013-03-29/pdf/2013-07341.pdf

Routine uses of the information in this record may include disclosure to:

1) law enforcement, in the event that the system of records indicates a violation or potential violation of law;
2) a federal, state, local, or international agency, in response to its request;
3) a contractor of the USPTO having need for the information in order to perform a contract;
4) the Department of Justice for determination of whether the Freedom of Information Act (FOIA) requires disclosure of the record;
5) a Member of Congress submitting a request involving an individual to whom the record pertains, when the individual has requested the Member's assistance with respect to the subject matter of the record;
6) a court, magistrate, or administrative tribunal, in the course of presenting evidence, including disclosures to opposing counsel in the course of settlement negotiations;
7) the Administrator, General Services Administration (GSA), or their designee, during an inspection of records conducted by GSA under authority of 44 U.S.C. 2904 and 2906, in accordance with the GSA regulations and any other relevant (i.e., GSA or Commerce) directive, where such disclosure shall not be used to make determinations about individuals;
8) 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));
9) the Office of Personnel Management (OPM) for personnel research purposes; and
10) the Office of Management and Budget (OMB) for legislative coordination and clearance.

If you do not furnish the information requested on this form, the USPTO may not be able to process and/or examine your submission, which may result in termination of proceedings, abandonment of the application, and/or expiration of the patent.

Notice of Allowability	Application No. 18/154,998	Applicant(s) CHUNG et al.	
	Examiner YAN XIAO	Art Unit 1642	AIA (FITF) Status Yes

-- 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 11/14/2023.
   ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

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 See Continuation Sheet . As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see **http://www.uspto.gov/patents/init_events/pph/index.jsp** or send an inquiry to **PPHfeedback@uspto.gov.**

4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

   Certified copies:

   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. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   ☐ 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).

6. ☐ 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. ☑ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date _____.
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material _____.
4. ☑ Interview Summary (PTO-413), Paper No./Mail Date. _____.
5. ☑ Examiner's Amendment/Comment
6. ☐ Examiner's Statement of Reasons for Allowance
7. ☐ Other _____.

/YAN XIAO/
Primary Examiner, Art Unit 1642

Continuation of 3. The allowed claim(s) is/are: 54-56,59-66 and 78-79

Notice of Pre-AIA or AIA Status

1. The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

EXAMINER'S AMENDMENT

2. The amendments/arguments filed 11/13/2023 and 11/14/2023, have been entered.

3. 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 Mr. Paul Zarek on 11/21/2023.

4. The application has been amended as follows:

In the claims:

Claims 67-79 have been replaced with the following amended claims:

Claims 67-77. (Canceled)

Claim 78. (Currently Amended) The modified antibody of claim [[77]]56, wherein the bio-orthogonal click chemistry group is a group capable of participating in a click chemistry reaction selected from Huisgen 1,3-dipolar cycloaddition and Diels-Alder reaction.

Claim 79. (Currently Amended) The modified antibody of claim [[77]]56, wherein the bio-orthogonal click chemistry functional group is selected from the group consisting

of an azide group, a tetrazine group, a dibenzocyclooctyne group, and a norbornene group.

5. Any comments considered necessary by applicant must be submitted no later than the payment of the Issue Fee and, to avoid processing delays, should preferably accompany the Issue Fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

Conclusion

6. Claims 54-56, 59-66 and 78-79 have been allowed.

7. Claims 54-56, 59-66 and 78-79 have been renumbered as claims 1-13 respectively.

8. Any inquiry concerning this communication or earlier communications from the examiner should be directed to YAN XIAO whose telephone number is (571)270-3578. The examiner can normally be reached M-F 8-5 EST.

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at http://www.uspto.gov/interviewpractice.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Misook Yu can be reached on 571-272-0839. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of published or unpublished applications may be obtained from Patent Center. Unpublished application information in Patent Center is available to registered users. To file and manage patent submissions in Patent Center, visit: https://patentcenter.uspto.gov. Visit https://www.uspto.gov/patents/apply/patent-center for more information about Patent Center and https://www.uspto.gov/patents/docx for information about filing in DOCX format. For additional questions, contact the Electronic Business Center (EBC) at 866-217-9197

(toll-free). If you would like assistance from a USPTO Customer Service Representative, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/YAN XIAO/
Primary Examiner, Art Unit 1642

Examiner-Initiated Interview Summary	**Application No.** 18/154,998	**Applicant(s)** CHUNG et al.		
	Examiner YAN XIAO	**Art Unit** 1642	**AIA (First Inventor to File) Status** Yes	**Page** 1 of 1

All Participants (applicant, applicants representative, PTO personnel)	**Title**	**Type**
YAN XIAO	Primary Examiner	Telephonic
Paul Zarek	Agent	

Date of Interview: 21 November 2023

Issues Discussed:

Other

Mr. Zarek authorized entry of the proposed examiner's amendment.

/YAN XIAO/ Primary Examiner, Art Unit 1642	

Applicant is reminded that a complete written statement as to the substance of the interview must be made of record in the application file. It is the applicants responsibility to provide the written statement, unless the interview was initiated by the Examiner and the Examiner has indicated that a written summary will be provided. See MPEP 713.04
Please further see:
MPEP 713.04
Title 37 Code of Federal Regulations (CFR) § 1.133 Interviews, paragraph (b)
37 CFR § 1.2 Business to be transacted in writing

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.

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	18/154,998	CHUNG et al.
	Examiner	Art Unit
	YAN XIAO	1642

CPC - Searched*		
Symbol	**Date**	**Examiner**
A61K 47/6803	08/09/2023	YX
A61K 47/6855	08/09/2023	YX
A61K 47/6889	08/09/2023	YX
A61P 35/00	08/09/2023	YX
C07D 207/46	08/09/2023	YX
C07K 16/32	08/09/2023	YX

CPC Combination Sets - Searched*		
Symbol	**Date**	**Examiner**

US Classification - Searched*			
Class	**Subclass**	**Date**	**Examiner**
424	133.1	08/09/2023	YX

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

Search Notes		
Search Notes	**Date**	**Examiner**
Search through PE2ESearch and STN	08/09/2023	YX
Performed and recorded inventors search	08/09/2023	YX
STIC (please see PE2E-DAV for sequences search results)	08/04/2023	YX
Interference search through PE2ESearch	11/20/2023	YX

/YAN XIAO/ Primary Examiner, Art Unit 1642	

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	18/154,998	CHUNG et al.
	Examiner	Art Unit
	YAN XIAO	1642

Interference Search			
US Class/CPC Symbol	**US Subclass/CPC Group**	**Date**	**Examiner**
424	133.1	11/20/2023	YX
A61K	47/6803, 47/6855, 47/6889	11/20/2023	YX
A61P	35/00	11/20/2023	YX
C07D	207/46	11/20/2023	YX
C07K	16/32	11/20/2023	YX
PGPUB, USPAT	lysine at position 248, biorthogonal, terminal bio-reactive functional group, inventors	11/20/2023	YX
Sequence search (interference database)	see PE2E-DAV	11/20/2023	YX

/YAN XIAO/ Primary Examiner, Art Unit 1642	

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	18/154,998	CHUNG et al.
	Examiner	**Art Unit**
	YAN XIAO	1642

CPC								
Symbol							**Type**	**Version**
A61K	/	47	/	6803			F	2017-08-01
A61K	/	47	/	6889			I	2017-08-01
A61K	/	47	/	6855			I	2017-08-01
A61P	/	35	/	00			I	2018-01-01
C07D	/	207	/	46			I	2013-01-01
C07K	/	16	/	32			I	2013-01-01

CPC Combination Sets								
Symbol					**Type**	**Set**	**Ranking**	**Version**
	/		/					

NONE		**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	13	
/YAN XIAO/ Primary Examiner, Art Unit 1642	21 November 2023	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	None

U.S. Patent and Trademark Office

Part of Paper No.: 20231120

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	18/154,998	CHUNG et al.
	Examiner	**Art Unit**
	YAN XIAO	1642

INTERNATIONAL CLASSIFICATION				
CLAIMED				
A61K	/	47	/	6803
A61K	/	47	/	6889
A61K	/	47	/	6855
A61P	/	35	/	00
C07D	/	207	/	46
C07K	/	16	/	32

NON-CLAIMED				
	/		/	

US ORIGINAL CLASSIFICATION	
CLASS	**SUBCLASS**
424	133.1

CROSS REFERENCES(S)						
CLASS	**SUBCLASS (ONE SUBCLASS PER BLOCK)**					

NONE (Assistant Examiner)	(Date)	**Total Claims Allowed:** 13	
/YAN XIAO/ Primary Examiner, Art Unit 1642 (Primary Examiner)	21 November 2023 (Date)	O.G. Print Claim(s) 1	O.G. Print Figure None

U.S. Patent and Trademark Office

Part of Paper No.: 20231120

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	18/154,998	CHUNG et al.
	Examiner	**Art Unit**
	YAN XIAO	1642

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIMS

Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original
1	54	10	65												
2	55	11	66												
3	56	12	78												
4	59	13	79												
5	60														
6	61														
7	62														
8	63														
9	64														

NONE (Assistant Examiner)	(Date)	**Total Claims Allowed:** 13	
/YAN XIAO/ Primary Examiner, Art Unit 1642 (Primary Examiner)	21 November 2023 (Date)	O.G. Print Claim(s) 1	O.G. Print Figure None

U.S. Patent and Trademark Office

Part of Paper No.: 20231120

Bibliographic Data

Application No: 18/154,998

Foreign Priority claimed: ○ Yes ◉ No

35 USC 119 (a-d) conditions met: ☐ Yes ☑ No ☐ Met After Allowance

Verified and Acknowledged: /YAN XIAO/ Examiner's Signature | Initials

Title: SITE-SPECIFIC ANTIBODY CONJUGATION AND ANTIBODY-DRUG CONJUGATE AS SPECIFIC EMBODIMENT THEREOF

FILING or 371(c) DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
01/16/2023	424	1642	18896-000072-US-COA
RULE			

APPLICANTS

AbTis Co., Ltd., Gyeonggi-do, KOREA, REPUBLIC OF

INVENTORS

Sang Jeon CHUNG, Gyeonggi-do, KOREA, REPUBLIC OF

Ju Hwan KIM, Gyeonggi-do, KOREA, REPUBLIC OF

Young Geun LEE, Gyeonggi-do, KOREA, REPUBLIC OF

Tae Jin LEE, Gyeonggi-do, KOREA, REPUBLIC OF

Jin Woo SEO, Gyeonggi-do, KOREA, REPUBLIC OF

CONTINUING DATA

This application is a CON of 16980503 09/14/2020

16980503 is a 371 of PCT/KR20/03282 03/09/2020

PCT/KR20/03282 has PRO of 62815557 03/08/2019

OK/Y.X./

FOREIGN APPLICATIONS

IF REQUIRED, FOREIGN LICENSE GRANTED**

04/11/2023

**** SMALL ENTITY ****

STATE OR COUNTRY

KOREA, REPUBLIC OF

ADDRESS

Harness Dickey (St. Louis)

7700 Bonhomme, Suite 400

St. Louis, MO 63105

UNITED STATES

FILING FEE RECEIVED

$2,856

PE2E SEARCH - Search History (Prior Art)

There are no Prior Art searches to show.

PE2E SEARCH - Search History (Interference)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	British Equivalents	Time Stamp
N1	1090	lysine WITH "248"	(US-PGPUB; USPAT)	ADJ	ON	ON	2023/11/20 11:25 AM
N2	11	N1 AND (bio-orthogonal OR biorthogonal OR terminal bio-reactive functional group)	(US-PGPUB; USPAT)	ADJ	ON	ON	2023/11/20 11:33 AM
N3	203589	(A61K47/6803 OR A61K47/6855 OR A61K47/6889 OR A61P35/00 OR C07D207/46 OR C07K16/32).cpc.	(US-PGPUB; USPAT)	ADJ	ON	ON	2023/11/20 11:33 AM
N4	13558	424/133.1	(US-PGPUB; USPAT)	ADJ	ON	ON	2023/11/20 11:35 AM
N5	8	N2 AND (N3 OR N4)	(US-PGPUB; USPAT)	ADJ	ON	ON	2023/11/20 11:37 AM
N6	0	N5 AND @PY<="2019"	(US-PGPUB; USPAT)	ADJ	ON	ON	2023/11/20 11:38 AM
N7	15	CHUNG, Sang Jeon .IN.	(US-PGPUB; USPAT)	ADJ	ON	ON	2023/11/20 11:39 AM
N8	28	Lee, Young Geun .IN.	(US-PGPUB; USPAT)	ADJ	ON	ON	2023/11/20 11:55 AM
N9	575	Lee, Tae Jin .IN.	(US-PGPUB; USPAT)	ADJ	ON	ON	2023/11/20 11:55 AM
N10	23	Seo, Jin Woo .IN.	(US-PGPUB; USPAT)	ADJ	ON	ON	2023/11/20 11:55 AM
N11	61	Kim, Ju Hwan .IN.	(US-PGPUB; USPAT)	ADJ	ON	ON	2023/11/20 11:55 AM
N12	4	N7 AND lysine .CLM.	(US-PGPUB; USPAT)	ADJ	ON	ON	2023/11/20 11:56 AM
N13	2	N8 AND lysine .CLM.	(US-PGPUB; USPAT)	ADJ	ON	ON	2023/11/20 11:57 AM
N14	2	N9 AND lysine .CLM.	(US-PGPUB; USPAT)	ADJ	ON	ON	2023/11/20 11:57 AM
N15	2	N10 AND lysine .CLM.	(US-PGPUB; USPAT)	ADJ	ON	ON	2023/11/20 11:58 AM
N16	2	N11 AND lysine .CLM.	(US-PGPUB; USPAT)	ADJ	ON	ON	2023/11/20 11:58 AM

Used in Lieu of PTO/SB/08A/B
(Based on PTO 10-07 version)

Substitute for form 1449/PTO				Complete if Known	
INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	18/154,998-Conf. #5143
				Filing Date	January 16, 2023
				First Named Inventor	Sang Jeon CHUNG
				Art Unit	1642
(Use as many sheets as necessary)				Examiner Name	Y. Xiao
Sheet	1	of	1	Attorney Docket Number	18896-000072-US-COA

U.S. PATENT DOCUMENTS					
Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (*if known*)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	AA	US-2021/0139541-A1	05-13-2021	MATSUDA et al.	

FOREIGN PATENT DOCUMENTS						
Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]-Number[4]-Kind Code[5] (*if known*)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
	BA	WO-2020/184944-A1	09-17-2020	Abtis Co Ltd		

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. * CITE NO.: Those application(s) which are marked with an single asterisk (*) next to the Cite No. are not supplied (under 37 CFR 1.98(a)(2)(iii)) because that application was filed after June 30, 2003 or is available in the IFW. [1] Applicant's unique citation designation number (optional). [2] See Kinds Codes of USPTO Patent Documents at www.USPTO.gov or MPEP 901.04. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [6] Applicant is to place a check mark here if English language Translation is attached.

NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	CA	Office Action from corresponding Australian Patent Application No. 2020234394 issued on 26 September 2023.	
	CB	TAMURA, T., et al.; "Rapid labelling and covalent inhibition of intracellular native proteins using ligand-directed N-acyl-N-alkyl sulfonamide", Nature Communications", 2018, 9, 1870, pp. 1-12.	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1]Applicant's unique citation designation number (optional). [2]Applicant is to place a check mark here if English language Translation is attached.

Examiner Signature	/YAN XIAO/	Date Considered	11/20/2023

KL/ei

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /Y.X./

Used in Lieu of PTO/SB/08A/B
(Based on PTO 10-07 version)

Substitute for form 1449/PTO				Complete if Known	
INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	18/154,998-Conf. #5143
				Filing Date	January 16, 2023
				First Named Inventor	Sang Jeon CHUNG
				Art Unit	1642
(Use as many sheets as necessary)				Examiner Name	Y. Xiao
Sheet	1	of	1	Attorney Docket Number	18896-000072-US-COA

U.S. PATENT DOCUMENTS					
Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (*if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear

FOREIGN PATENT DOCUMENTS						
Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]-Number[4]-Kind Code[5] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
	BA**	KR-10-2020-0002858-A	01-08-2020	Ajinomoto KK		√

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. ** CITE NO.: Those document(s) which are marked with an double asterisk (**) next to the Cite No. are not supplied because they were previously cited by or submitted to the Office in a prior application relied upon in this application for an earlier filing date under 35 U.S.C. 120. [1] Applicant's unique citation designation number (optional). [2] See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [6] Applicant is to place a check mark here if English language Translation is attached.

NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	CA**	Notice of Allowance from corresponding Korean Application No. 10-2020-0029224, dated 25 July 2023.	√

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1]Applicant's unique citation designation number (optional). [2]Applicant is to place a check mark here if English language Translation is attached.

Examiner Signature	/YAN XIAO/	Date Considered	11/20/2023

63282689 KL/aa

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /Y.X./

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application No.:	18/154,998
Filing Date:	16 January 2023
Applicant:	Sang Jeon CHUNG
Group Art Unit:	1642
Confirmation No.:	5143
Examiner:	Yan XIAO
Title:	SITE-SPECIFIC ANTIBODY CONJUGATION AND ANTIBODY-DRUG CONJUGATE AS SPECIFIC EMBODIMENT THEREOF
Attorney Docket:	18896-000072-US-COA

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

14 November 2023

SUPPLEMENTAL RESPONSE TO OFFICE ACTION DATED 16 AUGUST 2023

This paper is a supplemental response to Applicant's reply to the Office Action dated 16 August 2023 filed on 13 November 2023 in the above referenced application. This response is timely and no fee is believed due. If a fee is due, authorization is hereby provided to charge any fee due to Deposit Account No. **08-0750**.

* * *

AMENDMENTS to the specification begin on page 2 of this paper.

AMENDMENTS to the claims begin on page 3 of this paper.

REMARKS on the present amendment begin on page 10 of this paper.

AMENDMENTS TO THE SPECIFICATION

Please replace the existing specification with the attached substitute specification. As required by 37 C.F.R. §1.121(b)(3), a substitute specification is attached hereto in both "marked-up" and "clean" form in compliance with 37 C.F.R. §1.52, §1.115, §1.125 and §1.215. Applicant states in accordance with 37 C.F.R. §1.125(b) that the substitute specification includes no new matter.

AMENDMENT TO THE CLAIMS

The following listing of claims will replace all prior versions and listings of claims in the application.

1.-53. (Cancelled)

54. (Previously Presented) A modified antibody or its fragment comprising bio-orthogonal click chemistry functional group,

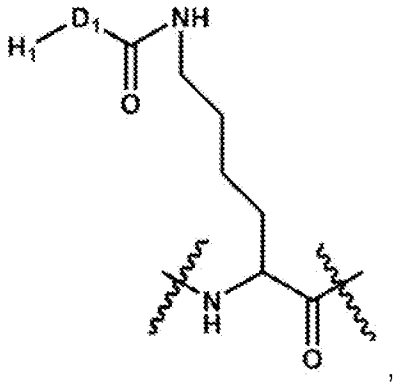
wherein the modified antibody comprises one or more modified heavy chain,

wherein each of the modified heavy chain comprises a modified lysine residue at 248 position of heavy chain amino acid sequence, wherein the 248 position is determined by an EU numbering system, and

wherein the modified lysine residue comprises a bio-orthogonal click chemistry functional group at terminal end, and

wherein the modified lysine residue does not comprise a terminal bio-reactive functional group selected from terminal hydroxy group, terminal carboxyl group, terminal thiol group, terminal amino group, and terminal aldehyde group.

55. (Previously Presented) The modified antibody of claim 54, wherein the modified lysine residue has following structure:



,

wherein H_1 is a group having the bio-orthogonal click chemistry functional group, and

wherein D_1 is bond, or is selected from C_{1-4} alkylene, C_{2-4} alkenylene, C_{2-4} alkynylene and C_{3-8} cycloalkylene.

56. (Previously Presented) The modified antibody of claim 55, wherein D_1 is bond, or is selected from unsubstituted C_{1-4} alkylene, unsubstituted C_{2-4} alkenylene, unsubstituted C_{2-4} alkynylene and unsubstituted C_{3-8} cycloalkylene.

57. (Cancelled)

58. (Cancelled)

59. (Currently Amended) The modified antibody of claim 54, wherein the modified heavy chain comprises an amino acid sequence having a structure of formula 8-2:

[formula 8-2]

G—P—S—V—F—L—F—P—P—K—P—(K)'—D—T—L—M—I (SEQ ID NO: **21** **[[22]]**),

wherein K' is the modified lysine residue.

60. (Previously Presented) The modified antibody of claim 55, wherein D_1 comprises one or more heteroatoms.

61. (Previously Presented) The modified antibody of claim 55,wherein D_1 is $-[CH_2]_a-O-[CH_2]_b-$,

wherein each of a and b is independently selected from 0 to 4, and each of a and b is integer, and

wherein sum of a and b is 0 or more and 4 or less.

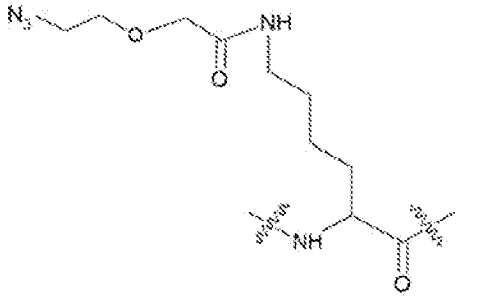
62. (Previously Presented) The modified antibody of claim 55, wherein D_1 is $-CH_2CH_2OCH_2-$ or $-CH_2OCH_2-$.

63. (Previously Presented) The modified antibody of claim 54, wherein the modified antibody comprises two modified heavy chains.

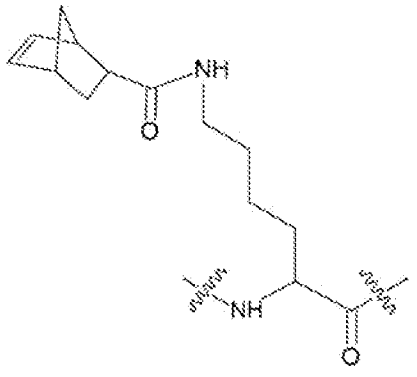
64. (Previously Presented) The modified antibody of claim 54, wherein the bio-orthogonal click chemistry functional group is a group capable of participating in a click chemistry reaction selected from Huisgen 1,3-dipolar cycloaddition and Diels-Alder reaction.

65. (Previously Presented) The modified antibody of claim 54, wherein the bio-orthogonal click chemistry functional group is selected from azide group, tetrazine group, dibenzocyclooctyne group, and norbornene group.

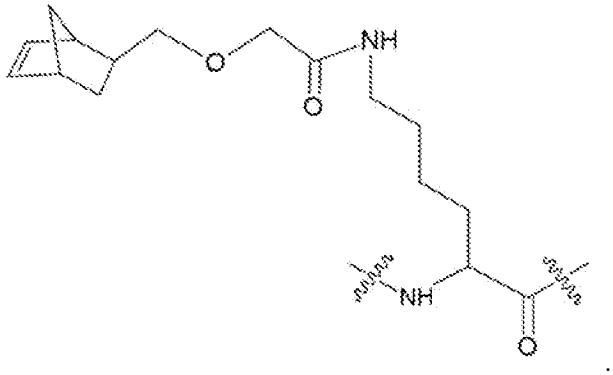
66. (Previously Presented) The modified antibody of claim 54, wherein the modified lysine residue has following structure:



,



, or



67. (Withdrawn) A method for preparing antibody-payload conjugate, comprising:

contacting a modified antibody or its fragment comprising a first click chemistry functional group with a payload comprising a cargo unit and a second click chemistry functional group,

wherein the antibody-payload conjugate is formed by the click chemistry reaction between the first click chemistry functional group of the modified antibody and the second click chemistry functional group of the payload,

wherein the modified antibody comprises:

one or more modified heavy chain,

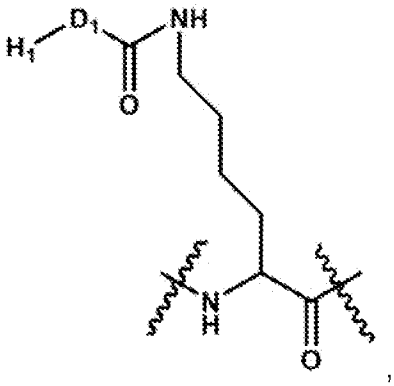
wherein each of the modified heavy chain comprises a modified lysine residue at 248 position of heavy chain amino acid sequence, wherein the 248 position is determined based on EU numbering system,

wherein the modified lysine residue comprises the first click chemistry functional group which is a bio-orthogonal click chemistry functional group at terminal end,

wherein the antibody-payload conjugate comprises an antibody unit derived from the modified antibody and a payload unit derived from the payload,

wherein the payload unit of the antibody-payload conjugate is linked to the antibody unit through K248 of the antibody unit.

68. (Withdrawn) The method of claim 67, wherein the modified lysine residue has the following structure:



,

wherein H_1 is a group having the bio-orthogonal click chemistry functional group,

wherein D_1 is bond, or is selected from C_{1-4} alkylene, C_{2-4} alkenylene, C_{2-4} alkynylene and C_{3-8} cycloalkylene.

69. (Withdrawn) The method of claim 68, wherein D_1 is bond, or is selected from unsubstituted C_{1-4} alkylene, unsubstituted C_{2-4} alkenylene, unsubstituted C_{2-4} alkynylene and unsubstituted C_{3-8} cycloalkylene.

70. (Withdrawn) The method of claim 68, wherein the modified lysine residue does not comprise terminal bio-reactive group selected from terminal hydroxy group, terminal carboxyl group, terminal thiol group, terminal amino group, and terminal aldehyde group.

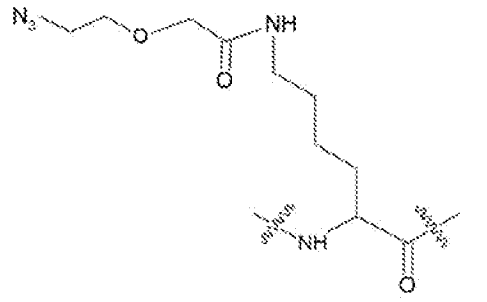
71. (Withdrawn) The method of claim 68, wherein D_1 comprises one or more heteroatoms.

72. (Withdrawn) The method of claim 69, wherein D_1 is $-CH_2CH_2OCH_2-$ or $-CH_2OCH_2-$.

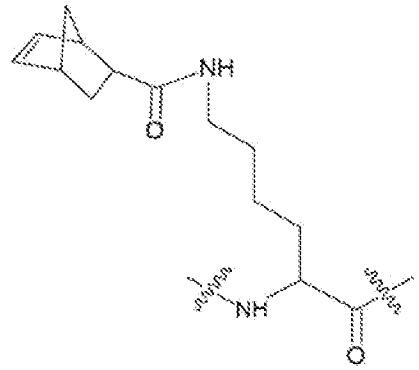
73. (Withdrawn) The method of claim 68, wherein the bio-orthogonal click chemistry functional group is a group capable of participating in a click chemistry reaction selected from Huisgen 1,3-dipolar cycloaddition and Diels-Alder reaction.

74. (Withdrawn) The method of claim 68, wherein the bio-orthogonal click chemistry functional group is selected from azide group and norbornene group.

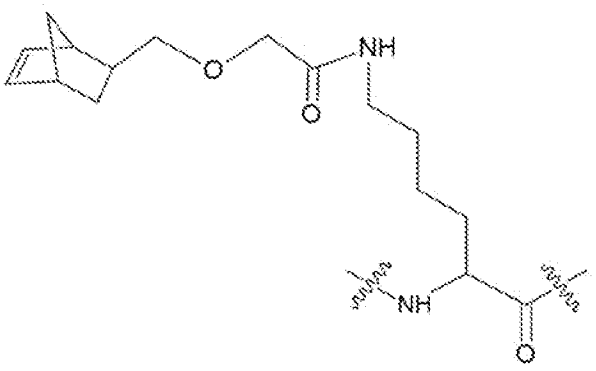
75. (Withdrawn) The method of claim 68, wherein the modified lysine residue has following structure:



,



, or



.

76. (Withdrawn) The method of claim 68, wherein the cargo unit comprises a carrier moiety, a fluorescent moiety, a drug moiety, or a radioactive moiety.

77. (Previously Presented) The modified antibody of claim 56, wherein H_1 is the bio-orthogonal click chemistry functional group.

78. (Previously Presented) The modified antibody of claim 77, wherein the bio-orthogonal click chemistry group is a group capable of participating in a click chemistry reaction selected from Huisgen 1,3-dipolar cycloaddition and Diels-Alder reaction.

79. (Previously Presented) The modified antibody of claim 77, wherein the bio-orthogonal click chemistry functional group is selected from the group consisting of an azide group, a tetrazine group, a dibenzocyclooctyne group, and a norbornene group.

STATUS OF THE CLAIMS

Following the amendment as requested herein, Claims 54, 55, and 58-79 are pending in the present application. Of the pending claims, Claims 67-78 are withdrawn. Of the pending claims, Claims 54-56 and 59-65 are rejected.

Claims 54, 59, and 65 were amended in Applicant's previous reply.

Claim 59 is amended here to correct the SEQ ID NO associated with the recited sequence.

No new matter is added. Applicant respectfully requests entry of these amendments and favorable consideration of the pending claims.

SUPPLEMENTAL RESPONSE TO OFFICE ACTION DATED 16 AUGUST 2023

1. Sequence listing

Submitted with this response is an amended sequence listing to include SEQ ID NOs: 27-46 for the sequence described in Table 1 on pages 28-30. The revised sequence listing includes no new matter, as required by 37 CFR §§ 1.821(e), 1.821(f), 1.821(g), 1.824(a)-(d), 1.825(b), or 1.825(d). Applicant requests the revised sequence listing be entered into the application.

2. Amended specification

Submitted with this response is a revised substitute specification to include SEQ ID NOs for the sequences disclosed in Table 1 the specification. Since Table 1 in the originally filed specification disclosed the specific sequences, no new matter has been added.

3. Conclusion

This response supplements Applicant's response to the non-final Office Action filed on 13 November 2023. Applicant respectfully submits that the previous response fully addresses all outstanding objections and rejections.

It is believed that all of the stated grounds of rejections are properly traversed, accommodated or rendered moot herein. Applicant therefore respectfully requests that the Examiner reconsider and withdraw all presently outstanding objection and rejections. It is believed that a full and complete response has been made to the outstanding Office Action and that the application is in condition for allowance. Thus, prompt and favorable consideration of this amendment is respectfully requested.

Respectfully submitted,

Dated: 14 November 2023

By: / Paul Zarek /
Paul Zarek, Reg. No. 75,190

HARNESS, DICKEY & PIERCE, P.L.C.
7700 Bonhomme, Suite 400
St. Louis, MO 63105
314-446-7668 (telephone)
314-726-7501 (facsimile)

UNITED STATES
PATENT AND TRADEMARK OFFICE

P.O. Box 1450
Alexandria, VA 22313 - 1450
www.uspto.gov

ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
18/154,998	**11/14/2023 03:56:25 PM Z ET**	**18896-000072-US-COA**

Title of Invention

SITE-SPECIFIC ANTIBODY CONJUGATION AND ANTIBODY-DRUG CONJUGATE AS SPECIFIC EMBODIMENT THEREOF

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	5143	FILED BY	Erica Imergoot
PATENT CENTER #	63246166	FILING DATE	01/16/2023
CUSTOMER #	28997	FIRST NAMED INVENTOR	Sang Jeon CHUNG
CORRESPONDENCE ADDRESS	-	AUTHORIZED BY	Paul Zarek

Documents

TOTAL DOCUMENTS: 7

DOCUMENT		PAGES	DESCRIPTION	SIZE (KB)
000072uscoa_SupplementalResponseToNonFinalOfficeActionDated16August2023.pdf		11	-	206 KB
000072uscoa_SupplementalResponseToNonFinalOfficeActionDated16August2023-SA...pdf	(1-1)	1	Supplemental Response or Supplemental Amendment	82 KB
000072uscoa_SupplementalResponseToNonFinalOfficeActionDated16August2023-SPEC.pdf	(2-2)	1	Specification	79 KB

000072uscoa_SupplementalResponseToNonFinalOfficeActionDated16August2023-CLM.pdf	(3-9)	7	Claims	187 KB
000072uscoa_SupplementalResponseToNonFinalOfficeActionDated16August2023-REM.pdf	(10-11)	2	Applicant Arguments/Remarks Made in an Amendment	83 KB
000072uscoa_SubstituteSpecification_Clean.pdf		171	Specification	6413 KB
000072uscoa_SubstituteSpecification_MarkedUp.pdf		171	Specification	6443 KB
000072uscoa_SequenceListing.xml		-	Sequence Listing Supp (XML File)	80 KB

Warning: Please note that ST.23 and ST.25 sequence listings are required for applications with a filing date before July 1, 2022.

Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)
000072uscoa_SupplementalResponseToNonFinalOfficeActionDated16August2023.pdf	EA16CC813386B8C604A6BB635FDCF05960CA8AD7AEB53973B68C101E6A00079DAE236D9430DDE33999CA9DB88E10D479A40857BFFB3E218826A74BD43C1F729D
000072uscoa_SupplementalResponseToNonFinalOfficeActionDated16August2023-SA...pdf	78536D4AD55924FE6E2B873EAFDAD5EA35FBDCD2EB389EC28924FC039DD76B3EA7A9F5F79A2EE44B2AD4308963EA1B2CC4533B3841586275AB2C3B2B877E0E4D
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000072uscoa_SupplementalResponseToNonFinalOfficeActionDated16August2023-CLM.pdf	09BDEAF5944F7B0166D79C8F4A7197D988D52F4E337B9A2979AB1A8EFE31451B0DF12C6615F8020CC627FA549CF26DCBEC4AE364A9AD102F6E5F6235BB5DB477
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New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for 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.

PTO/SB/06 (09-11)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 18/154,998	Filing Date 01/16/2023	☐ To be Mailed

ENTITY: ☐ LARGE ☑ SMALL ☐ MICRO

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED		(Column 2) NUMBER EXTRA		RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A		N/A		N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A		N/A		N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A		N/A		N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =		*		x $40 =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =		*		x $ 192 =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).					
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))						
* If the difference in column 1 is less than zero, enter "0" in column 2.					TOTAL	

APPLICATION AS AMENDED - PART II

		(Column 1)		(Column 2)	(Column 3)			
AMENDMENT	11/13/2023	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 24	Minus	** 23	= 1		x $40 =	40
	Independent (37 CFR 1.16(h))	* 2	Minus	*** 3	= 0		x $ 192 =	0
	☐ Application Size Fee (37 CFR 1.16(s))							
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							
							TOTAL ADD'L FEE	40

		(Column 1)		(Column 2)	(Column 3)			
AMENDMENT	11/14/2023	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 24	Minus	** 23	= 1		x $40 =	40
	Independent (37 CFR 1.16(h))	* 2	Minus	*** 3	= 0		x $ 192 =	0
	☐ Application Size Fee (37 CFR 1.16(s))							
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							
							TOTAL ADD'L FEE	40

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.	LIE
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".	/YOLANDA CHADWICK/
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".	
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.	

This collection of information is required by 37 CFR 1.16. 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, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

SITE-SPECIFIC ANTIBODY CONJUGATION AND ANTIBODY-DRUG CONJUGATE AS SPECIFIC EMBODIMENT THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation application of U.S. Patent Application No. 16/980,503, filed on 14 September 2020, which is a national phase application of PCT Application No. PCT/KR2020/003282, filed on March 9, 2020, which claims priority to U.S. Provisional Patent Application No. 62/815,557, filed on March 8, 2019. The entire disclosure of the applications identified in this paragraph is incorporated herein by reference.

FIELD

[0002] The present invention relates to technology for selectively labeling a certain amino acid residue of an antibody with substances (low-molecular-weight compounds, synthetic polymers, biopolymers (i.e., peptides, carbohydrates, proteins, and the like)) or linking a molecule (hereinafter referred to as a "cargo") to be delivered to a certain target cell or tissue to the antibody. Also, the present invention relates to technology for labeling a certain site of an antibody with a desired number of substances or linking a desired number of cargos to certain amino acid residues of the antibody. In addition, the present invention encompasses an antibody complex prepared by the method or a method using a fragment complex of the antibody.

BACKGROUND

[0003] Antibodies are biomolecules that have a function of recognizing certain molecules, and have been used for various industrial applications. For example, an antibody may be used to detect or screen for a certain substance and check a route through which the certain substance moves in the body or cells. Also, the antibody may be used for therapeutic purposes by inducing an immune response against the certain substance.

[0004] To expand the functions of such an antibody, there has been attempts to improve the antibody's ability. Typically, there has been an attempt to label an antibody with foreign substances in order to supplement or expand the antibody's functions. Typically, when an antibody is labeled with a fluorescent substance, the antibody may be used for a fluorescence assay, or when an antibody is labeled with an agent for treating a certain disease, the antibody may be used to maximize a therapeutic effect of the antibody. These attempts and technology are generally referred to as "antibody labeling." The present invention relates to a novel method

of labeling an antibody.

[0005] In the beginning, the antibody labeling was achieved by randomly attaching a foreign substance to an antibody. However, this method has a lot of problems. The antibodies thus prepared have a problem in that they have poor homogeneity. These antibodies have a problem in that their effects remain uneven because there are a difference in the number of substances attached to each of the antibodies and a difference in binding sites for the antibodies. This problem is a great barrier to the development of antibody-drug conjugate (ADC) technology that requires high safety and reproducibility.

[0006] Also, the method has a problem in that an antibody's ability to recognize may be significantly degraded. An antibody consists of an Fab domain including an antigen-binding domain that recognizes an antigen, and an Fc domain involved in the crystallization of the antibody. Random labeling resulted in a highly degraded antibody's ability to recognize by allowing a foreign substance to bind to an antigen-binding domain of an antibody or a site adjacent to the antigen-binding domain.

[0007] Therefore, there has been a demand for technology for uniformly labeling an antibody in a site-specific manner in the related art. Although some techniques were developed, e.g., genetically modulating or modifying an antibody and the like, most of them are ineffective in technical and economic aspects. Accordingly, the present invention is designed to solve the problems, and thus is directed to technology capable of specifically linking a certain substance (or a “moiety”) to a certain site of an antibody without any additional modulation of the antibody and delivering a certain substance (a drug or a labeled substance) into cells or tissues using the antibody as well.

SUMMARY

Technical Problem

[0008] The present invention provides technology for specifically transferring a chemical functional group to a certain site of an antibody. In one specific embodiment, the present invention provides technology for specifically transferring a chemical functional group to lysine 246 in the antibody. In another specific embodiment, the present invention provides technology for specifically transferring a chemical functional group to lysine 248 in the antibody. In still another specific embodiment, the present invention provides technology for specifically transferring a chemical functional group to lysine 246 and 248 in the antibody.

[0009] The present invention provides technology capable of linking a desired number of chemical functional groups to an antibody. In one specific embodiment, the present invention

provides an antibody or a fragment thereof, which has two certain moieties bound thereto. In another specific embodiment, the present invention provides an antibody or a fragment thereof, which has four certain moieties bound thereto.

[0010] The present invention provides technology for specifically linking a cargo moiety to a certain site of an antibody. In one specific embodiment, the present invention provides technology for specifically linking a cargo moiety to lysine 246 in the antibody. In another specific embodiment, the present invention provides technology for specifically linking a cargo moiety to lysine 248 in the antibody. In still another specific embodiment, the present invention provides technology for specifically linking a cargo moiety to lysine 246 and 248 in the antibody.

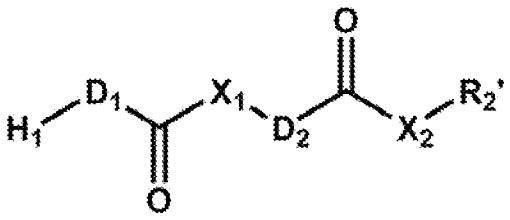
[0011] The present invention provides technology for linking a desired number of cargo moieties to an antibody. In one specific embodiment, the present invention provides an antibody or a fragment thereof, which has two cargo moieties bound thereto. In another specific embodiment, the present invention provides an antibody or a fragment thereof, which has four cargo moieties bound thereto.

[0012] The present invention provides a method of using the aforementioned antibody or antibody fragment. In one specific embodiment, the present invention provides a method of treating a certain disease using an antibody-drug complex.

Technical Solution

[0013] The present application provides A compound of Formula 2:

[0014] [formula 2]



[0015] wherein, H_1 is a first click chemistry functional group, D_1 is selected from a covalent bond, $C_{1\sim4}$alkylene, $C_{2\sim4}$alkenylene, $C_{2\sim4}$alkynlene, and $C_{3\sim8}$cycloalkylene, X_1 is S, D_2 is selected from $C_{1\sim7}$alkylene, $C_{2\sim7}$alkenylene, $C_{2\sim7}$alkynylene, and $C_{3\sim8}$cycloalkynlene, X_2 is O, R_2' is N-succinimide, p-nitrophenyl, or pentafluorophenyl.

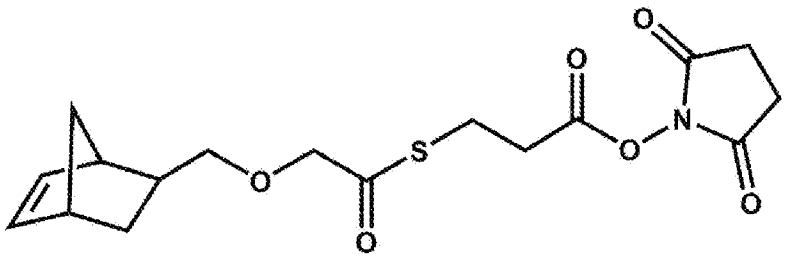
[0016] Also, the present application provides the compound wherein H_1 is selected from terminal alkyne, azide, strained alkyne, diene, dienophile, alkene, thiol, and tetrazine. Furthermore, the present application provides the compound wherein H_1 is selected from norbornene, tetrazine, azide and dibenzocyclooctyne-amine.

[0017] Additionally, the present application provides the compound wherein R_2' is N-

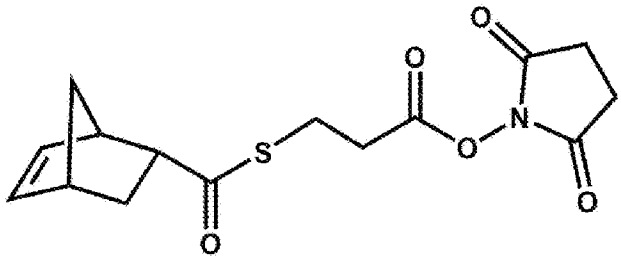
succinimide.

[0018] Additionally, the present application provides the compound wherein the formula 2 is formula 2-1, 2-2, or 2-3:

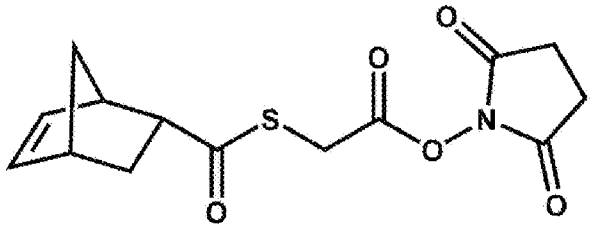
[0019] [formula 2-1]



[0020] [formula 2-2]



[0021] [formula 2-3]

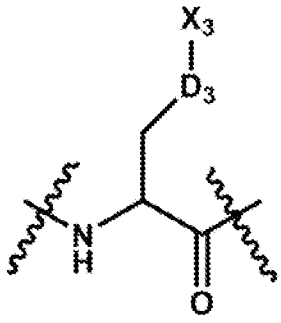


[0022] the present application provides a peptide of formula 4-2:

[0023] [formula 4-2]

$$(Xaa)_{1-3}\text{-}C\text{-}(Xaa)_2\text{-}H\text{-}Xa_1\text{-}G\text{-}Xa_2\text{-}L\text{-}V\text{-}Xa_3\text{-}C\text{-}(Xaa)_{1-3}$$ (SEQ ID NO: 6)

[0024] wherein, each Xaa is independently any amino acid residue that is not a cysteine residue, C is a cysteine residue, H is a histidine residue, G is a glycine residue, Xa_2 is a glutamic acid residue or an asparagine residue, L is a leucine residue, V is a valine residue, Xa_3 is selected from a tryptophan residue, a naphthylalanine residue, and a phenylalanine residue, and Xa_1 is



, wherein D_3 is a covalent bond or $C_{1\sim3}$alkylene and X_3 is NH_2, wherein the peptide consists of 13 to 17 amino acid residues, wherein the peptide exhibits the activity of binding to human immunoglobulin G (IgG), and wherein the cysteine residue that is between two to four amino acids from the N-terminus of formula 4-2 and the cysteine residue that is between two to four amino acids from the C-terminus of formula 4-2 are optionally linked.

[0025] Also, the present application provides the peptide wherein D_3 is a covalent bond, methylene, or ethylene

[0026] Additionally, the present application provides the peptide wherein the formula 4-2 is formula 4-6:

[0027] [formula 4-6]

$$D\text{-}C\text{-}A\text{-}W\text{-}H\text{-}Xa_1\text{-}G\text{-}E\text{-}L\text{-}V\text{-}W\text{-}C\text{-}T$$ (SEQ ID NO: 10)

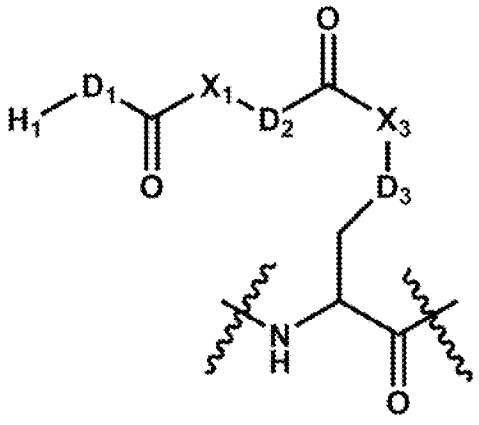
[0028] wherein, D is an aspartic acid residue, A is an alanine residue, E is a glutamic acid residue, W is a tryptophan residue, and T is a threonine residue.

[0029] The present application provides a peptide-compound conjugate of formula 6-2:

[0030] [formula 6-2]

$$(Xaa)_{1\text{-}3}\text{-}C\text{-}(Xaa)_2\text{-}H\text{-}(Xa_1)'\text{-}G\text{-}Xa_2\text{-}L\text{-}V\text{-}Xa_3\text{-}C\text{-}(Xaa)_{1\text{-}3}$$ (SEQ ID NO: 15)

[0031] wherein, each Xaa is independently any amino acid residue that is not a cysteine residue, C is a cysteine residue, H is a histidine residue, G is a glycine residue, Xa_2 is a glutamic acid residue or an asparagine residue, L is a leucine residue, V is a valine residue, Xa_3 is selected from a tryptophan residue, a naphthylalanine residue, and a phenylalanine residue, and



[0032] $(Xa_1)'$ is , wherein H_1 is a first click chemistry functional group, D_1 is selected from a covalent bond, $C_{1\sim4}$alkylene, $C_{2\sim4}$alkenylene, $C_{2\sim4}$alkynylene, and $C_{3\sim8}$cycloalkynlene, X_1 is S, D_2 is selected from $C_{1\sim7}$alkylene, $C_{2\sim7}$alkenylene, $C_{2\sim7}$alkynylene, and $C_{3\sim8}$cycloalkynlene, D_3 is a covalent bond, or $C_{1\sim3}$alkylene, and X_3 is NH, wherein the peptide consists of 13 to 17 amino acid residues, wherein the peptide exhibits the activity of binding to human immunoglobulin G (IgG), and wherein the cysteine residue that is between two to four amino acids from the N-terminus of formula 6-2 and the cysteine residue that is between two to four amino acids from the C-terminus of formula 6-2 are optionally linked.

[0033] Also, the present application provides the peptide-compound conjugate wherein a distance from a beta carbon of the $(Xa_1)'$ to a first carbonyl carbon of the $(Xa_1)'$ is less than approx. 11.668Å.

[0034] Additionally, the present application provides the peptide-compound conjugate wherein D_2 is $C_y$alkylene, $C_y$alkenylene, or $C_y$alkynylene, D_3 is $C_x$alkylene, wherein y is an integer greater than or equal to 1, and $1 \leq x+y \leq 5$.

[0035] Additionally, the present application provides the peptide-compound conjugate wherein a distance from a beta carbon of the $(Xa_1)'$ to a first carbonyl carbon of the $(Xa_1)'$ is greater than approx. 16.208Å.

[0036] Additionally, the present application provides the peptide-compound conjugate wherein D_2 is $C_y$alkylene, $C_y$alkenylene, or $C_y$alkynylene, and D_3 is $C_x$alkylene, wherein y is an integer greater than or equal to 1, and $9 \leq x+y \leq 12$. Furthermore, the present application provides the peptide-compound conjugate wherein D_2 is $C_y$alkenylene, or $C_y$alkynylene.

[0037] Additionally, the present application provides the peptide-compound conjugate wherein a distance from a beta carbon of the $(Xa_1)'$ to a first carbonyl carbon of the $(Xa_1)'$ is approx. 11.668Å to approx. 16.208Å.

[0038] Additionally, the present application provides the peptide-compound conjugate wherein D_2 is $C_y$alkylene, $C_y$alkenylene, or $C_y$alkynylene, and D_3 is $C_x$alkylene, wherein y is an

integer greater than or equal to 1, and $6 \leq x+y \leq 8$.

[0039] Additionally, the present application provides the peptide-compound conjugate wherein the formula 6-2 is formula 6-3:

[0040] [formula 6-3]

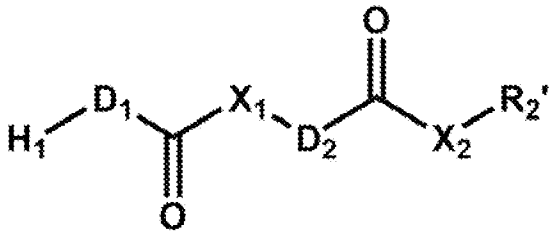
D-C-A-W-H-$(Xa_1)'$-G-E-L-V-W-C-T (SEQ ID NO: 16)

[0041] wherein, D is an aspartic acid residue, A is an alanine residue, E is a glutamic acid residue, W is a tryptophan residue, T is a threonine residue.

[0042] Additionally, the present application provides the peptide-compound conjugate wherein D_1 is a covalent bond and D_2 is methylene.

[0043] The present application provides a method for preparing an agent for transferring a first click chemical functional group to an antibodycomprising reacting a compound of formula 2:

[0044] [formula 2]

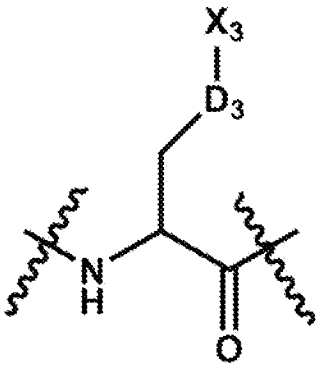


[0045] wherein, H_1 is a first click chemistry functional group, D_1 is selected from a covalent bond, $C_{1\sim4}$alkylene, $C_{2\sim4}$alkenylene, $C_{2\sim4}$alkynylene, and $C_{3\sim8}$cycloalkynlene, X_1 is S, D_2 is selected from $C_{1\sim7}$alkylene, $C_{2\sim7}$alkenylene, $C_{2\sim7}$alkynylene, and $C_{3\sim8}$cycloalkynlene, X_2 is O, R_2' is N-succinimide, p-nitrophenyl, or pentafluorophenyl, with a peptide of formula 4-2:

[0046] [formula 4-2]

$(Xaa)_{1-3}$-C-$(Xaa)_2$-H-Xa_1-G-Xa_2-L-V-Xa_3-C-$(Xaa)_{1-3}$ (SEQ ID NO: 6)

[0047] wherein, each Xaa is independently any amino acid residue that is not a cysteine residue, C is a cysteine residue, H is a histidine residue, G is a glycine residue, Xa_2 is a glutamic acid residue or an asparagine residue, L is a leucine residue, V is a valine residue, Xa_3 is selected from a tryptophan residue, a naphthylalanine residue, and a phenylalanine residue, and



[0048] Xa_1 is , wherein D_3 is a covalent bond or $C_{1\sim3}$alkylene and X_3 is NH_2, wherein the peptide consists of 13 to 17 amino acid residues, wherein the peptide exhibits the activity of binding to human immunoglobulin G (IgG), and wherein the cysteine residue that is between two to four amino acids from the N-terminus of formula 4-2 and the cysteine residue that is between two to four amino acids from the C-terminus of formula 4-2 are optionally linked.

[0049] Also, the present application provides the method for preparing an agent for transferring a first click chemical functional group to an antibodywherein D_2 is $C_y$alkylene, $C_y$alkenylene, or $C_y$alkynylene, and D_3 is $C_x$alkylene, wherein y is an integer greater than or equal to 1, and $1\leq x+y\leq5$, characterized in that the agent for transferring a first click chemical functional group to an antibodyprepared by the method can react with an antibody to deliver the first click chemistry functional group specifically to a 248 a lysine residue of an Fc domain of the antibody.

[0050] Additionally, he present application provides the method for preparing an agent for transferring a first click chemical functional group to an antibodywherein D_2 is $C_y$alkylene, $C_y$alkenylene, or $C_y$alkynylene, and D_3 is $C_x$alkylene, wherein y is an integer greater than or equal to 1, and $1\leq x+y\leq5$, characterized in that the agent for transferring a first click chemical functional group to an antibodyprepared by the method can react with an antibody to deliver the first click chemistry functional group specifically to a 248 a lysine residue of an Fc domain of the antibody.

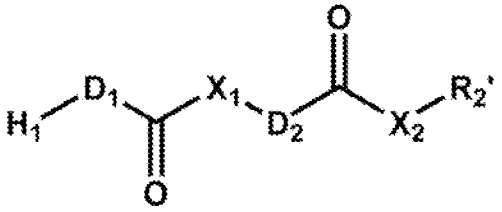
[0051] Additionally, the present application provides the method for preparing an agent for transferring a first click chemical functional group to an antibodywherein D_2 is $C_y$alkylene, $C_y$alkenylene, or $C_y$alkynylene, and D_3 is $C_x$alkylene, wherein y is an integer greater than or equal to 1, and $9\leq x+y\leq12$, characterized in that the agent for transferring a first click chemical functional group to an antibodyprepared by the method can react with an antibody to deliver the first click chemistry functional group specifically to a 246 a lysine residue of an Fc domain of the antibody.

[0052] Additionally, the present application provides the method for preparing an agent for transferring a first click chemical functional group to an antibodywherein D_2 is $C_y$alkylene, $C_y$alkenylene, or $C_y$alkynylene, and D_3 is $C_x$alkylene, wherein y is an integer greater than or equal

to 1, and 6≤x+y≤8, characterized in that the agent for transferring a first click chemical functional group to an antibodyprepared by the method can react with an antibody to deliver the first click chemistry functional group selectively to a 246 a lysine residue or a 248 a lysine residue of an Fc domain of the antibody

[0053] The present application provides a kit for preparing a first click chemistry functional group transferring to an antibody comprising a compound of formula 2:

[0054] [formula 2]

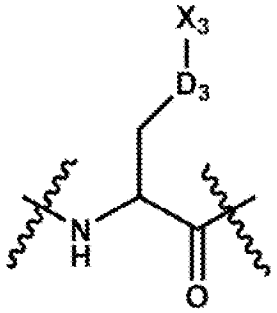


[0055] wherein, H_1 is a first click chemistry functional group, D_1 is selected from a covalent bond, $C_{1\sim4}$alkylene, $C_{2\sim4}$alkenylene, $C_{2\sim4}$alkynylene, and $C_{3\sim8}$cycloalkynlene, X_1 is S, D_2 is selected from $C_{1\sim7}$alkylene, $C_{2\sim7}$alkenylene, $C_{2\sim7}$alkynylene, and $C_{3\sim8}$cycloalkynlene, X_2 is O, R_2' is N-succinimide, p-nitrophenyl, or pentafluorophenyl; and a peptide of formula 4-2:

[0056] [formula 4-2]

$$(Xaa)_{1\text{-}3}\text{-}C\text{-}(Xaa)_2\text{-}H\text{-}Xa_1\text{-}G\text{-}Xa_2\text{-}L\text{-}V\text{-}Xa_3\text{-}C\text{-}(Xaa)_{1\text{-}3}$$ (SEQ ID NO: 6)

[0057] wherein, each Xaa is independently any amino acid residue that is not a cysteine residue, C is a cysteine residue, H is a histidine residue, G is a glycine residue, Xa_2 is a glutamic acid residue or an asparagine residue, L is a leucine residue, V is a valine residue, Xa_3 is selected from a tryptophan residue, a naphthylalanine residue, and a phenylalanine residue, and

[0058] Xa_1 is 

, wherein D_3 is a covalent bond or $C_{1\sim3}$alkylene and X_3 is NH_2, wherein the peptide consists of 13 to 17 amino acid residues, wherein the peptide exhibits the activity of binding to human immunoglobulin G (IgG), and wherein the cysteine residue that is between two to four amino acids from the N-terminus of formula 4-2 and the cysteine residue that is between two to four amino acids from the C-terminus of formula 4-2 are optionally linked..

[0059] The present application provides an antibody or a fragment thereof comprising one or more amino acid sequence selected from formula 8-1, formula 8-2, and formula 8-3:

[0060] [formula 8-1] (SEQ ID NO: 20)

G—P—S—V—F—L—F—P—P—(K)'—P—K—D—T—L—M—I

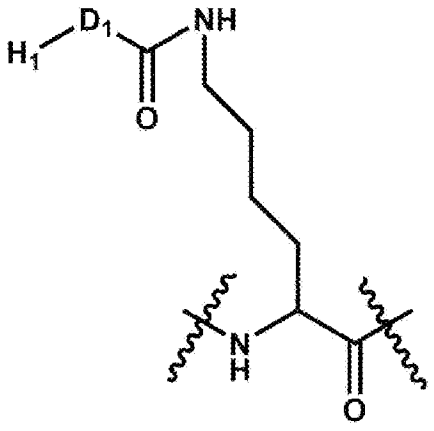
[0061] [formula 8-2] (SEQ ID NO: 21)

G—P—S—V—F—L—F—P—P—K—P—(K)'—D—T—L—M—I

[0062] [formula 8-3] (SEQ ID NO: 22)

G—P—S—V—F—L—F—P—P—(K)'—P—(K)'—D—T—L—M—I

[0063] wherein, G is a glycine residue, P is a proline residue, S is a serine residue, V is a valine residue, F is a phenylalanine residue, L is a leucine residue, K is a lysine residue, D is an aspartic acid residue, T is a threonine residue, M is a methionine residue, I is an isoleucine residue, and

[0064] (K)' is 

, wherein H_1 is a first click chemistry functional group, D_1 is selected from a covalent bond, $C_{1\sim4}$alkylene, $C_{2\sim4}$alkenylene, $C_{2\sim4}$alkynylene, and $C_{3\sim8}$cycloalkynlene.

[0065] Also, the present application provides the antibody or a fragment thereof wherein D_1 is a covalent bond.

[0066] Additionally, the present application provides the antibody or a fragment thereof comprising the amino acid sequence of formula 8-1 and not comprising the amino acid sequence of formula 8-2 and 8-3. Furthermore, the present application provides the antibody or a fragment thereof comprising the amino acid sequence of formula 8-1 in both two Fc domains.

[0067] Additionally, the present application provides the antibody or a fragment thereof comprising the amino acid sequence of formula 8-2 and not comprising the amino acid sequence of formula 8-1 and 8-3. Furthermore, the present application provides the antibody or a fragment thereof comprising the amino acid sequence of formula 8-2 in both two Fc domains

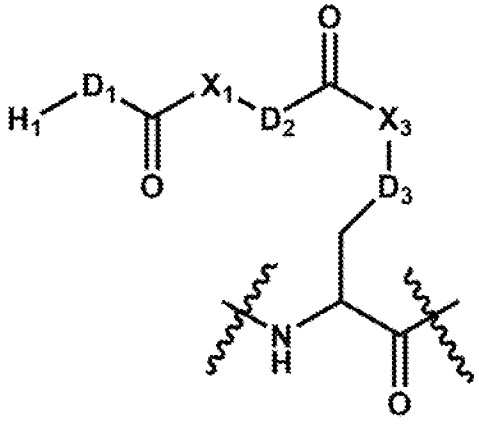
[0068] Additionally, the present application provides the antibody or a fragment thereof comprising the amino acid sequence of formula 8-3 and not comprising the amino acid sequence of formula 8-1 and 8-2. Furthermore, the present application provides the antibody or a fragment thereof comprising the amino acid sequence of formula 8-3 in both two Fc domains.

[0069] The present application provides a method for preparing an antibody or a fragment thereof comprising first click chemistry functional group comprising reacting a peptide-compound conjugate of formula 6-2:

[0070] [formula 6-2]

$$(Xaa)_{1\text{-}3}\text{-}C\text{-}(Xaa)_2\text{-}H\text{-}(Xa_1)'\text{-}G\text{-}Xa_2\text{-}L\text{-}V\text{-}Xa_3\text{-}C\text{-}(Xaa)_{1\text{-}3}$$ (SEQ ID NO: 15)

[0071] wherein, each Xaa is independently any amino acid residue that is not a cysteine residue, C is a cysteine residue, H is a histidine residue, G is a glycine residue, Xa_2 is a glutamic acid residue or an asparagine residue, L is a leucine residue, V is a valine residue, Xa_3 is selected from a tryptophan residue, a naphthylalanine residue, and a phenylalanine residue, and

[0072] $(Xa_1)'$ is  , wherein H_1 is a first click chemistry functional group, D_1 is selected from a covalent bond, $C_{1\sim4}$alkylene, $C_{2\sim4}$alkenylene, $C_{2\sim4}$alkynylene, and $C_{3\sim8}$cycloalkynlene, X_1 is S, D_2 is selected from $C_{1\sim7}$alkylene, $C_{2\sim7}$alkenylene, $C_{2\sim7}$alkynylene, and $C_{3\sim8}$cycloalkynlene, D_3 is a covalent bond or $C_{1\sim3}$alkylene and X_3 is NH, wherein the peptide consists of 13 to 17 amino acid residues, wherein the peptide exhibits the activity of binding to human immunoglobulin G (IgG), and wherein the cysteine residue that is between two to four amino acids from the N-terminus of formula 6-2 and the cysteine residue that is between two to four amino acids from the C-terminus of formula 6-2 are optionally linked, with an antibody or a fragment thereof.

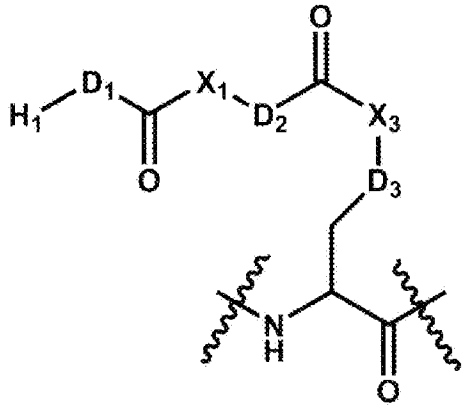
[0073] The present application provides a kit for preparing an antibody or a fragment thereof comprising first click chemistry functional group comprising

[0074] a peptide-compound conjugate of formula 6-2:

[0075] [formula 6-2]

$$(Xaa)_{1-3}\text{-}C\text{-}(Xaa)_2\text{-}H\text{-}(Xa_1)'\text{-}G\text{-}Xa_2\text{-}L\text{-}V\text{-}Xa_3\text{-}C\text{-}(Xaa)_{1-3}$$ (SEQ ID NO: 15)

[0076] wherein, each Xaa is independently any amino acid residue that is not a cysteine residue, C is a cysteine residue, H is a histidine residue, G is a glycine residue, Xa_2 is a glutamic acid residue or an asparagine residue, L is a leucine residue, V is a valine residue, W is a tryptophan residue, and



[0077] $(Xa_1)'$ is , wherein H_1 is a first click chemistry functional group, D_1 is selected from a covalent bond, $C_{1\sim4}$alkylene, $C_{2\sim4}$alkenylene, $C_{2\sim4}$alkynylene, and $C_{3\sim8}$cycloalkynlene, X_1 is S, D_2 is selected from $C_{1\sim7}$alkylene, $C_{2\sim7}$alkenylene, $C_{2\sim7}$alkynylene, and $C_{3\sim8}$cycloalkynlene, D_3 is a covalent bond or $C_{1\sim3}$alkylene and X_3 is NH, wherein the peptide consists of 13 to 17 amino acid residues, wherein the peptide exhibits the activity of binding to human immunoglobulin G (IgG), and wherein the cysteine residue that is between two to four amino acids from the N-terminus of formula 6-2 and the cysteine residue that is between two to four amino acids from the C-terminus of formula 6-2 are optionally linked; and an antibody or a fragment thereof.

[0078] The present application provides a compound of formula 9:

[0079] [formula 9]

$$C_m\text{——}H_2$$

[0080] wherein, C_m is a cargo moiety, H_2 is a second click chemistry functional group.

[0081] The present application provides a method for preparing an antibody-drug conjugate comprising reacting an antibody or a fragment thereof comprising one or more amino acid sequence selected from formula 8-1, formula 8-2, and formula 8-3:

[0082] [formula 8-1] (SEQ ID NO: 20)

$$G\text{—}P\text{—}S\text{—}V\text{—}F\text{—}L\text{—}F\text{—}P\text{—}P\text{—}(K)'\text{—}P\text{—}K\text{—}D\text{—}T\text{—}L\text{—}M\text{—}I$$

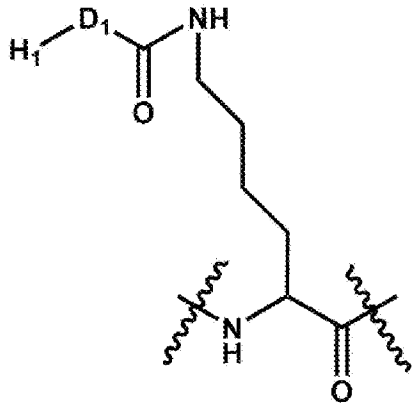
[0083] [formula 8-2] (SEQ ID NO: 21)

G—P—S—V—F—L—F—P—P—K—P—(K)'—D—T—L—M—I

[0084] [formula 8-3] (SEQ ID NO: 22)

G—P—S—V—F—L—F—P—P—(K)'—P—(K)'—D—T—L—M—I

[0085] wherein, G is a glycine residue, P is a proline residue, S is a serine residue, V is a valine residue, F is a phenylalanine residue, L is a leucine residue, K is a lysine residue, D is an aspartic acid residue, T is a threonine residue, M is a methionine residue, I is an isoleucine residue, and



[0086] (K)' is , wherein H_1 is a first click chemistry functional group, D_1 is selected from a covalent bond, $C_{1\sim4}$alkylene, $C_{2\sim4}$alkenylene, $C_{2\sim4}$alkynylene, and $C_{3\sim8}$cycloalkynlene, with a compound of formula 9:

[0087] [formula 9]

C_m——H_2

[0088] wherein, C_m is a cargo moiety, H_2 is a second click chemistry functional group which is complementary with the first click chemistry functional group.

[0089] The present application provides a kit for preparing an antibody-drug conjugate comprising an antibody or a fragment thereof comprising one or more amino acid sequence selected from formula 8-1, formula 8-2, and formula 8-3:

[0090] [formula 8-1] (SEQ ID NO: 20)

G—P—S—V—F—L—F—P—P—(K)'—P—K—D—T—L—M—I

[0091] [formula 8-2] (SEQ ID NO: 21)

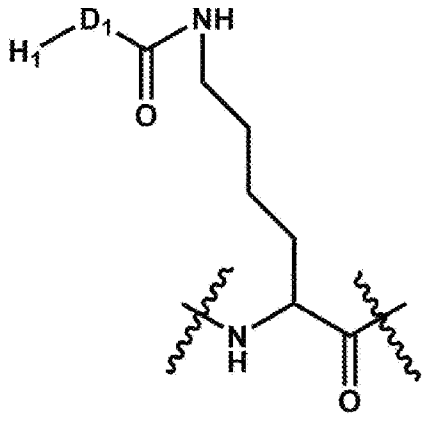
G—P—S—V—F—L—F—P—P—K—P—(K)'—D—T—L—M—I

[0092] [formula 8-3] (SEQ ID NO: 22)

G—P—S—V—F—L—F—P—P—(K)'—P—(K)'—D—T—L—M—I

[0093] wherein, G is a glycine residue, P is a proline residue, S is a serine residue, V is a

valine residue, F is a phenylalanine residue, L is a leucine residue, K is a lysine residue, D is an aspartic acid residue, T is a threonine residue, M is a methionine residue, I is an isoleucine residue, and

[0094] (K)' is  , wherein H_1 is a first click chemistry functional group, D_1 is selected from a covalent bond, $C_{1\sim4}$alkylene, $C_{2\sim4}$alkenylene, $C_{2\sim4}$alkynylene, and $C_{3\sim8}$cycloalkynlene; and a compound of formula 9:

[0095] [formula 9]



[0096] wherein, C_m is a cargo moiety, H_2 is a second click chemistry functional group which is complementary with the first click chemistry functional group.

[0097] The present application provides an antibody or a fragment thereof comprising one or more amino acid sequence selected from formula 10-1, formula 10-2, and formula 10-3:

[0098] [formula 10-1] (SEQ ID NO: 23)

G—P—S—V—F—L—F—P—P—(K)"-P—K—D—T—L—M—I

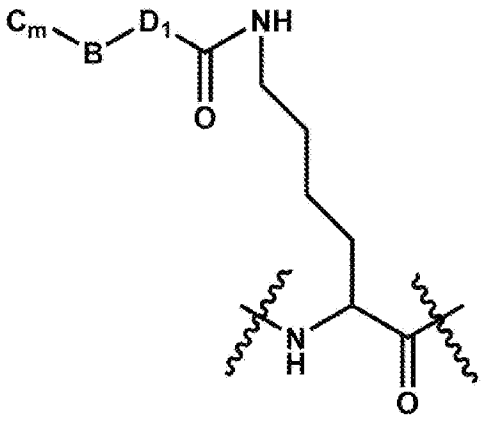
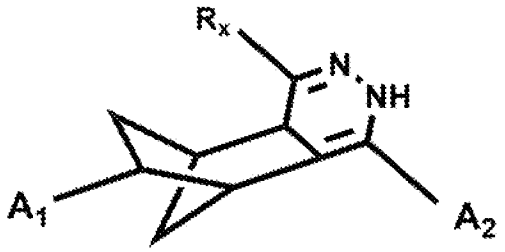
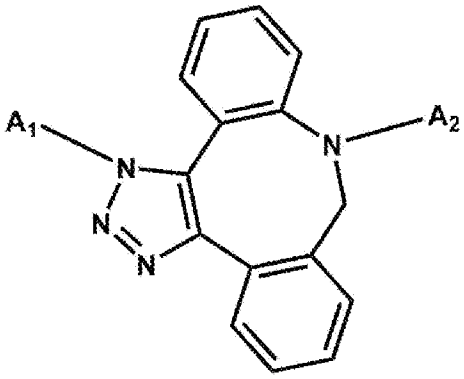
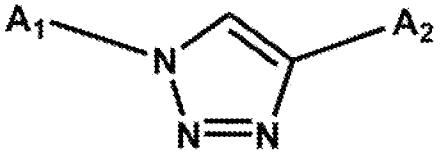
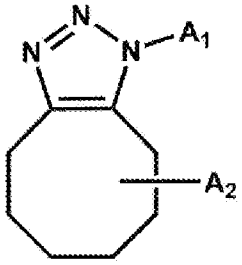
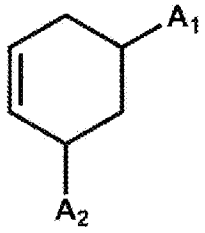
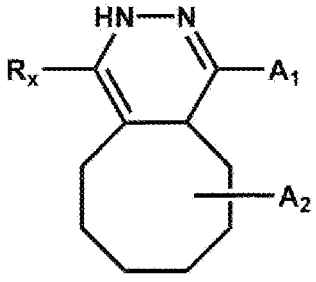
[0099] [formula 10-2] (SEQ ID NO: 24)

G—P—S—V—F—L—F—P—P—K—P—(K)"-D—T—L—M—I

[0100] [formula 10-3] (SEQ ID NO: 25)

G—P—S—V—F—L—F—P—P—(K)"-P—(K)"-D—T—L—M—I

[0101] wherein, G is a glycine residue, P is a proline residue, S is a serine residue, V is a valine residue, F is a phenylalanine residue, L is a leucine residue, K is a lysine residue, D is an aspartic acid residue, T is a threonine residue, M is a methionine residue, I is an isoleucine residue, and

[0102] (K)" is  , wherein C_m is a cargo moiety, B is selected from  ,  ,  ,  ,  ,  , and  , wherein A_1 and A_2 is connected to the cargo moiety or D_1 and they are not both connected to same, R_x is selected from H, halogen, and $C_{1\sim3}$alkyl, D_1 is selected from a covalent bond, $C_{1\sim4}$alkylene, $C_{2\sim4}$alkenylene, $C_{2\sim4}$alkynylene, and $C_{3\sim8}$cycloalkynlene.

[0103] Also, the present application provides the antibody or a fragment thereof comprising the amino acid sequence of formula 10-1 and not comprising the amino acid sequence of formula 10-2 and 10-3. Furthermore, the present application provides the antibody or a

fragment thereof comprising the amino acid sequence of formula 10-1 in both two Fc domains.

[0104] Additionally, the present application provides the antibody or a fragment thereof comprising the amino acid sequence of formula 10-2 and not comprising the amino acid sequence of formula 10-1 and 10-3. Furthermore, the present application provides the antibody or a fragment thereof comprising the amino acid sequence of formula 10-2 in both two Fc domains.

[0105] Additionally, the present application provides the antibody or a fragment thereof comprising the amino acid sequence of formula 10-3 and not comprising the amino acid sequence of formula 10-1 and 10-2. Furthermore, the present application provides the antibody or a fragment thereof comprising the amino acid sequence of formula 10-3 in both two Fc domains.

[0106] Additionally, the present application provides the antibody or a fragment thereof wherein the cargo moiety comprises a drug moiety. Further, the present application provides the antibody or a fragment thereof wherein the cargo moiety comprises more than one drug moiety. Or further, the present application provides the antibody or a fragment thereof wherein the drug moiety is an anticancer agent. Furthermore, the present application provides the antibody or a fragment thereof wherein the anticancer agent is at least one selected from DM1, DM3, DM4, abrin, ricin A, pseudomonas exotoxin, cholera toxin, diphtheria toxin, tumor necrosis factor, α-interferon, β-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, a cytokine, an apoptotic agen, an anti-angiogenic agent, a lymphokine, taxane, a DNA-alkylating agent, anthracycline, tubulysin analogs, duocarmycin analogs, auristatin E, auristatin F, maytansinoid, a cytotoxic agent comprising a reactive polyethylene glycol moiety, taxon, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, t. colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoid, procaine, tetracaine, lidocaine, propranolol, puromycin, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine, mechlorethamine, thiotepachlorambucil, meiphalan, carmustine, lomustine, cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, cisplatin, dactinomycin, bleomycin, anthramycin, calicheamicin, Gemcitabine, bendamustine, bortezomib, carboplatin, cabazitaxel, dasatinib, docetaxel, epirubicin, erlotinib, everolimus, gemcitabine, gefitinib, idarubicin, imatinib, hydroxyurea, lapatinib, leuprorelin, melphalan, nedaplatin, nilotinib, oxaliplatin, pazopanib, pemetrexed, picoplatin, romidepsin, satraplatin, sorafenib, vemurafenib, sunitinib, teniposide, triplatin, and vinorelbine.

[0107] The present application provides a pharmaceutical composition for treating cancer comprising aforementioned antibody-drug conjugate

[0108] Also, the present application provides the pharmaceutical composition for treating

cancer wherein the cancer is selected from bladder cancer, bone cancer, brain tumor, breast cancer, heart cancer, cervical cancer, colorectal cancer, rectal cancer, esophageal cancer, fibrosarcoma, gastric cancer, gastrointestinal cancer, head and neck cancer, Kaposi sarcoma, renal cancer, leukemia, liver cancer, lung cancer, lymphoma, melanoma, myeloma, ovarian cancer, pancreatic cancer, penile cancer, prostate cancer, testicular germ cell cancer, thymoma and thymic carcinoma.

[0109] Additionally, the present application provides the pharmaceutical composition for treating cancer wherein the cancer is breast cancer.

[0110] The present application provides a method for treating cancer comprising administering a pharmaceutical composition comprising aforementioned antibody-drug conjugate to a subject.

[0111] Also, the present application provides the method for treating cancer wherein the cancer is selected from bladder cancer, bone cancer, brain tumor, breast cancer, heart cancer, cervical cancer, colorectal cancer, rectal cancer, esophageal cancer, fibrosarcoma, gastric cancer, gastrointestinal cancer, head and neck cancer, Kaposi sarcoma, renal cancer, leukemia, liver cancer, lung cancer, lymphoma, melanoma, myeloma, ovarian cancer, pancreatic cancer, penile cancer, prostate cancer, testicular germ cell cancer, thymoma and thymic carcinoma.

[0112] Furthermore, the present application provides the method for treating cancer wherein the cancer is breast cancer.

[Advantageous Effects]

[0113] An antibody product according to the present invention can have a certain number of chemical functional groups or cargo moieties labeled at a certain site thereof. Therefore, the present invention can provide an antibody product having high uniformity. Also, the present invention can provide an antibody product whose antibody functions are not degraded. That is, the present invention can provide an antibody product whose antibody binding affinity and half-life are not degraded. The present invention is of great significance as being the first technology allowing site-specific labeling of an antibody without any complicated processes.

BRIEF DESCRIPTION OF THE DRAWINGS

[0114] FIG. 1 shows a partial sequence of an Fc domain **of SEQ ID NO: 1** and numbers in the sequence numbered according to the EU numbering system.

[0115] FIG. 2 shows positions of lysine residues in an Fc domain including lysine 246 and 248.

[0116] FIG. 3 and FIG. 4 show the topology between SSFI and the Fc domain.

[0117] FIG. 5 shows Xa_1 of the SSFI and lysine 246 and 248 in the Fc domain.

[0118] FIG. 6 shows a distance between an amine group of the lysine 246 and a beta carbon of the Xa_1 of the SSFI.

[0119] FIG. 7 shows a distance between an amine group of the lysine 248 and a beta carbon of the Xa_1 of the SSFI.

[0120] FIG. 8 shows a structure of R_1'-L_2-SSFI and a distance (L_c) between a beta carbon and a first carbonyl carbon of (Xa_1)'.

[0121] FIG. 9 shows the topology between the R1'-L2-SSFI and the Fc domain so that a side chain of (Xa_1)' has a direction (a dotted arrow) parallel with the x axis on the drawing.

[0122] FIG. 10 shows the conditions used to allow the first carbonyl carbon to react well with lysine 248.

[0123] FIG. 11 shows the conditions used to allow the first carbonyl carbon to react well with lysine 246.

[0124] FIG. 12 shows the conditions used to allow the first carbonyl carbon to selectively react with lysine 246 or 248.

[0125] FIG. 13 shows an FcRn binding site and lysine 246 and 248 in the Fc domain.

[0126] FIG. 14 shows the analysis of a binding structure between SSFI and the Fc domain in comparison with a binding site between Fc and of FcRn.

[0127] FIG. 15 shows a method of synthesizing Compound I.

[0128] FIG. 16 shows the results of confirming a structure of Compound I by means of mass spectrometry.

[0129] FIG. 17 shows a method of synthesizing Compound II.

[0130] FIG. 18 shows the results of confirming a structure of Compound II by means of mass spectrometry.

[0131] FIG. 19 shows a method of synthesizing Compound III.

[0132] FIG. 20 shows the results of confirming a structure of Compound III by means of mass spectrometry.

[0133] FIG. 21 and FIG. 22 show a method of synthesizing Compound IV.

[0134] FIG. 23 shows the results of confirming a structure of Compound IV by means of mass spectrometry.

[0135] FIG. 24 shows the results of confirming a structure of SSFI (6Lys) by means of mass spectrometry.

[0136] FIG. 25 shows the results of confirming a structure of SSFI (6Orn) by means of

mass spectrometry.

[0137] FIG. 26 shows the results of confirming a structure of SSFI (6Dab) by means of mass spectrometry.

[0138] FIG. 27 shows the results of confirming a structure of SSFI (6Dap) by means of mass spectrometry.

[0139] FIG. 28 shows the results of confirming a structure of DD2 by means of mass spectrometry.

[0140] FIG. 29 shows the results of confirming a structure of DD3 by means of mass spectrometry.

[0141] FIG. 30 shows the results of confirming a structure of DD4 by means of mass spectrometry.

[0142] FIG. 31 shows the results of confirming a structure of DD5 by means of mass spectrometry.

[0143] FIG. 32 shows the results of confirming a structure of DD6 by means of mass spectrometry.

[0144] FIG. 33 shows the results of confirming a structure of Compound I-SSFI (6Lys) by means of mass spectrometry.

[0145] FIG. 34 shows the results of confirming a structure of Compound II-SSFI (6Lys) by means of mass spectrometry.

[0146] FIG. 35 shows the results of confirming a structure of Compound III-SSFI (6Lys) by means of mass spectrometry.

[0147] FIG. 36 shows the results of confirming a structure of Compound III-SSFI (6Orn) by means of mass spectrometry.

[0148] FIG. 37 shows the results of confirming a structure of Compound III-SSFI (6Dab) by means of mass spectrometry.

[0149] FIG. 38 shows the results of confirming a structure of Compound III-SSFI (6Dap) by means of mass spectrometry.

[0150] FIG. 39 shows the results of confirming a structure of Compound IV-SSFI (6Dap) by means of mass spectrometry.

[0151] FIG. 40 shows the results of observing a binding reaction using trastuzumab and Compound I-SSFI (6Lys) by means of HIC-HPLC.

[0152] FIG. 41 shows the results of observing a binding reaction using trastuzumab and Compound II-SSFI (6Lys) by means of HIC-HPLC.

[0153] FIG. 42 shows a reaction of an antibody with Compound III-SSFI (6Dap, Dab, Orn, or Lys).

[0154] FIG. 43 shows a structure of Ab (246/248)-Norbornene as the final product.

[0155] FIG. 44 shows the results of observing a binding reaction using trastuzumab and Compound III-SSFI (6Dap) by means of HIC-HPLC.

[0156] FIG. 45 shows the results of observing a binding reaction using trastuzumab and Compound III-SSFI (6Dab) by means of HIC-HPLC.

[0157] FIG. 46 shows the results of observing a binding reaction using trastuzumab and Compound III-SSFI (6Orn) by means of HIC-HPLC.

[0158] FIG. 47 shows the results of observing a binding reaction using trastuzumab and Compound III-SSFI (6Lys) by means of HIC-HPLC.

[0159] FIG. 48 shows the results of observing a binding reaction using trastuzumab and Compound IV-SSFI (6Dap) by means of HIC-HPLC.

[0160] FIG. 49 and FIG. 50 show an increase of molecular weight spectra by antibody-norbornene binding.

[0161] FIG. 51, FIG. 52, FIG. 53 and FIG. 54 show the MS/MS chromatogram results of trastuzumab and antibody-norbornene complexes.

[0162] FIG. 55 shows the sequence matching results by means of the MS/MS spectrum.

[0163] FIG. 56 shows the mass spectrum of trastuzumab measured to confirm a trastuzumab-azide structure.

[0164] FIG. 57 shows the mass spectrum of a trastuzumab-azide complex measured to confirm the trastuzumab-azide structure.

[0165] FIG. 58 shows the results of confirming a structure of tetrazine-DM1 by means of mass spectrometry.

[0166] FIG. 59 shows a structure of a Compound III-SSFI (6Dap)-based trastuzumab-DM1 conjugate.

[0167] FIG. 60 shows the results of observing a formation reaction of a trastuzumab-DM1 conjugate by means of HIC-HPLC.

[0168] FIG. 61 and FIG. 62 show an increase of molecular weight spectra by norbornene-tetrazine-DM1 binding.

[0169] FIG. 63 shows structures of three designs of payloads bound to an antibody labeled with norbornene.

[0170] FIG. 64 shows the results of analyzing antigen binding affinity of an antibody-drug

conjugate.

[0171] FIG. 65, FIG. 66 and FIG. 67 show the results of analyzing serum stability of the antibody-drug conjugate.

[0172] FIG. 68, FIG. 69 and FIG. 70 show the results of evaluating a medicinal effect of the antibody-drug conjugate at a cellular level.

[0173] FIG. 71 and FIG. 72 show the results of evaluating a medicinal effect of the antibody-drug conjugate at an animal level.

[0174] FIG. 73 shows the results of a pharmacokinetics test on the antibody-drug conjugate.

DETAILED DESCRIPTION

[0175] Definitions

[0176] Unless otherwise defined, all technical and scientific terms used in the present invention have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. The following references are provided to give a number of common definitions of the terms used in the present invention to those skilled in the related art: Singleton *et al.*, Dictionary of Microbiology and Molecular Biology (2nd ed. 1994); The Cambridge Dictionary of Science and Technology (Walker ed., 1988); The Glossary of Genetics, 5th Ed., R. Rieger *et al.* (eds.), Springer Verlag (1991); and Hale & Marham, The Harper Collins Dictionary of Biology (1991). Unless otherwise clearly specified, the following terms used in the present invention shall have the meanings ascribed to them as follows.

[0177] In some embodiments, a chemical structure is disclosed together with the corresponding chemical name. When the terms contradict or conflict with each other, the chemical structure takes precedence over the chemical name to understand the meaning of a compound through the chemical structure.

[0178] The term “hetero” used in the present invention refers to a compound or a group of compounds including at least one heteroatom. The term “heteroatom” refers to an atom other than a carbon or hydrogen atom, and, for example, includes B, Si, N, P, O, S, and Se. Preferably, the heteroatom includes, among others, polyvalent elements such as N, O, and S, or monovalent elements such as F, Cl, Br, and I, but the present invention is not limited thereto.

[0179] The term “lower” used in the present invention is used to modify hydrocarbons, for example, alkylenes, and the like, and thus means that the corresponding hydrocarbon has 6 or less carbon atoms. For example, a C_{1-6} linear or branched alkyl group are referred to by another

name such as a “lower alkyl” group.

[0180] The term “oxy” used in the present invention refers to a secondary radical (-O-) of an oxygen atom.

[0181] The term “alkyl” or “alkane” refers to a linear or branched non-aromatic hydrocarbon that is completely saturated. Unless otherwise defined, a linear or branched alkyl group typically has a 1 to approximately 20 carbon atoms, preferably 1 to approximately 10 carbon atoms. The linear and branched alkyl group includes methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, tert-butyl, pentyl, hexyl, pentyl, and octyl.

[0182] The term “alkenyl” or “alkene” refers to a linear or branched non-aromatic hydrocarbon that contains at least one double bond. Unless otherwise defined, a linear or branched alkenyl group typically has 1 to approximately 20 carbon atoms, preferably 1 to approximately 10 carbon atoms.

[0183] The term “alkynyl” or “alkyne” refers to a linear or branched non-aromatic hydrocarbon that contains at least one triple bond. Unless otherwise defined, a linear or branched alkynyl group typically has 1 to approximately 20 carbon atoms, preferably 1 to approximately 10 carbon atoms.

[0184] The term “cycloalkane” or “cycloalkyl” group refers to a completed saturated cyclic hydrocarbon. The “cycloalkyl” includes monocyclic and polycyclic rings. Unless otherwise defined, a monocyclic cycloalkyl group generally has 3 to approximately 10 carbon atoms, more generally 3 to 8 carbon atoms. Rings other than the first ring of the polycyclic cycloalkyl may be selected from saturated, unsaturated, and aromatic rings. The cycloalkyl includes a bicyclic molecule that contains 1, 2, or 3 or more atoms shared between two rings. The term “fused cycloalkyl” refers to a polycyclic cycloalkyl in which one ring shares two adjacent atoms with another ring. Rings other than the first ring of the fused polycyclic cycloalkyl may be selected from saturated, unsaturated, and aromatic rings.

[0185] The term “cycloalkyne” or “cycloalkynyl” refers to a cyclic hydrocarbon that contains at least one triple bond, which is also referred to as a “strained alkyne.” The “cycloalkynyl” includes monocyclic and polycyclic rings. Unless otherwise defined, a monocyclic cycloalkynyl generally has 3 to approximately 10 carbon atoms, more generally 3 to 8 carbon atoms. Rings other than the first ring of the polycyclic cycloalkynyl may be selected from saturated, unsaturated and aromatic rings. The cycloalkynyl is a bicyclic molecule that contains 1, 2, or 3 or more atoms shared between two rings. The term “fused cycloalkynyl” refers to a polycyclic cycloalkynyl in which one ring shares two adjacent atoms with another ring. Rings other than the first ring of the fused polycyclic cycloalkynyl may be selected from saturated,

unsaturated, and aromatic rings.

[0186] The term “alkylene” used as a molecule itself or used as part of another molecule refers to a divalent radical derived from an alkane. For example, the group includes -CH_2CH_2-, and - $CH_2CH_2CH_2CH_2$-, all of which contain 10 or less carbon atoms, but the present invention is not limited thereto. The term “lower alkylene” refers to a short alkylene group that generally has 6 or less carbon atoms. Unless stated otherwise, the term “alkylene” is intended to encompass groups represented by the “heteroalkylene” in the present invention.

[0187] The term “heteroalkylene” refers to a divalent radical derived from a heteroalkyl, and, for example, includes -CH_2-CH_2-S-CH_2-CH_2-, and -CH_2-S-CH_2-CH_2-NH-CH_2-, but the present invention is not limited thereto. A heteroalkylene group may contain the same or different heteroatoms at each end or all ends of a chain thereof (including an alkyleneoxy, an alkylenedioxy, an alkyleneamino, an alkylenediamino, an aminooxyalkylene, and the like, but the present invention is not limited thereto). Further, an indication of connection of both ends of the chain is independent of the arrangement of groups of a formula. For example, the formula -$C(O)_2$R’- refers to both -$C(O)_2$R’- and -R’$(O)_2$C-.

[0188] The term “alkenylene” used as a molecule itself or used as part of another molecule refers to a divalent radical derived from an alkene. For example, the group includes -CH=CH-, -CH_2CH=CHCH_2-, and -CH=CH-CH=CH-, all of which have 10 or less carbon atoms, but the present invention is not limited thereto. Unless stated otherwise, the term “alkenylene” is intended to encompass heteroalkenylenes in the present invention.

[0189] The term “alkynylene” used as a molecule itself or used as part of another molecule refers to a divalent radical derived from an alkyne. For example, the group includes -C≡C-, - CH_2C≡CCH_2-, and -C≡C-C≡C-, all of which have 10 or less carbon atoms, but the present invention is not limited thereto. Unless stated otherwise, the term “alkynylene” is intended to encompass heteroalkynylenes in the present invention.

[0190] The term “cycloalkylene” used as a molecule itself or used as part of another molecule refers to a divalent radical derived from a cycloalkene. Unless stated otherwise, the “cycloalkylene” is intended to encompass heterocycloalkylenes in the present invention.

[0191] The term “alkylene” used in this specification, examples, and the claims is intended to encompass both “unsubstituted alkylene” and “substituted alkylene.” Among these, the latter refers to an alkylene group that has a substituent replacing a hydrogen atom on one or more carbon atoms of the hydrocarbon. Unless otherwise clearly specified, the substituent may, for example, include a halogen, a hydroxyl group, a carbonyl group (for example, carboxyl, alkoxycarbonyl, formyl, or acyl), a thiocarbonyl group (for example, thioester, thioacetate, or

thioformate), an alkoxy group, a phosphoryl group, a phosphate group, a phosphonate group, a phosphinate group, an amino group, an amido group, an amidine group, an imine group, a cyano group, a nitro group, an azido group, a sulfhydryl group, an alkylthio group, a sulfate group, a sulfonate group, a sulfamoyl group, a sulfonamido group, a sulfonyl group, a heterocyclyl group, an aralkyl group, or an aromatic or heteroaromatic group. When properly substituted, it may be understood by those skilled in the related art that a substituted residue on a hydrocarbon chain may itself be substituted. For example, the substituent of the substituted alkylene may include substituted and unsubstituted amino, azido, imino, amido, phosphoryl (including phosphonates and phosphinates), sulfonyl (including sulfates, sulfonamidos, sulfamoyls, and sulfonates), and silyl groups, and may also include ethers, alkylthios, carbonyls (including ketones, aldehydes, carboxylates, and esters), -CF3, -CN, and equivalents thereof. Exemplary substituted alkyls are as described below. The cycloalkylene may be further substituted with an alkyl, an alkenyl, an alkoxy, an alkylthio, an aminoalkyl, a carbonyl-substituted alkyl, -CF3, -CN, and equivalents thereof. This content is also equally applicable to the alkenylene and the alkynylene.

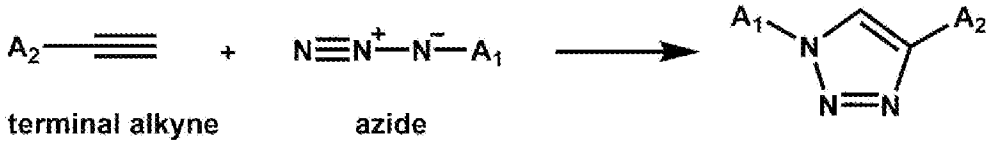
[0192] When used together with a residue such as an alkylene, an alkenylene, or an alkynylene, the term "C_{x-y}" is, for example, intended to encompass residues containing x to y carbon atoms in the chain thereof. For example, the term "C_{x-y} alkylene" refers to a substituted or unsubstituted, linear or branched alkylene group that contains x to y carbon atoms in the chain thereof. For example, it is meant to exemplarily include a haloalkylene group such as difluoromethylene, 2,2,2-trifluoroethylene, and the like. The C_0 alkylene refers to a covalent bond. The terms "C_{2-y} alkenylene" and "C_{2-y} alkynylene" refers to a substituted or unsubstituted unsaturated aliphatic residue to which a definition of length and possible substitution is applied as described in the definition of the alkylene. However, this means that each contains at least one double or triple bond.

[0193] The term "click-chemistry reaction" is used as a chemical concept introduced by K. Barry Sharpless of the Scripps Research Institute to explain complementary chemical functional groups and a chemical reaction designed so that two molecules can rapidly and stably form a covalent bond. The click-chemistry reaction does not refer to a certain reaction, but refers to a concept of such a rapid and stable reaction. In any embodiment, the click-chemistry reaction must be modular, wide in scope, give high yields, generate only insignificant by-products, be stereospecific, physiologically stable, driven by a thermodynamic driving force (for example, greater than 84 kJ/mol), and/or have high atom economy. Some reactions are known to satisfy the requirements:

[0194] (1) Huisgen 1,3-dipolar cycloaddition (for example, including a Cu(I)-catalyzed

cycloaddition reaction, which is often commonly referred to as a "click reaction"; see Tornoe *et al.*, Journal of Organic Chemistry (2002) 67: 3057-3064): Copper or ruthenium is generally used as a catalyst;

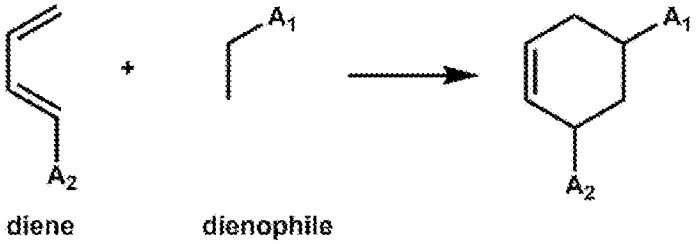
[0195] [Schematic diagram of Huisgen 1,3-dipolar cycloaddition]



1,3-dipolar cycloaddition

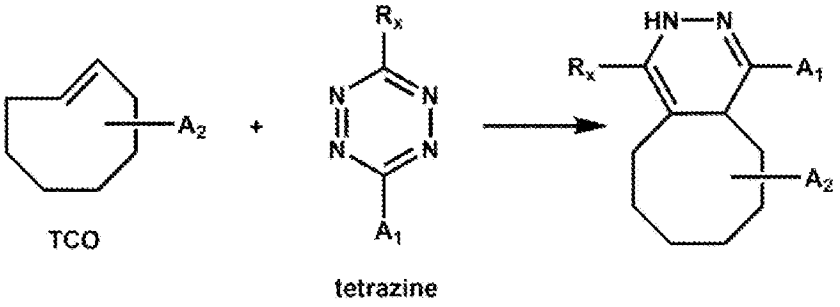
[0196] (2) Diels-Alder reaction, for example cycloaddition (for example, strain-promoted cycloaddition (SPAAC)) including a normal electron-demand Diels-Alder reaction and an inverse electron-demand Diels-Alder reaction, but the present invention is not limited thereto);

[0197] [Schematic diagram of Diels-Alder reaction]

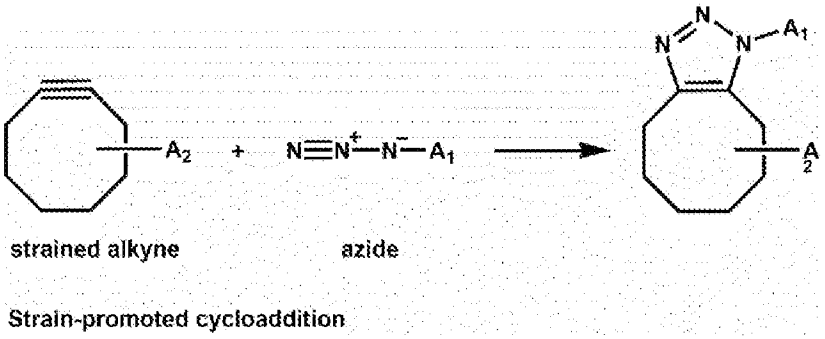


Diels-Alder reaction

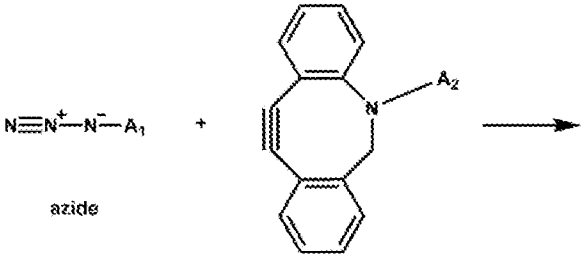
[0198] [Example of Diels-Alder reaction; TCO and tetrazine]

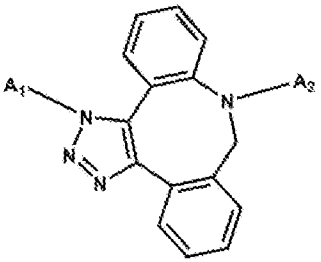


[0199] [Schematic diagram of strain-promoted cycloaddition]



[0200] [Example of strain-promoted cycloaddition; Azide and DBCO]



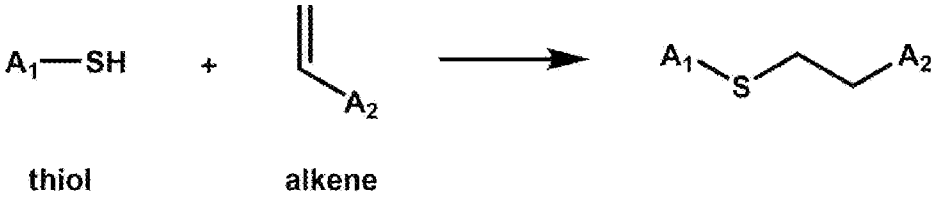


[0201] (3) Nucleophilic addition to small strained rings such as epoxides and aziridines;

[0202] (4) Nucleophilic addition to activated carbonyl groups;

[0203] (5) Addition to carbon-carbon double bonds or triple bonds.

[0204] [Addition of thiol and alkene]



[0205] The term “click-chemistry functional group” used in the present invention refers to a functional group that participates in a click-chemistry reaction. For example, a strained alkyne (for example, a cyclooctyne) corresponds to a click-chemistry functional group. In general, the click-chemistry reaction requires at least two molecules, each of which contains click-chemistry functional groups complementary to each other. In this way, a pair of click-chemistry functional

groups having reactivity with each other is often referred to as "partner click-chemistry functional groups" in the present invention. In strain-promoted cycloaddition of a cyclooctyne with an azide, for example, the azide is a partner click-chemistry functional group for the cyclooctyne and other alkynes. Exemplary click-chemistry functional groups used in the present invention include a terminal alkyne, an azide, a strained alkyne, a diene, a dienophile, a trans-cyclooctene, an alkene, a thiol, and a tetrazine, but the present invention is not limited thereto. Other click-chemistry functional groups are known to those skilled in the related art.

[0206] The term "leaving group" used in the present invention has the same concept as well known to those skilled in the related art (Advanced Organic Chemistry: reactions, mechanisms and structure- Fourth Edition of Jerry March, John Wiley and Sons Ed.; 1992, pages 351-357), and refers to a chemical functional group linked to any reactant, which moves when the reactant is subjected to a substitution reaction (displacement reaction), for example, a nucleophilic substitution reaction. A good leaving group refers to a leaving group that easily moves during the nucleophilic substitution reaction. Exemplary good leaving groups include a halogen (F, Cl, Br, and I), a tosylate, a mesylate, a triflate, an acetate, a trifluoromethylacetate, a camphorsulfonate, 2-thioxobenzo[d]thiazol-3(2H)-yl, N-hydrosuccinimide, N-aryloxide, and an aryloxide substituted with one or more electron-withdrawing groups (EWGs), but the present invention is not limited thereto. For example, the aryloxide substituted with one or more electron-withdrawing groups include 2-nitrophenoxide, 4-nitrophenoxide, 2,4-dinitrophenoxide, pentafluorophenoxide, 2-chloro-4-nitrophenoxide, 2,4-dichlorophenoxide, and 2,4,6-chlorophenoxide, and the electron-withdrawing group, for example, includes a halogen (F, Cl, Br, or I), $-NO_2$, -CN, $-C(O)(C_{1-6}$ alkyl), -C(O)(aryl), $-C(O)O(C_{1-6}$ alkyl), -C(O)O(aryl), and the like.

[0207] The term "interactome" used in the present invention refers to a protein or a peptide that participates in a protein-protein interaction when there is a protein-protein interaction (PPI) between proteins or between peptides. For example, a chaperone protein and its passenger protein are mutual interactomes. The "protein-protein interaction" means that two or more proteins or peptide molecules interact to come into physical contact with each other with high specificity. In this case, the causative interaction includes an electromagnetic force, a hydrogen bond, and a hydrophobic interaction, but the present invention is not limited thereto.

[0208] The term "antibody interactome" used in the present invention refers to an interactome for an antibody including immunoglobulins. Exemplary antibody interactomes are listed in Table 1. Among these, peptides such as Fc-III, and the like are known to have binding activity for an Fc domain of an immunoglobulin. In this case, the peptides are also referred to as "Fc interactomes."

<Table 1> List of SSAIs

Peptide	Binding Constant	Binding Capacity	Elution pH	Remarks
PAM	K_d = 0.3μM	n.a.	3 or 9	Dendrimer comb. library SpA mimic
D-PAM	n.a.	50	3.5	
D-PAM-Φ	n.a.	10	4	
TWKTSRSIF	n.a.	n.a.	n.a.	Phage display library SpA mimic
FGRLYSSAIRY	n.a.	n.a.	n.a.	
Fc-III (DeLano et al.) Fc-III-(Sepharose)	K_d = 16nM	26.6mg/μmol	3.5	Phage display cyclic peptide library SpA mimic
FcBP-2	K_d = 1.6nM	n.a.	n.a.	Bicyclic peptide
Fc-III-4C	K_d = 2.5nM	n.a.	3.5	Bicyclic peptide
EPHRSTLTALL	n.a.	320μg/g	n.a.	Phage display library SpA mimic
APAR	K_d = 94nM	9.1	n.a.	Comb. tetrapeptide library
FcRM	K_d = 20μM	n.a.	2.7	Synthetic cyclic peptide library, Fcγ-receptor mimic

<Table 1> (Continued)

Peptide	Binding Constant	Binding Capacity	Elution pH	Remarks
FWRGWV	K_d = 10μM	28.4	4	Comb. library (one-bead-one-peptide) SpA mimic
HYFKFD	K_d = 11μM	27.0	n.a.	
HFRRHL	K_d = 29μM	33.6	n.a.	
FWOrGWV	K_d = 106μM	72	n.a.	
DAAG	K_a = 7.9x10^{5}M^{-1}	36.2	3.6	Comb. library SpA mimic
DAAG	Ka = 2.6x10^{5}M^{-1}	49.6	3.6	
cyclo[(Ne-Ac)KA)-FWHYFK-Lact-E]	n.a.	n.a.	3.5	Cyclic peptide
cyclo[(Ne-Ac)-Dap(A)-FWHYFK-Lact-E]	n.a.	n.a.	3.5	
cyclo[Link-M-WPRHYK]	K_d = 7.6μM	19.7	4.0	mRNA display library SpA mimic
NKFRGRYK	K_a = 3.6x10^{6}M^{-1}	DBC-4.9	4.0	Spot peptide array Fcv mimic
NARKFYKG	K_a = 8.5x10^{6}M^{-1}	DBC-5.0	4.0	

<Table 1> (Continued)

Peptide	Binding Constant	Binding Capacity	Elution pH	Remarks
FYWHCLDE(1)	K_d = 1.5μM	104.3	6.0	Biomimetic design strategy Fc-binder (SpA mimic)
FYCHWALE(2)	K_d = 6.1μM	87.6	6.0	
FYCHTIDE(3)	K_d = 5.7μM	63.7	6.0	Dual affinity ligand
Dual 1/3 (2:1)	K_d = 0.69μM	137.9	6.0	
ERGW	K_d = 0.5mM	n.a.	n.a.	Computer design strategy Fc-binder
KHRFNKD	K_d = 20nM	n.a.	n.a.	Phage-display library SpA mimic

* Maximal binding capacity (q_m) in mg/mL. DBC: Dynamic binding capacity. The depicted values are towards IgG and may not be directly compared to each other because they were obtained at different conditions or by different methods. n.a.: Not available.

[0209] According to the present invention, the term “antibody” refers to an immunoglobulin molecule or a fragment thereof. Immunoglobulins are generally well known, and have an ability

to specifically bind to a certain antigen. However, because the antibody according to the present invention is a concept that also encompasses a fragment thereof, the antibody does not have to show binding ability to a certain antigen as in case of the Fc fragment. In addition to the naturally occurring immunoglobulins, the antibody is also intended to encompass all recombinant proteins, fusion proteins, chimeric proteins, human immunoglobulins, non-human animal immunoglobulins, and the like, which have the same or similar structures.

[0210] According to the present invention, the term "conjugate" refers to a heterogeneous molecule made when conjugate partners are taken together to form a covalent bond. The covalent bond may be preferably formed by means of a click-chemistry reaction. The term "conjugate partner" refers to each of molecules intended to form a conjugate. In this case, when one wishing to perform conjugation intends to link a certain molecule to any other molecule, the certain molecule may be generally referred to as a "target molecule" or "target protein," and the any other molecule may be generally referred to as a "cargo molecule" or "cargo moiety" for the sake of convenience.

[0211] According to the present invention, the term "carrier moiety" refers to a portion of molecule that constitutes a conjugate, that is, a molecule that functions to enhance serum stability of a molecule linked thereto or extend the half-life of the molecule. Molecules that may be used as the carrier moiety are well known in the related art. Representative examples of the carrier moiety include albumin, gelatin, elastin (including tropoelastin), an elastin-derived polypeptide (α-elastin and elastin-like polypeptides (ELPs)), gliadin, legumin, zein, a soy protein (for example, a soy protein isolate (SPI)), a milk protein, a whey protein, bilirubin, and the like, but the present invention is not limited thereto.

[0212] According to the present invention, the term "fluorescent moiety" is intended to encompass dyes or dye reagents used for fluorescence. Molecules that may be used as the dyes and dye reagents are well known in the related art. Representative examples of the fluorescent moiety are as listed in Table 2, but the present invention is not limited thereto (Immunotech-Coulter Corp. catalog, "Cytometry Monoclonal Reagent Guide," 8/95, p.3).

<Table 2> List of fluorescent moieties

Fluorochrome	Maximum Absorbance	Excitation at 488 nm	Maximum Emission	Fluorescence
Fluorescein isothiocyanate (FITC)	495 nm	Yes	525 nm	Green
Phycoerythrin (PE)	488; 565 nm	Yes	575 nm	Orange-red
Energy Coupled Dye [ECD] (PE-Texas-Red)	488; 565 nm	Yes	610-635 nm	Red
Phycoerythrin-Cyanin 5 (PE-Cy5)	488; 565; 652 nm	Yes	670 nm	Deep-red

[0213] According to the present invention, the term "drug moiety" refers to a molecule that has a therapeutic effect on any disease. The drug moiety according to the present invention includes those known to a person having ordinary skill in the art as being effective against any disease. Typically, the drug moiety having an anti-cancer effect includes DM1, DM3, DM4, Abrin, Ricin A, a *Pseudomonas* exotoxin, a *Cholera* toxin, a *Diphtheria* toxin, a tumor necrosis factor, α interferon, β interferon, a nerve growth factor, a platelet-derived growth factor, a tissue plasminogen activator, a cytokine, an apoptosis-inducing agent, an anti-angiogenic agent, a lymphokine, taxane, a DNA-alkylating agent, anthracyclin, a Tubulysin analogue, a duocarmycin analogue, auristatin E, auristatin F, a maytansinoid, a cytotoxic agent including a reactive polyethylene glycol residue, taxon, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, T. Colchicine, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dihydrotestosterone, a glucocorticoid, procaine, tetracaine, lidocaine, propranolol, puromycin, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine, mechlorethamine, thiotepa, chlorambucil, Meiphalan, carmustine, lomustine, cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, cisplatin, dactinomycin, bleomycin, anthramycin, calicheamicin, abiraterone, bendamustine, bortezomib, carboplatin, cabazitaxel, dasatinib, doxetaxel, epirubicin, erlotinib, everolimus, gemcitabine, gefitinib, idarubicin, imatinib, hydroxyurea, lapatinib, leuprorelin, melphalan, nedaplatin, nilotinib, oxaliplatin, pazopanib, pemetrexed, picoplatin, romidepsin, satraplatin, sorafenib, vemurafenib, sunitinib, teniposide, triplatin, and vinorelbine, but the present invention is not limited thereto.

[0214] According to the present invention, the term "radioactive moiety" refers to a moiety including a radioisotope. Radioisotopic labeling is useful for diagnostic imaging and radiotherapy. Representative radioactive moieties include ^{18}F, ^{11}C, ^{125}I, ^{123}I, ^{124}I, ^{131}I, and ^{99m}Tc, but the present invention is not limited thereto.

[0215] The term "pharmaceutically acceptable carrier" may be used in the sense of including an excipient, a diluent, or an adjuvant. The carrier may be, for example, selected from the group consisting of lactose, dextrose, sucrose, sorbitol, mannitol, xylitol, erythritol, maltitol, starch, acacia gum, alginate, gelatin, calcium phosphate, calcium silicate, cellulose, methyl cellulose, polyvinyl pyrrolidone, water, physiological saline, a buffer such as PBS, methylhydroxy benzoate, propylhydroxy benzoate, talc, magnesium stearate, and mineral oil. The carrier may include a filler, an anti-agglomerating agent, a lubricant, a wetting agent, a flavoring agent, an emulsifying agent, a preservative, or a combination thereof.

[0216] The term "pharmaceutically acceptable salt" refers to a salt in which biological effectiveness and properties of a proteome and a compound according to the present invention are preserved and that is not undesirable in biological or other aspects. In many cases, the proteome and compound of the present invention may form an acidic and/or basic salt in the presence of a charged group, for example, a charged amino and/or carboxyl group or the likes. A pharmaceutically acceptable acid addition salt may be prepared from an inorganic and organic acid, and a pharmaceutically acceptable base addition salt may be prepared from an inorganic and organic base.

[0217] The term "treatment" refers to an approach to obtain beneficial or desirable clinical outcomes. For the object of the present invention, non-limiting examples of the beneficial or desirable clinical outcomes include palliation of symptoms, reduction of a disease range, stabilization (i.e., not worsening) of a disease state, delay of progression or reduction of a progression rate of a disease, (partially or totally) improvement or temporal palliation and mitigation of a disease condition, and whether detectable or not. The treatment denotes all therapeutic treatments, and prophylactic or preventative methods. The treatments include treatments required for disorders to be prevented and already-occurring disorders. "Palliating" a disease means that a range of disease conditions and/or undesirable clinical signs are lowered and/or a time course of progression of the disease is delayed or extended, compared to when the disease is not treated.

[0218] The "therapeutically effective amount" (or "effective amount") refers to a sufficient amount of an active ingredient, for example, an agent according to the present invention, to achieve the treatment when administered to a subject or a patient. Therefore, what constitutes a therapeutically effective amount of a composition according to the present invention may be easily determined by those skilled in the related art. In the context of vision therapy, the "therapeutically effective amount" is an amount that causes an objectively measured change in one or more parameters associated with the treatment of eye diseases or conditions, which

include an increase or decrease in expression of one or more genes associated with the eye diseases or conditions, an induction of apoptosis or other cell death pathways, a clinical improvement in symptoms, a decrease in abnormal neovascularization or inflammation, and the like. Of course, the therapeutically effective amount may vary depending on a certain subject and condition to be treated, the weight and age of a subject, the severity of a disease condition, a certain compound to be selected, a subsequent dosing schedule, the administration time adjustment, a mode of administration, and the like, all of which may be easily determined by those skilled in the related art. In the context of combination therapy, it should be understood that what constitutes a therapeutically effective amount of a certain active ingredient may be different from what constitutes a therapeutically effective amount of an active ingredient that is administered for monotherapy (that is, a treatment regimen using one chemical entity as the active ingredient).

[0219] The “subject” or “patient” refers to an animal in need of treatment which may be achieved by the molecule of the present invention. The animal to be treated according to the present invention includes a vertebrate. Particularly preferred examples of the animal include mammals, for example, bovine, canine, equine, feline, ovine, porcine, and primate animals (including human and non-human primates).

[0220] The term “about” or “approximately” refers to an amount, level, value, number, frequency, percentage, dimension, size, quantity, weight, or length that varies by 30, 25, 20, 25, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1% with respect to the reference amount, level, value, number, frequency, percentage, dimension, size, quantity, weight, or length.

[0221] 1. Antibody

[0222] It should be understood that the description of the present invention is intended to provide a description of the structure, academic system, and physiological action of an antibody provided to aid in understanding the present invention, and the antibody falling within the scope of rights of the present invention is not intended to limit the description of the present invention.

[0223] An antibody consists of two heavy chains and two light chains as known in the art. When an antibody is simply divided in a functional aspect, the antibody is divided into a fragment antigen-binding variable region (Fab) including a light chain and a fragment crystallizable region composed as a portion of a heavy chain. The Fab includes a paratope that binds to an antigen, and refers to a region that allows an antibody to have specific binding activity for an antigen as known in the art. As the Fc domain is a ligand for an Fc receptor (FcR) in cells, it plays an important role in inducing an immune response. Also, the Fc domain plays an important role in extending the half-life of an antibody by binding to a neonatal Fc receptor so that the antibody can be repeatedly internalized into the cells.

[0224] From these facts, it is possible to deduce some desirable directivity of labeling an antibody: (1), First, it is desirable to label an antibody at a position spaced apart from a paratope of the antibody. When the labeling is performed on the paratope or at a position adjacent to the paratope, a binding affinity of the antibody for an antigen may be significantly lowered. (2) Second, it is desirable to label an antibody at a position spaced apart from a recognition site of FcR including FcRn. When the labeling is performed on the recognition site of the receptor or at a position adjacent to the recognition site, an immune response-inducing function of the antibody may be reduced, or the half-life of the antibody may be shortened. Information on the binding activity motif of the Fc domain may be found in DeLano, W.L. (2000): Convergent Solutions to Binding at a Protein-Protein Interface; Science, 287(5456), 1279-1283., and W. Lance Martin *et al*. (2001), Molecular Cell, Vol. 7, 867-877, April, 2001.

[0225] In the present invention, when reference to an amino acid sequence of the Fc domain of the antibody is made, numbers in the sequence are numbered according to the EU numbering system unless stated otherwise. The EU numbering system has been widely used as a sequencing system for the Fc domain after research on the sequence of IgG, as described in Edelman GM, *et al.*, The covalent structure of an entire gamma-G immunoglobulin molecule; Proc. Natl. Acad. Sci. USA., 1969 May; 63(1): 78-85.

[0226] 1.1. Search for desirable labeling site

[0227] A labeling site of an antibody may be designed in consideration of the criteria for the labeling site, that is, (1) a site spaced apart from a paratope; and (2) a site spaced apart from a recognition site of FcR including FcRn. Examples of the amino acids used in a bioconjugation reaction typically include lysine, cysteine, and tyrosine. Lysine 246 (Lys_{246}) and lysine 248 (Lys_{248}) present in an Fc domain of the antibody are residues that satisfy all the requirements, and thus are both desirable labeling sites. A sequence of the Fc domain including the Lys_{246} and Lys_{248} residues is GPSVFLFPPKPKDTLMI, and the sequence and numbers in the sequence numbered according to the EU numbering system are shown in FIG. 1 (FIG. 1, SEQ ID NO: 1).

[0228] In specific embodiments, the antibody according to the present invention may include a sequence of SEQ ID NO: 1 or derivatives thereof. Also, the antibody according to the present invention may include a derivative of SEQ ID NO: 1 in which lysine 246 is substituted. In addition, the antibody according to the present invention may include a derivative of SEQ ID NO: 1 in which lysine 248 is substituted. Furthermore, the antibody according to the present invention may include a derivative of SEQ ID NO: 1 in which lysine 246 and 248 are substituted.

[0229] The sequence of SEQ ID NO: 1 or derivatives thereof include sequences mutated in an acceptable range. In specific embodiments, the mutated sequences may have a homology

that is greater than or equal to approximately 90%, approximately 85%, approximately 80%, approximately 75%, or approximately 70% with respect to the sequence of SEQ ID NO: 1 or derivatives thereof. In specific embodiment specified below, it should be understood that the derivatives of SEQ ID NO: 1 represented by Formulas 7-1 to 7-3, 8-1 to 8-3, and 10-1 to 10-3 also include sequences mutated in an acceptable range.

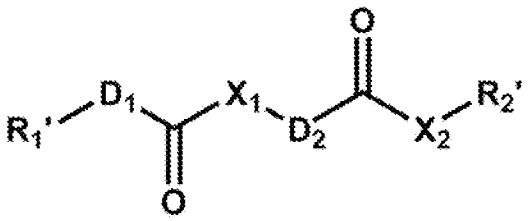
[0230] FIG. 2 shows positions of lysine residues in an Fc domain including lysine 246 and 248.

[0231] 2. Linker (R_1'-L_1)

[0232] The present invention discloses a novel compound capable of being used to label an antibody. For the sake of convenience, such a compound is herein referred to as a linker, which is indicated by the symbol "R_1'-L_1."

[0233] The present invention provides a compound having a structure of the following Formula 1:

[0234] [Formula 1]



[0235] wherein R_1' is a first chemical functional group,

[0236] D_1 is any alkylene, alkenylene, or alkynylene,

[0237] X_1 is an element that is more electronegative than carbon,

[0238] D_2 is any alkylene, alkenylene, or alkynylene,

[0239] X_2 is an element that is more electronegative than carbon, and

[0240] R_2' is a second chemical functional group.

[0241] In Formula 1, a carbonyl group connected to D_1 is referred to as a first carbonyl group. Also, a carbonyl group connected to D_2 is referred to as a second carbonyl group.

[0242] In specific embodiments, R_1' may include a click-chemistry functional group. Also, the click-chemistry functional group may include one or more selected from an alkyne, an azide, a strained alkyne, a diene, a dienophile, an alkene, a thiol, and a tetrazine. Furthermore, the click-chemistry functional group may be selected from an azide, or a strained alkyne. Further, the click-chemistry functional group may be selected from an azide, or dibenzocyclooctyne-amine. Additionally, the click-chemistry functional group may be selected from a diene, or a dienophile. Further, the click-chemistry functional group may be selected from a tetrazine, or a norbornene.

Alternatively, the click-chemistry functional group may be selected from a tetrazine, or a trans-cyclooctene. In addition, R_1' may include two or more click-chemistry functional groups.

[0243] In other specific embodiments, R_1' may include a carrier moiety, a fluorescent moiety, a drug moiety, or a radioactive moiety. Also, R_1' may include a drug moiety. In addition, R_1' may include a VC linker. In other specific embodiments, R_1' may include an antibody or an analogue thereof, which includes a paratope.

[0244] In specific embodiments, D_1 may include any one selected from a covalent bond, a C_{1-4} alkylene, a C_{2-4} alkenylene, a C_{2-4} alkynylene, and a C_{3-8} cycloalkylene. Also, D_1 may be $-CH_2OCH_2-$. In addition, D_1 may be a covalent bond. When D_1 is a covalent bond, R_1' and the carbon of the first carbonyl group are directly connected to each other. Hereinafter, when describing a structure of a compound, all the alkylene, the alkenylene, the alkynylene, and the cycloalkylene are intended to include a heteroalkylene, a heteroalkenylene, a heteroalkynylene, and heterocycloalkylene, respectively, the contents of which are also specified in the section "Definitions."

[0245] In specific embodiments, X_1 may be NR_1, S, or O, wherein R_1 may be H, a halogen, or a substituted or unsubstituted C_{1-3} alkylene. Also, X_1 may be S. X_1 may attract electrons from the carbon of the first carbonyl group to activate the first carbonyl group.

[0246] In specific embodiments, D_2 may include any one selected from a C_{1-7} alkylene, a C_{2-7} alkenylene, a C_{2-7} alkynylene, and a C_{3-8} cycloalkylene. Also, D_2 may be a C_{1-2} alkylene. Furthermore, D_2 may be methylene.

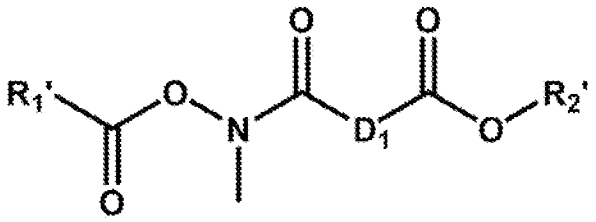
[0247] In specific embodiments, X_2 may be NR_1, S, or O, wherein R_1 may be H, a halogen, or a substituted or unsubstituted C_{1-3} alkylene. Also, X_2 may be O. X_2 may attract electrons from the carbon of the second carbonyl group to activate the second carbonyl group.

[0248] In specific embodiments, R_2' may be a halogen, N-succinimide, p-nitrophenyl, or pentafluorophenyl.

[0249] In specific embodiments, R_2' and X_2 taken together may form a leaving group. For example, X_2 may be O, and R_2' may be N-succinimide, p-nitrophenyl, or pentafluorophenyl. Also, R_2' may be N-succinimide. When a good leaving group is connected to the second carbonyl group, reactivity of the second carbonyl group may be enhanced. For example, an N-hydroxysuccinimide ester (an NHS ester) is known to show very high reactivity.

[0250] The present invention provides a compound having a structure of the following Formula 1-2:

[0251] [Formula 1-2]



[0252] wherein R_1' is a first chemical functional group,

[0253] D_1 is any alkylene, alkenylene, or alkynylene, and

[0254] R_2' is a second chemical functional group.

[0255] In specific embodiments, R_1' may include a click-chemistry functional group. Also, the click-chemistry functional group may include one or more selected from an alkyne, an azide, a strained alkyne, a diene, a dienophile, an alkene, a thiol, and a tetrazine. Furthermore, the click-chemistry functional group may be selected from an azide, or a strained alkyne. Further, the click-chemistry functional group may be selected from an azide, or dibenzocyclooctyne-amine. Additionally, the click-chemistry functional group may be selected from a diene, or a dienophile. Further, the click-chemistry functional group may be selected from a tetrazine, or a norbornene. Alternatively, the click-chemistry functional group may be selected from a tetrazine, or a trans-cyclooctene. In addition, R_1' may include two or more click-chemistry functional groups.

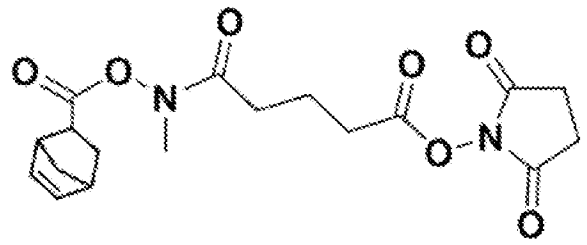
[0256] In other specific embodiments, R_1' may include a carrier moiety, a fluorescent moiety, a drug moiety, or a radioactive moiety. Also, R_1' may include a drug moiety. In addition, R_1' may include a VC linker. In other specific embodiments, R_1' may include an antibody or an analogue thereof, which includes a paratope.

[0257] In specific embodiments, D_1 may include any one selected from a C_{1-7} alkylene, a C_{2-7} alkenylene, a C_{2-7} alkynylene, and a C_{3-8} cycloalkylene. Also, D_1 may be a C_{1-2} alkylene. Furthermore, D_1 may be methylene.

[0258] In specific embodiments, R_2' and O taken together may form a leaving group. In this case, R_2' may be N-succinimide, p-nitrophenyl, or pentafluorophenyl. Also, R_2' may be N-succinimide.

[0259] In specific embodiments, the compound represented by Formula 1-2 may have a structure of the following Formula 1-3:

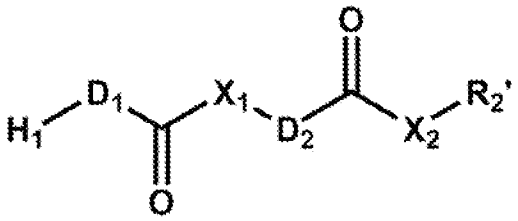
[0260] [Formula 1-3]



[0261] 2.1. Linker including first click-chemistry functional group (H_1-L_1)

[0262] The present invention provides a compound having a structure of the following Formula 2:

[0263] [Formula 2]



[0264] wherein H_1 is a first click-chemistry functional group,

[0265] D_1 is any alkylene, alkenylene, or alkynylene,

[0266] X_1 is an element that is more electronegative than carbon,

[0267] D_2 is any alkylene, alkenylene, or alkynylene,

[0268] X_2 is an element that is more electronegative than carbon, and

[0269] R_2' is a second chemical functional group. The linker having the structure of Formula 2 is herein referred to as a "linker including a first click-chemistry functional group," which is indicated by the symbol "H_1-L_1."

[0270] In Formula 2, a carbonyl group connected to D_1 is referred to as a first carbonyl group. Also, a carbonyl group connected to D_2 is referred to as a second carbonyl group.

[0271] In specific embodiments, H_1 may include any one selected from an alkyne, an azide, a strained alkyne, a diene, a dienophile, an alkene, a thiol, and a tetrazine. Furthermore, H_1 may be an azide, or a strained alkyne. Further, H_1 may be an azide, or dibenzocyclooctyne-amine. Additionally, H_1 may be a diene, or a dienophile. Further, H_1 may be a tetrazine, or a norbornene. Alternatively, H_1 may be a tetrazine, or a trans-cyclooctene.

[0272] In specific embodiments, D_1 may include any one selected from a covalent bond, a C_{1-4} alkylene, a C_{2-4} alkenylene, a C_{2-4} alkynylene, and a C_{3-8} cycloalkylene. Also, D_1 may be $-CH_2OCH_2-$. In addition, D_1 may be a covalent bond.

[0273] In specific embodiments, X_1 may be NR_1, S, or O, wherein R_1 may be H, a halogen,

or a substituted or unsubstituted C_{1-3} alkylene. Also, X_1 may be S.

[0274] In specific embodiments, D_2 may include any one selected from a C_{1-7} alkylene, a C_{2-7} alkenylene, a C_{2-7} alkynylene, and a C_{3-8} cycloalkylene. Also, D_2 may be a C_{1-2} alkylene. Furthermore, D_2 may be methylene.

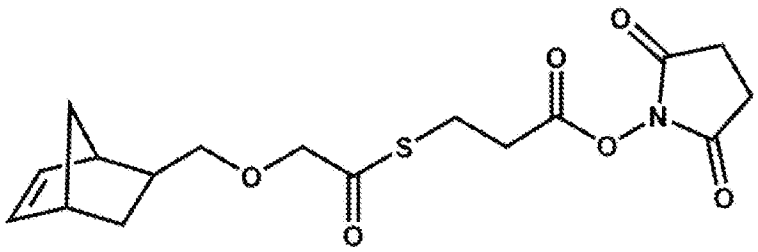
[0275] In specific embodiments, X_2 may be NR_1, S, or O, wherein R_1 may be H, a halogen, or a substituted or unsubstituted C_{1-3} alkylene. Also, X_2 may be O.

[0276] In specific embodiments, R_2' may be a halogen, N-succinimide, p-nitrophenyl, or pentafluorophenyl.

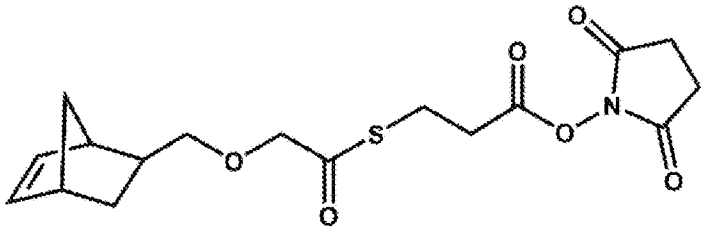
[0277] In specific embodiments, R_2' and X_2 taken together may form a leaving group. For example, X_2 may be O, and R_2' may be N-succinimide, p-nitrophenyl, or pentafluorophenyl. Also, R_2' may be N-succinimide.

[0278] In specific embodiments, the compound represented by Formula 2 may have any one structure selected from the following Formulas 2-1 to 2-3:

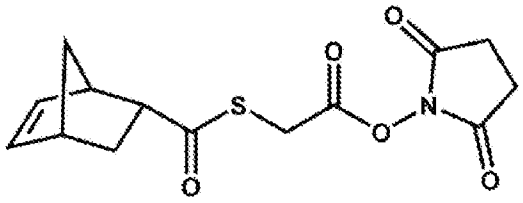
[0279] [Formula 2-1]



[0280] [Formula 2-2]



[0281] [Formula 2-3]

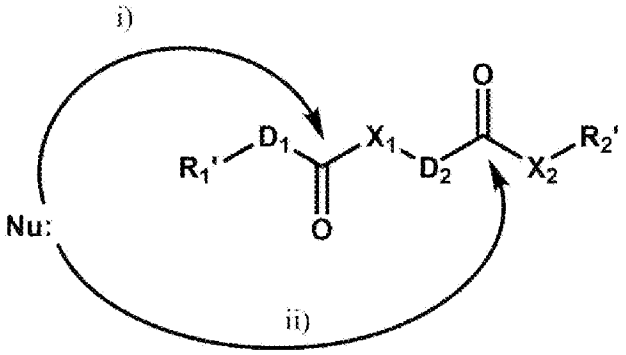


[0282] 2.2. The position at which a substitution reaction occurs may be specifically determined due to the difference in reactivity between the first carbonyl group and the second carbonyl group

[0283] When the linkers of Formulas 1 and 2, and sub-examples thereof are designed, sites for substitution reaction may be specified based on the design of X_1, X_2, and R_2'.

[0284] The linker disclosed in the present invention functions to transfer R_1' to a target molecule by means of a substitution reaction occurring between the activated first and/or second carbonyl groups. Such a substitution reaction is schematically shown as in the following Scheme 1.

[0285] [Scheme 1]



[0286] In Scheme 1, the target molecule is designated "Nu:" for the sake of convenience. The Nu: that serves as a nucleophile because it has an unshared electron pair causes a nucleophilic acyl substitution reaction with the first carbonyl group and/or second carbonyl group to form a bond with a linker. In the nucleophilic acyl substitution reaction, the reactivity of the carbonyl group may be determined by the basicity of the leaving group. Therefore, the reactivity of the carbonyl group is known to increase in the order of the carboxylate, the amide, the carboxylic acid, the ester, the thioester, and the acyl phosphate. Also, the carbonyl group such as an NHS ester, or the like is known to have high reactivity because the carbonyl group forms a very stable leaving group.

[0287] In specific embodiments, X_1 and X_2 may be elements that are more electronegative than carbon. Also, X_1 and X_2 may be NR_1, S, or O, wherein R_1 may be H, a halogen, or a substituted or unsubstituted C_{1-3} alkylene. Because X_1 and X_2 taken together with the residue(s) to which they are attached may form a leaving group, the carbonyl group may be activated.

[0288] In this case, the activation of the carbonyl group may optionally allow Nu: to i) preferentially react with the first carbonyl group or ii) preferentially react with the second carbonyl group. This tendency of reaction may be determined depending on a difference in reactivity between the first carbonyl group and the second carbonyl group. For example, when the basicity of a leaving group including X_1 is lower than that of a leaving group including X_2, the first carbonyl group may react first. In another embodiment, when the basicity of the leaving group including

X_2 is lower than that of the leaving group including X_1, the second carbonyl group may react first.

[0289] In preferred embodiments, the reactivity of the second carbonyl group is preferably higher than that of the first carbonyl group in the linker according to the present invention. According to the present invention, a selective reaction was achieved by allowing X_2-R_2' connected to the second carbonyl group to form a good leaving group, and designing the first carbonyl group to be an amide, a thioester, an ester, and the like, which show mild reactivity. For example, in the case of the linker of Formula 1-3, the second carbonyl group may be an NHS ester, thereby allowing it to react faster than the first carbonyl group (a thioester).

[0290] The prior art disclosed in Publication Nos. US 2018/0141976 A1 and WO 2018/199337 A1 is similar to the present invention in terms of the form of an agent for transferring a first chemical functional group to an antibody (see section 4 below), but is different from the present invention in that the cross-linker includes two NHS esters. The two carbonyl groups of the cross-linker have the same reactivity, and it is difficult to prepare a desired agent for transferring a chemical functional group with high yield. Also, the cross-linker has a high probability of reacting with two SSFIs due to high reactivity of the NHS esters. According to the present invention, such problems have been solved by designing the first carbonyl group to be a thioester, and the like, which have mild reactivity.

[0291] 3. Site-specific antibody interactome (SSAI)

[0292] According to the present invention, there is disclosed a novel peptide for bringing a molecule to be labeled into close contact with a certain site of an antibody. For the sake of convenience, such a peptide is herein referred to as a site-specific antibody interactome, which is indicated by the symbol "SSAI."

[0293] The SSAI provided according to the present invention may have binding activity for a certain site of an antibody.

[0294] In specific embodiments, the SSAI may have binding activity for an Fab domain of the antibody. In this case, the SSAI may preferably have binding activity for a site spaced apart from a paratope of the antibody.

[0295] In specific embodiments, the SSAI may have binding activity for the Fc domain of the antibody. In this case, the SSAI may preferably have binding activity for an FcRn binding site of the antibody or a site spaced apart from residues of the antibody which affects the FcRn binding site of the antibody.

[0296] 3.1: Site-specific Fc interactome (SSFI)

[0297] In the SSAI, a peptide having specific binding activity for the Fc domain is herein

referred to as a "site-specific Fc interactome", which is indicated by the symbol "SSFI."

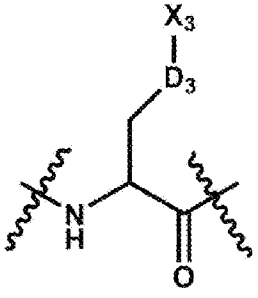
[0298] In specific embodiments, the SSFI may include an amino acid sequence represented by the following Formula 3:

[0299] [Formula 3]

$$(Xaa)_2\text{-}H\text{-}Xa_1\text{-}G\text{-}Xa_2\text{-}L\text{-}V\text{-}Xa_3 \quad \text{(SEQ ID NO: 3)}$$

[0300] wherein each Xaa is independently any amino acid except cysteine,

[0301] H is histidine, G is glycine, Xa_2 is glutamic acid or asparagine, L is leucine, V is valine, Xa_3 is selected from tryptophan, naphthylalanine, and phenylalanine, and

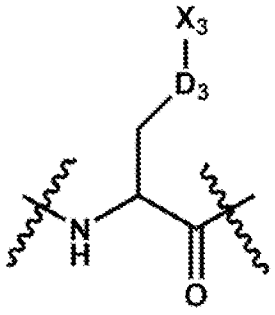
[0302] Xa_1 is  , wherein D_3 is a covalent bond or a $C_{1\text{-}3}$ alkylene, and X_3 is NH_2, OH, or SH. As an analogue of the sequence AWHLGELVW (SEQ ID NO: 2), which is a sequence found to have binding activity for the Fc domain in the articles "Dias, R. L. A., *et al* (2006), Protein Ligand Design: From Phage Display to Synthetic Protein Epitope Mimetics in Human Antibody Fc-Binding Peptidomimetics; Journal of the American Chemical Society, 128(8), 2726-2732; and DeLano, W.L., *et al.*, Convergent solutions to binding at a protein-protein interface; Science 2000, 287, 1279-1283," it has binding activity for the Fc domain. The Fc binding activity motif according to the present invention has characteristics as follows: 1) First, key residues having Fc binding activity are specified. 2) The motif is also designed to allow a nucleophilic substitution reaction with a linker by changing the 4th leucine of SEQ ID NO: 2 into Xa_1 having a free electron pair. $(X)_n$ means that it consists of n Xs, and $(X)_{n\text{-}m}$ means that it consist of n or more and m or less Xs. Hereinafter, the carbon connected to D_3 is referred to as "beta carbon (β-carbon)."

[0303] Also, the amino acid sequence of Formula 3 may have a structure represented by the following Formula 3-1:

[0304] [Formula 3-1]

$$A\text{-}W\text{-}H\text{-}Xa_1\text{-}G\text{-}Xa_2\text{-}L\text{-}V\text{-}Xa_3 \quad \text{(SEQ ID NO: 4)}$$

[0305] wherein A is alanine, H is histidine, G is glycine, Xa_2 is glutamic acid or asparagine, L is leucine, V is valine, Xa_3 is selected from tryptophan, naphthylalanine, and phenylalanine, and



[0306] Xa_1 is , wherein D_3 is a covalent bond or a C_{1-3} alkylene, and X_3 is NH_2, OH, or SH. In Formulas 3 and 3-1, X_3 may be NH_2. Alternatively, Xa_2 may be glutamic acid. Alternatively, Xa_3 may be tryptophan.

[0307] When the peptide including each of amino acid sequences of Formula 3 and sub-examples thereof is in a cyclic peptide form in which internal residues are connected to each other, the peptide is known to have better binding activity. The present invention provides a cyclic peptide including the amino acid sequence of Formula 3.

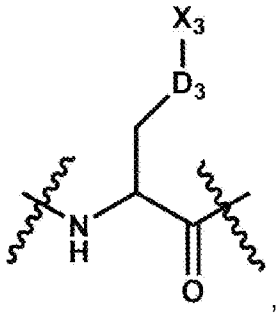
[0308] The present invention provides a cyclic peptide having a structure of the following Formula 4-1:

[0309] [Formula 4-1]

$$^{L}P\text{-}(Xaa)_2\text{-}H\text{-}Xa_1\text{-}G\text{-}Xa_2\text{-}L\text{-}V\text{-}Xa_3\text{-}^{D}P$$ (SEQ ID NO: 5)

[0310] wherein N-terminal ^{L}P and ^{D}P form a D-proline-L-proline template,

[0311] each Xaa is independently any amino acid except cysteine, H is histidine, G is glycine, Xa_2 is glutamic acid or asparagine, L is leucine, V is valine, Xa_3 is selected from tryptophan, naphthylalanine, and phenylalanine, and



[0312] Xa_1 is , wherein D_3 is a covalent bond or a C_{1-3} alkylene, and X_3 is NH_2, OH, or SH.

[0313] In specific embodiments, X_3 may be NH_2. In specific embodiments, $(X)_2$ may be AW. In specific embodiments, Xa_2 may be glutamic acid. In specific embodiments, Xa_3 may be tryptophan.

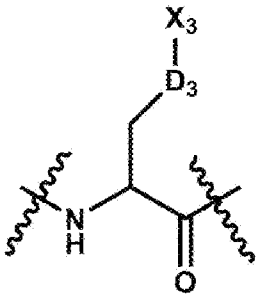
[0314] The present invention provides a cyclic peptide having a structure of the following Formula 4-2:

[0315] [Formula 4-2]

$$(Xaa)_{1-3}\text{-}C\text{-}(Xaa)_2\text{-}H\text{-}Xa_1\text{-}G\text{-}Xa_2\text{-}L\text{-}V\text{-}Xa_3\text{-}C\text{-}(Xaa)_{1-3}$$ (SEQ ID NO: 6)

[0316] wherein each Xaa is independently any amino acid except cysteine,

[0317] C is cysteine, H is histidine, G is glycine, Xa_2 is glutamic acid or asparagine, L is leucine, V is valine, Xa_3 is selected from tryptophan, naphthylalanine, and phenylalanine, and

[0318] Xa_1 is  , wherein D_3 is a covalent bond or a C_{1-3} alkylene, and X_3 is NH_2, OH, or SH.

[0319] In specific embodiments, the peptide may consist of 13 or more and 17 or less amino acid residues.

[0320] In specific embodiments, cysteine located 2 to 4 amino acids from the N-terminus and cysteine located 2 to 4 amino acids from the C-terminus may be optionally connected to each other.

[0321] In specific embodiments, X_3 may be NH_2. In specific embodiments, $(X)_2$ may be AW. In specific embodiments, Xa_2 may be glutamic acid. In specific embodiments, Xa_3 may be tryptophan.

[0322] In specific embodiments, one of the residues constituting the N-terminal $(X)_{1-3}$ and one of the residues constituting the C-terminal $(X)_{1-3}$ may be bound to each other. In one exemplary embodiment, the peptide of Formula 4-2 may have a structure of the following Formula 4-3:

[0323] [Formula 4-3]

$$^{L}P\text{-}D\text{-}C\text{-}(Xaa)_2\text{-}H\text{-}Xa_1\text{-}G\text{-}Xa_2\text{-}L\text{-}V\text{-}Xa_3\text{-}C\text{-}T\text{-}^{D}P$$ (SEQ ID NO: 7)

[0324] wherein N-terminal ^{L}P와 ^{D}P may form a D-proline-L-proline template.

[0325] In another exemplary embodiment, the peptide of Formula 4-2 may have a structure of the following Formula 4-4:

[0326] [Formula 4-4]

$$C\text{-}D\text{-}C\text{-}(Xaa)_2\text{-}H\text{-}Xa_1\text{-}G\text{-}Xa_2\text{-}L\text{-}V\text{-}Xa_3\text{-}C\text{-}T\text{-}C$$ (SEQ ID NO: 8)

[0327] wherein the N-terminal cysteine and the C-terminal cysteine may be connected to each other.

[0328] The present invention provides a cyclic peptide having a structure of the following Formula 4-5:

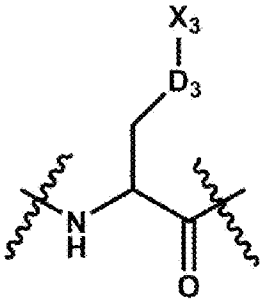
[0329] [Formula 4-5]

$$D - C - (Xaa)_2 - H - Xa_1 - G - Xa_2 - L - V - Xa_3 - C - T$$ (SEQ ID NO: 9)

[0330] wherein D is aspartic acid, T is threonine,

[0331] each Xaa is independently any amino acid except cysteine,

[0332] C is cysteine, H is histidine, G is glycine, Xa_2 is glutamic acid or asparagine, L is leucine, V is valine, Xa_3 is selected from tryptophan, naphthylalanine, and phenylalanine, and

[0333] Xa_1 is 

, wherein D_3 is a covalent bond or a C_{1-3} alkylene, and X_3 is NH_2, OH, or SH.

[0334] In specific embodiments, the peptide may consist of 13 or more and 17 or less amino acid residues.

[0335] In specific embodiments, cysteine located 2 amino acids from the N-terminus and cysteine located 2 amino acids from the C-terminus may be optionally connected to each other. In specific embodiments, X_3 may be NH_2. In specific embodiments, $(X)_2$ may be AW. In specific embodiments, Xa_2 may be glutamic acid. In specific embodiments, Xa_3 may be tryptophan.

[0336] In specific embodiments, the Formula 4-5 may be identical to the following Formula 4-6:

[0337] [Formula 4-6] (SEQ ID NO: 10)

[0338] $$D-C-A-W-H-Xa_1-G-E-L-V-W-C-T$$ (SEQ ID NO: 10)

[0339] wherein A is alanine, and E is glutamic acid.

[0340] The peptides having the structures of Formulas 4-1 to 4-6 may have binding activity for an antibody. Also, the peptides may have binding activity for immunoglobulin G (IgG). In addition, the peptides may have binding activity for an Fc domain of the antibody.

[0341] In specific embodiments, the N-terminus of the SSFI according to the present

invention may be succinylated. In specific embodiments, the SSFI according to the present invention may include a polar amino acid residue at the N-terminal $(X)_{1-3}$ thereof. In other specific embodiments, the SSFI according to the present invention may include a polar amino acid residue at the C-terminal $(X)_{1-3}$ thereof. In this case, the polar amino acid residue includes an acidic amino acid and a basic amino acid. Also, the polar amino acid residue may include glutamic acid or aspartic acid.

[0342] 3.2. Site-specific Fc interactome according to the present invention may be arranged with an Fc domain of an antibody with a specific topology.

[0343] In the present content, the compound of Formula 4-6 is provided as one example to aid in understanding the present invention, but the scope of the present invention is not limited thereto. It will be noted that the following description also applies to the compounds of Formulas 3, 3-1, and 4-1 to 4-6, and the compound of Formula 4-6 is merely provided as one example for the sake of convenience.

[0344] As previously described above, the SSFI according to the present invention has binding activity for the Fc domain of the antibody. In this case, the SSFI may be arranged with the Fc domain with a specific topology due to the interaction between amino acid residues. The representative interaction between the SSFI sequence according to the present invention and the Fc domain includes (1) a salt linkage of the SSFI with histidine 433 in the Fc domain, (2) a hydrogen bond of the SSFI with asparagine 434, (3) a salt linkage of the SSFI with glutamic acid 380, (4) a salt linkage of the SSFI with arginine 255, and the like. These interactions and specific topologies thus formed may be determined from the research results already known in the art (see DeLano, W.L., *et al.*, Convergent solutions to binding at a protein-protein interface; Science 2000, 287, 1279-1283).

[0345] When the SSFI according to the present invention is designed, it is important for the SSFI to form a stable topology with the Fc domain. This is because an agent for transferring a first chemical functional group to the antibody according to the present invention, and a labeling process using the same are designed based on the topology between the SSFI and the Fc domain found in research (see the following sections 5.2, 5.3, and 5.4). When the interaction between the SSFI and the Fc domain becomes unstable during the design of SSFI to disturb a topology between their molecules, it is unfavorable as the interaction may give a negative effect on the labeling process.

[0346] One embodiment of the design principle will be described with reference to an exemplary compound. The SSFI represented by Formula 4-6 has a structure as follows:

[0347] [Formula 4-6]

[0348] D-C-A-W-H-Xa_1-G-E-L-V-W-C-T (SEQ ID NO: 10)

[0349] The results of simulating the topology between SSFI set forth in SEQ ID NO: 10 and the Fc domain based on the data in the articles, and the like are shown in FIGS. 3 and 4 (see DeLano, W.L., *et al.*, Convergent solutions to binding at a protein-protein interface; Science 2000, 287, 1279-1283). In this case, a histidine residue at position 5 in the SSFI forms a salt linkage with glutamic acid 380 in the Fc domain, indicating that this salt linkage has a significant effect on the topology between the SSFI and the Fc domain (see a dotted line in FIG. 4). Therefore, it is desirable that the histidine residue and its position are not changed during the design of the SSFI (see 'H' next to the residue Xa_1 in Formulas 3, 3-1, and 4-1 to 4-6). In addition, it was confirmed that glutamic acid 8 is a residue that shows electronegativity, and thus forms a salt linkage with arginine 255 in the Fc domain, which shows electropositivity, thereby exerting a significant effect on the topology between the SSFI and the Fc domain (see a dotted line in FIG. 4). Therefore, the corresponding residue is preferably an acidic amino acid that may correspond to glutamic acid, and may be replaced with asparagine (see the residue Xa_2 in Formulas 3, 3-1, and 4-1 to 4-6). When the amino acid residues are replaced with other amino acid residues or are substituted with any functional groups, this may have an effect on the intermolecular interaction, thereby exerting an effect on the topology between the SSFI and the Fc domain.

[0350] In addition, glycine at position 7 in the sequence of SEQ ID NO: 10 is a small amino acid that is required to form a bent structure of the SSFI. Therefore, it is desirable that the glycine residue and its position are not changed during the design of the SSFI (see 'G' between the residues Xa_1 and Xa_2 in Formulas 3, 3-1, and 4-1 to 4-6).

[0351] FIG. 5 shows a topology between lysine residues of the Fc domain and the SSFI. Based on the topology, it can be seen that the lysine residues of the Fc domain located closest to the residue Xa_1 are lysine 246 and 248 (FIG. 5).

[0352] A distance between an amine group of lysine 246 and the beta carbon of Xa_1 was measured. As a result, it was confirmed that the minimum distance is measured to be approximately 11.668 Å (hereinafter referred to as "$D_{246,min}$"), and the maximum distance is measured to be approximately 20.765 Å (hereinafter referred to as "$D_{246,max}$") as the bonds constituting a lysine branch rotate (FIG. 6).

[0353] A distance between an amine group of lysine 248 and the beta carbon of Xa_1 was measured. As a result, it was confirmed that the minimum distance is measured to be approximately 6.723 Å (hereinafter referred to as "$D_{248,min}$"), and the maximum distance is measured to be approximately 16.208 Å (hereinafter referred to as "$D_{248,max}$") as the bonds

constituting a lysine branch rotate (FIG. 7).

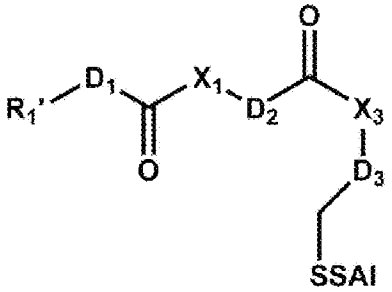
[0354] As will be described in the following section 5.3, the distance relationship may be an important consideration in the design of the linker and the SSFI, as well as the agent for transferring a first chemical functional group to an antibody.

[0355] 4. Agent for transferring first chemical functional group to antibody; Conjugate of R_1'-L_1 and SSAI (R_1'-L_2-SSAI)

[0356] According to the present invention, there is disclosed an agent for transferring a first chemical functional group to an antibody. Such a compound is herein indicated by the symbol "R_1'-L_2-SSAI." The compound is also referred to as a conjugate of R_1'-L_1 and SSAI (an R_1'-L_2-SSAI conjugate) depending on the structure thereof.

[0357] The present invention provides R_1'-L_2-SSAI having a structure of the following Formula 5:

[0358] [Formula 5]



[0359] wherein R_1' is a first chemical functional group,

[0360] D_1 is any alkylene, alkenylene, or alkynylene,

[0361] X_1 is an element that is more electronegative than carbon,

[0362] D_2 is any alkylene, alkenylene, or alkynylene,

[0363] D_3 is a covalent bond or a C_{1-3} alkylene,

[0364] X_3 is NH, O, or S, and

[0365] SSAI is a site-specific antibody interactome.

[0366] In specific embodiments, R_1' may include a click-chemistry functional group. Also, the click-chemistry functional group may include one or more selected from an alkyne, an azide, a strained alkyne, a diene, a dienophile, an alkene, a thiol, and a tetrazine. Furthermore, the click-chemistry functional group may be selected from an azide, or a strained alkyne. Further, the click-chemistry functional group may be selected from an azide, or dibenzocyclooctyne-amine. Additionally, the click-chemistry functional group may be selected from a diene, or a dienophile. Further, the click-chemistry functional group may be selected from a tetrazine, or a norbornene.

Alternatively, the click-chemistry functional group may be selected from a tetrazine, or a trans-cyclooctene. In addition, R_1' may include two or more click-chemistry functional groups.

[0367] In other specific embodiments, R_1' may include a carrier moiety, a fluorescent moiety, a drug moiety, or a radioactive moiety. Also, R_1' may include a drug moiety. In addition, R_1' may include a VC linker. In other specific embodiments, R_1' may include an antibody or an analogue thereof, which includes a paratope.

[0368] In specific embodiments, D_1 may include any one selected from a covalent bond, a C_{1-4} alkylene, a C_{2-4} alkenylene, a C_{2-4} alkynylene, and a C_{3-8} cycloalkylene. Also, D_1 may be $-CH_2OCH_2-$. In addition, D_1 may be a covalent bond.

[0369] In specific embodiments, X_1 may be NR_1, S, or O, wherein R_1 may be H, a halogen, or a substituted or unsubstituted C_{1-3} alkylene. Also, X_1 may be S.

[0370] In specific embodiments, D_2 may include any one selected from a C_{1-7} alkylene, a C_{2-7} alkenylene, a C_{2-7} alkynylene, and a C_{3-8} cycloalkylene. Also, D_2 may be a C_{1-2} alkylene. Furthermore, D_2 may be methylene.

[0371] In specific embodiments, X_3 may be NH.

[0372] In specific embodiments, the SSAI may be a peptide sequence having binding activity for Fab. In other specific embodiments, the SSAI may be a peptide sequence having binding activity for the Fc domain.

[0373] When the SSAI in Formula 5 is SSFI, this is indicated by the symbol "R_1'-L_2-SSFI." The R_1'-L_2-SSFI according to the present invention is produced by means of a nucleophilic substitution reaction of Xa_1 of the SSFI according to the present invention with a second carbonyl group of R_1'-L_1 (see the following section 4-2 and Scheme 2). Therefore, the R_1'-L_2-SSFI according to the present invention includes those in which Xa_1 of the SSFI in Formulas 3, 3-1, and 4-1 to 4-6 is substituted with (Xa_1)', but the present invention is not limited to the following exemplary embodiments thereof.

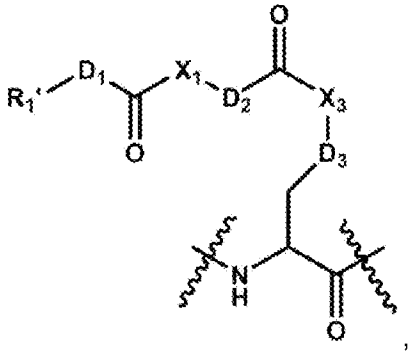
[0374] The present invention provides an R_1'-L_2-SSFI including an amino acid sequence of the following Formula 5-1:

[0375] [Formula 5-1]

[0376] $(Xaa)_2$-H-(Xa_1)'-G-Xa_2-L-V-Xa_3 (SEQ ID NO: 11)

[0377] wherein each Xaa is independently any amino acid except cysteine,

[0378] H is histidine, G is glycine, Xa_2 is glutamic acid or asparagine, L is leucine, V is valine, Xa_3 is selected from tryptophan, naphthylalanine, and phenylalanine, and



[0379] $(Xa_1)'$ is ,

[0380] wherein R_1' is a first chemical functional group,

[0381] D_1 is any alkylene, alkenylene, or alkynylene,

[0382] X_1 is an element that is more electronegative than carbon,

[0383] D_2 is any alkylene, alkenylene, or alkynylene,

[0384] D_3 is a covalent bond or a C_{1-3} alkylene, and

[0385] X_3 is NH, O, or S.

[0386] In specific embodiments, R_1' may include a click-chemistry functional group. Also, the click-chemistry functional group may include one or more selected from an alkyne, an azide, a strained alkyne, a diene, a dienophile, an alkene, a thiol, and a tetrazine. Furthermore, the click-chemistry functional group may be selected from an azide, or a strained alkyne. Further, the click-chemistry functional group may be selected from an azide, or dibenzocyclooctyne-amine. Additionally, the click-chemistry functional group may be selected from a diene, or a dienophile. Further, the click-chemistry functional group may be selected from a tetrazine, or a norbornene. Alternatively, the click-chemistry functional group may be selected from a tetrazine, or a trans-cyclooctene. In addition, R_1' may include two or more click-chemistry functional groups.

[0387] In other specific embodiments, R_1' may include a carrier moiety, a fluorescent moiety, or a drug moiety. Also, R_1' may include a VC linker. In addition, R_1' may include a radioactive moiety. Also, R_1' may include a drug moiety. In other specific embodiments, R_1' may include an antibody or an analogue thereof, which includes a paratope.

[0388] In specific embodiments, D_1 may include any one selected from a covalent bond, a C_{1-4} alkylene, a C_{2-4} alkenylene, a C_{2-4} alkynylene, and a C_{3-8} cycloalkylene. Also, D_1 may be $-CH_2OCH_2-$. In addition, D_1 may be a covalent bond.

[0389] In specific embodiments, X_1 may be NR_1, S, or O, wherein R_1 may be H, a halogen, or a substituted or unsubstituted C_{1-3} alkylene.

[0390] In specific embodiments, D_2 may include any one selected from a C_{1-7} alkylene, a C_{2-7} alkenylene, a C_{2-7} alkynylene, and a C_{3-8} cycloalkylene. Also, D_2 may be a C_{1-2} alkylene.

Furthermore, D_2 may be methylene.

[0391] In specific embodiments, X_3 may be NH.

[0392] In specific embodiments, $(X)_2$ may be AW. In specific embodiments, Xa_2 may be glutamic acid. In specific embodiments, Xa_3 may be tryptophan.

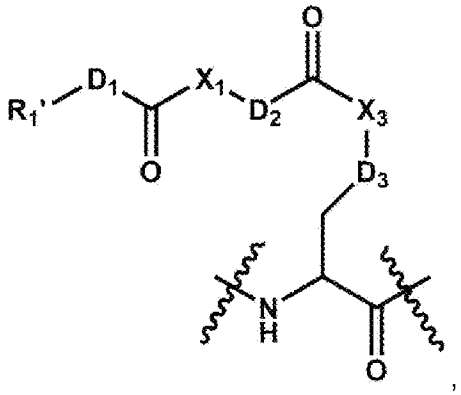
[0393] The present invention provides an R_1'-L_2-SSFI having a structure of the following Formula 5-2:

[0394] [Formula 5-2]

[0395] $\mathbf{(Xaa)_{1-3}\text{-}C\text{-}(Xaa)_2\text{-}H\text{-}(Xa_1)'\text{-}G\text{-}Xa_2\text{-}L\text{-}V\text{-}Xa_3\text{-}C\text{-}(Xaa)_{1-3}}$ (SEQ ID NO: 12)

[0396] wherein each Xaa is independently any amino acid except cysteine,

[0397] C is cysteine, H is histidine, G is glycine, Xa_2 is glutamic acid or asparagine, L is leucine, V is valine, Xa_3 is selected from tryptophan, naphthylalanine, and phenylalanine, and

[0398] (Xa_1)' is 

,

[0399] wherein R_1' is a first chemical functional group,

[0400] D_1 is any alkylene, alkenylene, or alkynylene,

[0401] X_1 is an element that is more electronegative than carbon,

[0402] D_2 is any alkylene, alkenylene, or alkynylene,

[0403] D_3 is a covalent bond or a C_{1-3} alkylene, and

[0404] X_3 is NH, O, or S.

[0405] In specific embodiments, R_1' may include a click-chemistry functional group. Also, the click-chemistry functional group may include one or more selected from an alkyne, an azide, a strained alkyne, a diene, a dienophile, an alkene, a thiol, and a tetrazine. Furthermore, the click-chemistry functional group may be selected from an azide, or a strained alkyne. Further, the click-chemistry functional group may be selected from an azide, or dibenzocyclooctyne-amine. Additionally, the click-chemistry functional group may be selected from a diene, or a dienophile. Further, the click-chemistry functional group may be selected from a tetrazine, or a norbornene.

Alternatively, the click-chemistry functional group may be selected from a tetrazine, or a trans-cyclooctene. In addition, R_1' may include two or more click-chemistry functional groups.

[0406] In other specific embodiments, R_1' may include a carrier moiety, a fluorescent moiety, a drug moiety, or a radioactive moiety. Also, R_1' may include a drug moiety. In addition, R_1' may include a VC linker. In other specific embodiments, R_1' may include an antibody or an analogue thereof, which includes a paratope.

[0407] In specific embodiments, D_1 may include any one selected from a covalent bond, a C_{1-4} alkylene, a C_{2-4} alkenylene, a C_{2-4} alkynylene, and a C_{3-8} cycloalkylene. Also, D_1 may be $-CH_2OCH_2-$. In addition, D_1 may be a covalent bond.

[0408] In specific embodiments, X_1 may be NR_1, S, or O, wherein R_1 may be H, a halogen, or a substituted or unsubstituted C_{1-3} alkylene.

[0409] In specific embodiments, D_2 may include any one selected from a C_{1-7} alkylene, a C_{2-7} alkenylene, a C_{2-7} alkynylene, and a C_{3-8} cycloalkylene. Also, D_2 may be a C_{1-2} alkylene. Furthermore, D_2 may be methylene.

[0410] In specific embodiments, Formula 5-2 may consist of 13 or more and 17 or less amino acid residues (including (Xa_1)').

[0411] In specific embodiments, cysteine located 2 to 4 amino acids from the N-terminus and cysteine located 2 to 4 amino acids from the C-terminus may be optionally connected to each other.

[0412] In specific embodiments, X_3 may be NH. In specific embodiments, $(X)_2$ may be AW. In specific embodiments, Xa_2 may be glutamic acid. In specific embodiments, Xa_3 may be tryptophan.

[0413] In specific embodiments, one of the residues constituting the N-terminal $(X)_{1-3}$ and one of the residues constituting the C-terminal $(X)_{1-3}$ may be bound to each other.

[0414] In specific embodiments, Formula 5-2 may be identical to Formula 5-3:

[0415] [Formula 5-3]

[0416] $D\text{-}C\text{-}A\text{-}W\text{-}H\text{-}(Xa_1)'\text{-}G\text{-}E\text{-}L\text{-}V\text{-}W\text{-}C\text{-}T$ (SEQ ID NO: 13)

[0417] wherein A is alanine, and E is glutamic acid.

[0418] The R_1'-L_2-SSAI or R_1'-L_2-SSFI having the structures of Formulas 5 and 5-1 to 5-3 may have binding activity for an antibody. Also, the R_1'-L_2-SSAI or R_1'-L_2-SSFI may have binding activity for immunoglobulin G (IgG). In addition, the R_1'-L_2-SSAI or R_1'-L_2-SSFI may have binding activity for an Fc domain of the antibody.

[0419] 4.1. Agent for transferring first click-chemistry functional group to antibody;

Conjugate of H_1-L_1 and SSAI (H_1-L_2-SSAI)

[0420] According to the present invention, there is disclosed an agent for transferring a first click-chemistry functional group to an antibody. Such a compound is herein indicated by the symbol "H_1-L_2-SSAI." The compound is also referred to as a conjugate of H_1-L_1 and SSAI (an H_1-L_2-SSAI conjugate) depending on the structure thereof. In this case, when the SSAI is SSFI, this is indicated by the symbol 'H_1-L_2-SSFI.'

[0421] The H_1-L_2-SSAI or H_1-L_2-SSFI according to the present invention includes those in which R_1' contains a click-chemistry functional group in the section '4. Agent for transferring first chemical functional group to antibody,' but the present invention is not limited to the following exemplary embodiments thereof.

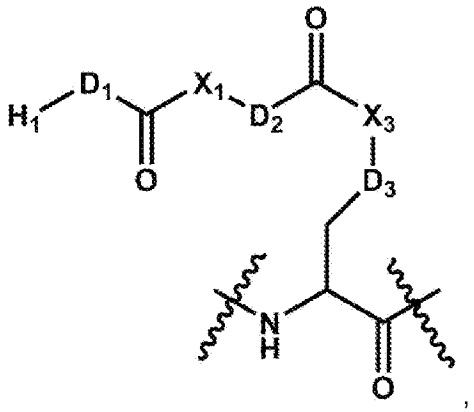
[0422] The present invention provides an H_1-L_2-SSFI including an amino acid sequence of the following Formula 6-1:

[0423] [Formula 6-1]

[0424] $\mathbf{(Xaa)_2\text{-}H\text{-}(Xa_1)'\text{-}G\text{-}Xa_2\text{-}L\text{-}V\text{-}Xa_3}$ (SEQ ID NO: 14)

[0425] wherein each Xaa is independently any amino acid except cysteine,

[0426] H is histidine, G is glycine, Xa_2 is glutamic acid or asparagine, L is leucine, V is valine, Xa_3 is selected from tryptophan, naphthylalanine, and phenylalanine, and

[0427] $(Xa_1)'$ is 

,

[0428] wherein H_1 is a first click-chemistry functional group,

[0429] D_1 is any alkylene, alkenylene, or alkynylene,

[0430] X_1 is an element that is more electronegative than carbon,

[0431] D_2 is any alkylene, alkenylene, or alkynylene,

[0432] D_3 is a covalent bond or a C_{1-3} alkylene, and

[0433] X_3 is NH, O, or S.

[0434] In specific embodiments, H_1 may include any one selected from an alkyne, an

azide, a strained alkyne, a diene, a dienophile, an alkene, a thiol, and a tetrazine. Furthermore, H_1 may be an azide, or a strained alkyne. Further, H_1 may be an azide, or dibenzocyclooctyne-amine. Additionally, H_1 may be a diene, or a dienophile. Further, H_1 may be a tetrazine, or a norbornene. Alternatively, H_1 may be a tetrazine, or a trans-cyclooctene.

[0435] In specific embodiments, D_1 may include any one selected from a covalent bond, a C_{1-4} alkylene, a C_{2-4} alkenylene, a C_{2-4} alkynylene, and a C_{3-8} cycloalkylene. Also, D_1 may be $-CH_2OCH_2-$. In addition, D_1 may be a covalent bond.

[0436] In specific embodiments, X_1 may be NR_1, S, or O, wherein R_1 may be H, a halogen, or a substituted or unsubstituted C_{1-3} alkylene.

[0437] In specific embodiments, D_2 may include any one selected from a C_{1-7} alkylene, a C_{2-7} alkenylene, a C_{2-7} alkynylene, and a C_{3-8} cycloalkylene. Also, D_2 may be a C_{1-2} alkylene. Furthermore, D_2 may be methylene.

[0438] In specific embodiments, X_3 may be NH.

[0439] In specific embodiments, $(X)_2$ may be AW. In specific embodiments, Xa_2 may be glutamic acid. In specific embodiments, Xa_3 may be tryptophan.

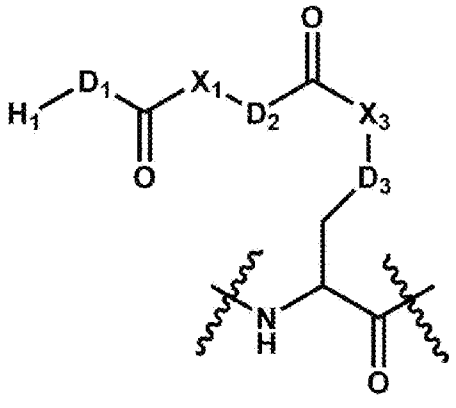
[0440] The present invention provides an H_1-L_2-SSFI having a structure of the following Formula 6-2:

[0441] [Formula 6-2]

[0442] $\mathbf{(Xaa)_{1-3}\text{-}C\text{-}(Xaa)_2\text{-}H\text{-}(Xa_1)'\text{-}G\text{-}Xa_2\text{-}L\text{-}V\text{-}Xa_3\text{-}C\text{-}(Xaa)_{1-3}}$ (SEQ ID NO: 15)

[0443] wherein each Xaa is independently any amino acid except cysteine,

[0444] C is cysteine, H is histidine, G is glycine, Xa_2 is glutamic acid or asparagine, L is leucine, V is valine, Xa_3 is selected from tryptophan, naphthylalanine, and phenylalanine, and

[0445] $(Xa_1)'$ is 

,

[0446] wherein H_1 is a first click-chemistry functional group,

[0447] D_1 is any alkylene, alkenylene, or alkynylene,

[0448] X_1 is an element that is more electronegative than carbon,

[0449] D_2 is any alkylene, alkenylene, or alkynylene,

[0450] D_3 is a covalent bond or a C_{1-3} alkylene, and

[0451] X_3 is NH, O, or S.

[0452] In specific embodiments, H_1 may include any one selected from an alkyne, an azide, a strained alkyne, a diene, a dienophile, an alkene, a thiol, and a tetrazine. Furthermore, H_1 may be an azide, or a strained alkyne. Further, H_1 may be an azide, or dibenzocyclooctyne-amine. Additionally, H_1 may be a diene, or a dienophile. Further, H_1 may be a tetrazine, or a norbornene. Alternatively, H_1 may be a tetrazine, or a trans-cyclooctene.

[0453] In specific embodiments, D_1 may include any one selected from a covalent bond, a C_{1-4} alkylene, a C_{2-4} alkenylene, a C_{2-4} alkynylene, and a C_{3-8} cycloalkylene. Also, D_1 may be $-CH_2OCH_2-$. In addition, D_1 may be a covalent bond.

[0454] In specific embodiments, X_1 may be NR_1, S, or O, wherein R_1 may be H, a halogen, or a substituted or unsubstituted C_{1-3} alkylene.

[0455] In specific embodiments, D_2 may include any one selected from a C_{1-7} alkylene, a C_{2-7} alkenylene, a C_{2-7} alkynylene, and a C_{3-8} cycloalkylene. Also, D_2 may be a C_{1-2} alkylene. Furthermore, D_2 may be methylene.

[0456] In specific embodiments, Formula 5-2 may consist of 13 or more and 17 or less amino acid residues (including $(Xa_1)'$).

[0457] In specific embodiments, cysteine located 2 to 4 amino acids from the N-terminus and cysteine located 2 to 4 amino acids from the C-terminus may be optionally connected to each other.

[0458] In specific embodiments, X_3 may be NH. In specific embodiments, $(X)_2$ may be AW. In specific embodiments, Xa_2 may be glutamic acid. In specific embodiments, Xa_3 may be tryptophan.

[0459] In specific embodiments, one of the residues constituting the N-terminal $(X)_{1-3}$ and one of the residues constituting the C-terminal $(X)_{1-3}$ may be bound to each other.

[0460] In specific embodiments, Formula 6-2 may be identical to Formula 6-3:

[0461] [Formula 6-3] (SEQ ID NO: 16)

[0462] $D\text{-}C\text{-}A\text{-}W\text{-}H\text{-}(Xa_1)'\text{-}G\text{-}E\text{-}L\text{-}V\text{-}W\text{-}C\text{-}T$ (SEQ ID NO: 16)

[0463] wherein A is alanine, and E is glutamic acid.

[0464] The H_1-L_2-SSAI or H_1-L_2-SSFI having the structures of Formulas 6-1 to 6-3 may have binding activity for an antibody. Also, the H_1-L_2-SSAI or H_1-L_2-SSFI may have binding

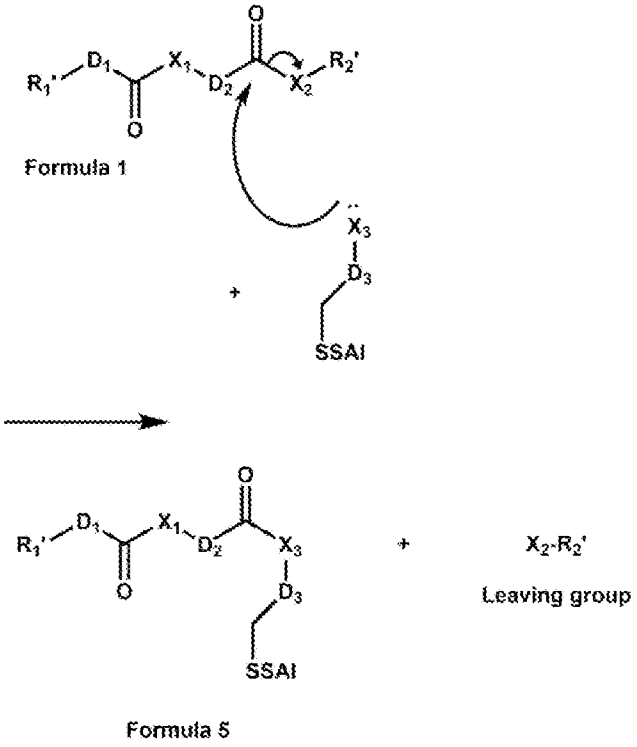
activity for immunoglobulin G (IgG). In addition, the H_1-L_2-SSAI or H_1-L_2-SSFI may have binding activity for an Fc domain of the antibody.

[0465] 4.2. Method of preparing agent for transferring first chemical functional group to antibody

[0466] According to the present invention, there are disclosed methods of preparing the R_1'-L_2-SSAI, the R_1'-L_2-SSFI, the H_1-L_2-SSAI, and the H_1-L_2-SSFI (hereinafter generally referred to as "R_1'-L_2-SSAI"). It will be noted that a description of the following preparation methods is provided to aid in understanding the present invention.

[0467] For example, a method of preparing a compound of Formula 5 will be described. In specific embodiments, the compound of Formula 5 may be prepared through a reaction of the following Scheme 2.

[0468] [Scheme 2]

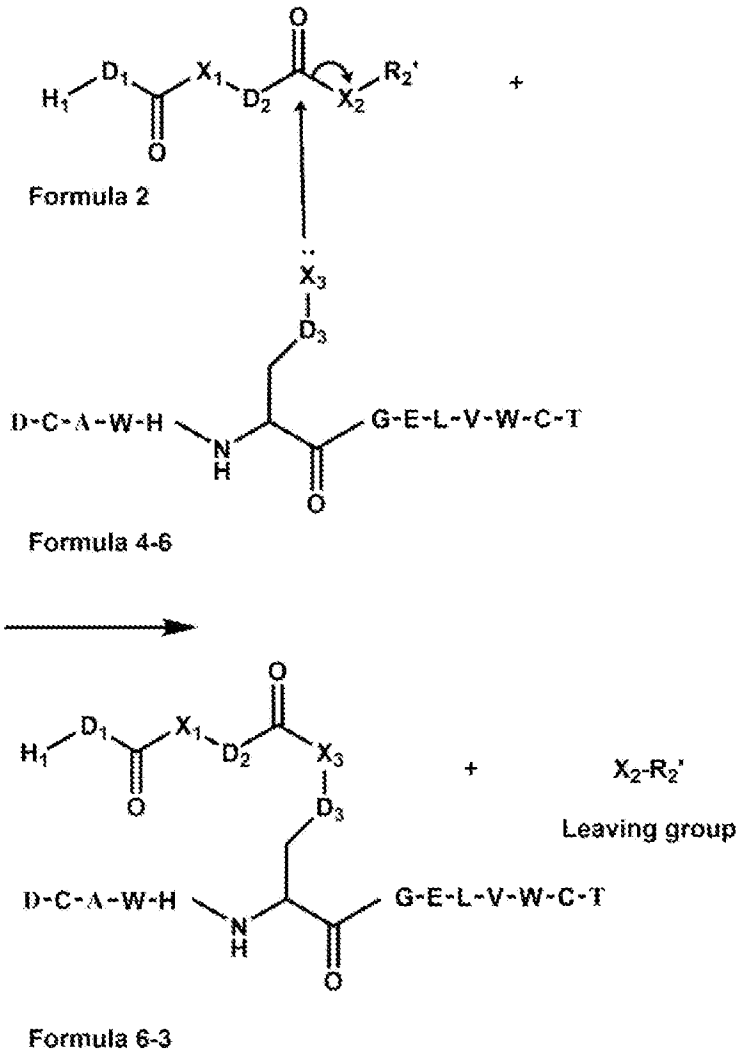


Formula 5

[0469] The R_1'-L_2-SSAI according to the present invention may be prepared by allowing a site-specific antibody interactome (SSAI) to react with the linker (R_1'-L_1) according to the present invention. The SSAI according to the present invention is designed to include a nucleophile X_3. The X_3 may attack an activated carbonyl group included in the linker to cause a nucleophilic substitution reaction. In this case, as the X_3 attacks the second carbonyl carbon, the R_1'-L_2-SSAI according to the present invention is prepared.

[0470] As a more specific example, a method of preparing a compound of Formula 6-3 will be described. In specific embodiments, the compound of Formula 6-3 may be prepared through a reaction of the following Scheme 3.

[0471] [Scheme 3]



[0472] The SSAI including the amino acid sequence of Formula 3 or 3-1 according to the present invention and having each of the structures of Formulas 4-1 to 4-6 includes an Xa_1 residue including a nucleophile X_3. As the X_3 attacks the second carbonyl group included in the linker, the R_1'-L_2-SSAI according to the present invention is prepared.

[0473] In specific embodiments, the basicity of the leaving group including X_2 may be lower than that of the leaving group including X_1 in the linker according to the present invention. In specific embodiments, the linker according to the present invention may allow X_2-R_2' connected to the second carbonyl group to form a good leaving group. Also, X_2 may be O, and R_2' may be

N-succinimide, p-nitrophenyl, or pentafluorophenyl. In this case, the reactivity of the second carbonyl group is higher than that of the first carbonyl group. Therefore, X_3 of the SSAI may specifically attack the second carbonyl group of the linker.

[0474] According to the present invention, there is disclosed a method of preparing an agent for transferring a first chemical functional group to an antibody.

[0475] The present invention provides a method of preparing an R_1'-L_2-SSAI, which comprises:

[0476] reacting a linker according to the present invention with a site-specific antibody interactome according to the present invention.

[0477] In specific embodiments, the linker may be any one selected from Formulas 1, 2, and 2-1 to 2-3.

[0478] In specific embodiments, the site-specific antibody interactome may include or have any one structure selected from Formulas 3, 3-1, and 4-1 to 4-6.

[0479] Also, the linker may have the structure of Formula 2, and the site-specific antibody interactome may have any one structure selected from Formulas 4-2 to 4-6. Furthermore, the site-specific antibody interactome may have the structure of Formula 4-6.

[0480] According to the present invention, there is disclosed a method of preparing an agent for transferring a first click-chemistry functional group to an antibody.

[0481] The present invention provides a method of preparing an H_1-L_2-SSAI, which comprises:

[0482] reacting a linker according to the present invention with a site-specific antibody interactome according to the present invention.

[0483] In specific embodiments, the linker may be any one selected from Formulas 2, and 2-1 to 2-3.

[0484] In specific embodiments, the site-specific antibody interactome may include or have any one structure selected from Formulas 3, 3-1, and 4-1 to 4-6.

[0485] Also, the linker may have the structure of Formula 2, and the site-specific antibody interactome may have any one structure selected from Formulas 4-2 to 4-6. Furthermore, the site-specific antibody interactome may have the structure of Formula 4-6.

[0486] According to the present invention, there is disclosed a kit for preparing an agent for transferring a first chemical functional group to an antibody.

[0487] The present invention provides a kit for preparing an agent for transferring a first chemical functional group to an antibody, which comprises the linker according to the present

invention and the site-specific antibody interactome according to the present invention.

[0488] In specific embodiments, the linker may be any one selected from Formulas 1, 2, and 2-1 to 2-3.

[0489] In specific embodiments, the site-specific antibody interactome may include or have any one structure selected from Formulas 3, 3-1, and 4-1 to 4-6.

[0490] Also, the linker may have the structure of Formula 2, and the site-specific antibody interactome may have any one structure selected from Formulas 4-2 to 4-6. Furthermore, the site-specific antibody interactome may have the structure of Formula 4-6.

[0491] According to the present invention, there is disclosed a kit for preparing an agent for transferring a first click-chemistry functional group to an antibody.

[0492] The present invention provides a kit for preparing an agent for transferring a first click-chemistry functional group to an antibody, which comprises a linker containing the first click-chemistry functional group according to the present invention, and a site-specific antibody interactome.

[0493] In specific embodiments, the linker may be any one selected from Formulas 2, and 2-1 to 2-3.

[0494] In specific embodiments, the site-specific antibody interactome may include or have any one structure selected from Formulas 3, 3-1, and 4-1 to 4-6.

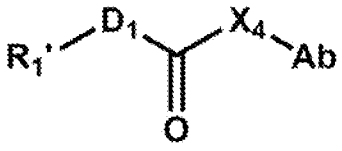
[0495] Also, the linker may have the structure of Formula 2, and the site-specific antibody interactome may have any one structure selected from Formulas 4-2 to 4-6. Furthermore, the site-specific antibody interactome may have the structure of Formula 4-6.

[0496] 5. Antibody containing first chemical functional group (R_1'-Ab)

[0497] According to the present invention, there is disclosed an antibody containing a first chemical functional group. Such a compound is herein indicated by the symbol "R_1'-Ab."

[0498] The present invention provides an R_1'-Ab represented by Formula 7:

[0499] [Formula 7]



[0500] wherein R_1' is a first chemical functional group,

[0501] D_1 is any alkylene, alkenylene, or alkynylene,

[0502] X_4 is NH, O, or S, and

[0503] Ab is an antibody.

[0504] In specific embodiments, R_1' may be a click-chemistry functional group. Also, R_1' may include any one selected from an alkyne, an azide, a strained alkyne, a diene, a dienophile, an alkene, a thiol, and a tetrazine. Furthermore, R_1' may be an azide, or a strained alkyne. Further, R_1' may be an azide, or dibenzocyclooctyne-amine. Additionally, R_1' may be a diene, or a dienophile. Further, R_1' may be a tetrazine, or a norbornene. Alternatively, R_1' may be a tetrazine, or a trans-cyclooctene.

[0505] In other specific embodiments, R_1' may include a carrier moiety, a fluorescent moiety, a drug moiety, or a radioactive moiety. Also, R_1' may include a drug moiety. In addition, R_1' may include a VC linker. In other specific embodiments, R_1' may include an antibody or an analogue thereof, which includes a paratope.

[0506] In specific embodiments, D_1 may include any one selected from a covalent bond, a C_{1-4} alkylene, a C_{2-4} alkenylene, a C_{2-4} alkynylene, and a C_{3-8} cycloalkylene. Also, D_1 may be $-CH_2OCH_2-$. In addition, D_1 may be a covalent bond.

[0507] In one specific embodiment, X_4 may be NH.

[0508] In specific embodiments, Ab may be a human antibody. In other specific embodiments, Ab may be a non-human animal antibody. In specific embodiments, Ab may be immunoglobulin G (IgG). In specific embodiments, Ab may be a whole antibody. In other specific embodiments, Ab may be a fragment of the antibody.

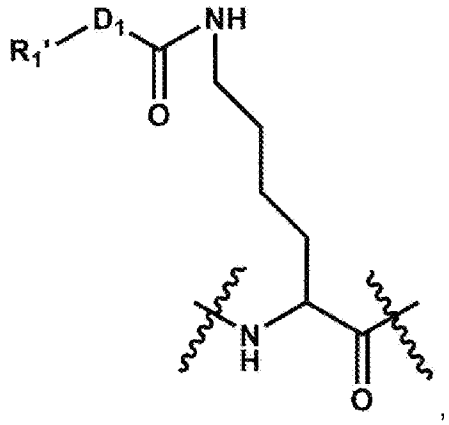
[0509] In specific embodiments, X_4 and Ab may be connected via an Fab domain of Ab. In other specific embodiments, X_4 and Ab may be connected via an Fc domain of Ab. Also, X_4 and Ab may be connected via lysine 246 or 248 in the Fc domain of Ab. Furthermore, X_4 and Ab may be connected via lysine 246 in the Fc domain of Ab. Further, X_4 and Ab may be connected via lysine 248 in the Fc domain of Ab. Alternatively, X_4 and Ab may be connected via lysine 246 and 248 in the Fc domain of Ab. In specific embodiments, X_4 and Ab may be connected via only one of two Fc domains of Ab. In other specific embodiments, X_4 and Ab may be connected via both of the two Fc domains of Ab.

[0510] The present invention provides an antibody or a fragment thereof, which includes an amino acid sequence of the following Formula 7-1:

[0511] [Formula 7-1]

[0512] G—P—S—V—F—L—F—P—P-(K)'-P—K—D—T—L—M—I (SEQ ID NO: 17)

[0513] wherein G is glycine, P is proline, S is serine, V is valine, F is phenylalanine, L is leucine, K is lysine, D is aspartic acid, T is threonine, M is methionine, I is isoleucine, and

[0514] (K)' is 

,

[0515] wherein R_1' is a first chemical functional group, and

[0516] D_1 is any alkylene, alkenylene, or alkynylene. The antibody or fragment thereof has R_1' connected via lysine 246 in an Fc domain thereof, or a site corresponding to the lysine 246.

[0517] In specific embodiments, R_1' may include a click-chemistry functional group. Also, the click-chemistry functional group may include one or more selected from an alkyne, an azide, a strained alkyne, a diene, a dienophile, an alkene, a thiol, and a tetrazine. Furthermore, the click-chemistry functional group may be selected from an azide, or a strained alkyne. Further, the click-chemistry functional group may be selected from an azide, or dibenzocyclooctyne-amine. Additionally, the click-chemistry functional group may be selected from a diene, or a dienophile. Further, the click-chemistry functional group may be selected from a tetrazine, or a norbornene. Alternatively, the click-chemistry functional group may be selected from a tetrazine, or a trans-cyclooctene. In addition, R_1' may include two or more click-chemistry functional groups.

[0518] In other specific embodiments, R_1' may include a carrier moiety, a fluorescent moiety, a drug moiety, or a radioactive moiety. Also, R_1' may include a drug moiety. In addition, R_1' may include a VC linker. In other specific embodiments, R_1' may include an antibody or an analogue thereof, which includes a paratope.

[0519] In specific embodiments, D_1 may include any one selected from a covalent bond, a C_{1-4} alkylene, a C_{2-4} alkenylene, a C_{2-4} alkynylene, and a C_{3-8} cycloalkylene. Also, D_1 may be $-CH_2OCH_2-$. In addition, D_1 may be a covalent bond.

[0520] In specific embodiments, the antibody may be a human antibody. In other specific embodiments, the antibody may be a non-human animal antibody. In specific embodiments, the antibody may be immunoglobulin G (IgG). In specific embodiments, the antibody may be a whole antibody. In other specific embodiments, the antibody may be a fragment of the antibody.

[0521] In specific embodiments, the antibody may include the amino acid sequence of

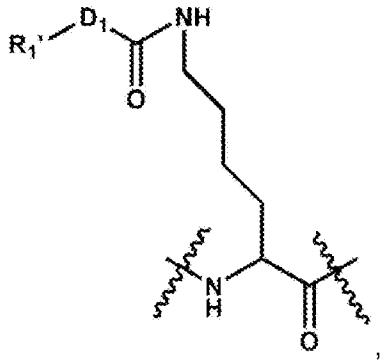
Formula 7-1 in only one of the two Fc domains thereof. In other specific embodiments, the antibody may include the amino acid sequence of Formula 7-1 in both of the two Fc domains thereof.

[0522] The present invention provides an antibody or a fragment thereof, which includes an amino acid sequence of the following Formula 7-2:

[0523] [Formula 7-2]

G—P—S—V—F—L—F—P—P—K—P-(K)'-D—T—L—M—I (SEQ ID NO: 18)

[0524] wherein G is glycine, P is proline, S is serine, V is valine, F is phenylalanine, L is leucine, K is lysine, D is aspartic acid, T is threonine, M is methionine, I is isoleucine, and

[0525] (K)' is 

,

[0526] wherein R_1' is a first chemical functional group, and

[0527] D_1 is any alkylene, alkenylene, or alkynylene. The antibody or fragment thereof has R_1' connected via lysine 248 in an Fc domain thereof, or a site corresponding to the lysine 248.

[0528] In specific embodiments, R_1' may include a click-chemistry functional group. Also, the click-chemistry functional group may include one or more selected from an alkyne, an azide, a strained alkyne, a diene, a dienophile, an alkene, a thiol, and a tetrazine. Furthermore, the click-chemistry functional group may be selected from an azide, or a strained alkyne. Further, the click-chemistry functional group may be selected from an azide, or dibenzocyclooctyne-amine. Additionally, the click-chemistry functional group may be selected from a diene, or a dienophile. Further, the click-chemistry functional group may be selected from a tetrazine, or a norbornene. Alternatively, the click-chemistry functional group may be selected from a tetrazine, or a trans-cyclooctene. In addition, R_1' may include two or more click-chemistry functional groups.

[0529] In other specific embodiments, R_1' may include a carrier moiety, a fluorescent moiety, a drug moiety, or a radioactive moiety. Also, R_1' may include a drug moiety. In addition, R_1' may include a VC linker. In other specific embodiments, R_1' may include an antibody or an analogue thereof, which includes a paratope.

[0530] In specific embodiments, D_1 may include any one selected from a covalent bond, a C_{1-4} alkylene, a C_{2-4} alkenylene, a C_{2-4} alkynylene, and a C_{3-8} cycloalkylene. Also, D_1 may be $-CH_2OCH_2-$. In addition, D_1 may be a covalent bond.

[0531] In specific embodiments, the antibody may be a human antibody. In other specific embodiments, the antibody may be a non-human animal antibody. In specific embodiments, the antibody may be immunoglobulin G (IgG). In specific embodiments, the antibody may be a whole antibody. In other specific embodiments, the antibody may be a fragment of the antibody.

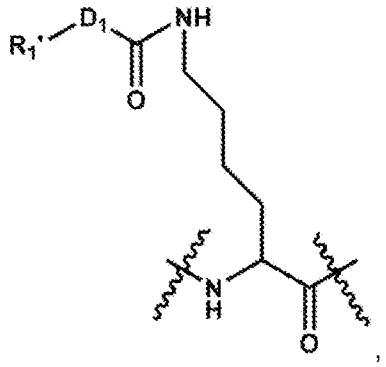
[0532] In specific embodiments, the antibody may include the amino acid sequence of Formula 7-2 in only one of the two Fc domains thereof. In other specific embodiments, the antibody may include the amino acid sequence of Formula 7-2 in both of the two Fc domains thereof.

[0533] The present invention provides an antibody or a fragment thereof, which includes an amino acid sequence of the following Formula 7-3:

[0534] [Formula 7-3] (SEQ ID NO: 19)

G—P—S—V—F—L—F—P—P–(K)'–P–(K)'–D—T—L—M—I (SEQ ID NO: 19)

[0535] wherein G is glycine, P is proline, S is serine, V is valine, F is phenylalanine, L is leucine, K is lysine, D is aspartic acid, T is threonine, M is methionine, I is isoleucine, and

[0536] (K)' is 

,

[0537] wherein R_1' is a first chemical functional group, and

[0538] D_1 is any alkylene, alkenylene, or alkynylene. The antibody or fragment thereof has R_1' connected via lysine 246 and 248 in an Fc domain thereof, or sites corresponding to the lysine 246 and 248.

[0539] In specific embodiments, R_1' may include a click-chemistry functional group. Also, the click-chemistry functional group may include one or more selected from an alkyne, an azide, a strained alkyne, a diene, a dienophile, an alkene, a thiol, and a tetrazine. Furthermore, the click-chemistry functional group may be selected from an azide, or a strained alkyne. Further, the click-chemistry functional group may be selected from an azide, or dibenzocyclooctyne-amine.

Additionally, the click-chemistry functional group may be selected from a diene, or a dienophile. Further, the click-chemistry functional group may be selected from a tetrazine, or a norbornene. Alternatively, the click-chemistry functional group may be selected from a tetrazine, or a trans-cyclooctene. In addition, R_1' may include two or more click-chemistry functional groups.

[0540] In other specific embodiments, R_1' may include a carrier moiety, a fluorescent moiety, a drug moiety, or a radioactive moiety. Also, R_1' may include a drug moiety. In addition, R_1' may include a VC linker. In other specific embodiments, R_1' may include an antibody or an analogue thereof, which includes a paratope.

[0541] In specific embodiments, D_1 may include any one selected from a covalent bond, a C_{1-4} alkylene, a C_{2-4} alkenylene, a C_{2-4} alkynylene, and a C_{3-8} cycloalkylene. Also, D_1 may be $-CH_2OCH_2-$. In addition, D_1 may be a covalent bond.

[0542] In specific embodiments, the antibody may be a human antibody. In other specific embodiments, the antibody may be a non-human animal antibody. In specific embodiments, the antibody may be immunoglobulin G (IgG). In specific embodiments, the antibody may be a whole antibody. In other specific embodiments, the antibody may be a fragment of the antibody.

[0543] In specific embodiments, the antibody may include the amino acid sequence of Formula 7-3 in only one of the two Fc domains thereof. In other specific embodiments, the antibody may include the amino acid sequence of Formula 7-3 in both of the two Fc domains thereof.

[0544] The present invention provides an antibody or a fragment thereof, which includes one or more amino acid sequences selected from Formulas 7-1, 7-2, and 7-3. In this case, the contents of the sequences of Formulas 7-1 to 7-3 are as described above.

[0545] In specific embodiments, D_1 may be a covalent bond.

[0546] In specific embodiments, the antibody or fragment thereof may include only the amino acid sequence of Formula 7-1 and may not include the amino acid sequences of Formulas 7-2 and 7-3. Also, the antibody or fragment thereof may include the amino acid sequence of Formula 7-1 in only one of the two Fc domains thereof. In addition, the antibody may include the amino acid sequence of Formula 7-1 in both of the two Fc domains thereof.

[0547] In other specific embodiments, the antibody or fragment thereof may include only the amino acid sequence of Formula 7-2 and may not include the amino acid sequences of Formulas 7-1 and 7-3. Also, the antibody or fragment thereof may include the amino acid sequence of Formula 7-2 in only one of the two Fc domains thereof. In addition, the antibody may include the amino acid sequence of Formula 7-2 in both of the two Fc domains thereof.

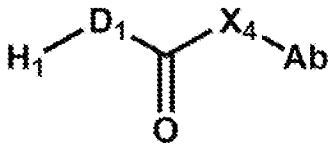
[0548] In specific embodiments, the antibody or fragment thereof may include only the amino acid sequence of Formula 7-3 and may not include the amino acid sequences of Formulas 7-1 and 7-2. Also, the antibody or fragment thereof may include the amino acid sequence of Formula 7-3 in only one of the two Fc domains thereof. In addition, the antibody may include the amino acid sequence of Formula 7-3 in both of the two Fc domains thereof.

[0549] 5.1. Antibody containing first click-chemistry functional group

[0550] According to the present invention, there is disclosed an antibody containing a first click-chemistry functional group. Such a compound is herein indicated by the symbol "H_1-Ab."

[0551] The present invention provides an H_1-Ab represented by Formula 8:

[0552] [Formula 8]



[0553] wherein H_1 is a first click-chemistry functional group,

[0554] D_1 is any alkylene, alkenylene, or alkynylene,

[0555] X_4 is NH, O, or S, and

[0556] Ab is an antibody.

[0557] In specific embodiments, H_1 may include any one selected from an alkyne, an azide, a strained alkyne, a diene, a dienophile, an alkene, a thiol, and a tetrazine. Furthermore, H_1 may be an azide, or a strained alkyne. Further, H_1 may be an azide, or dibenzocyclooctyne-amine. Additionally, H_1 may be a diene, or a dienophile. Further, H_1 may be a tetrazine, or a norbornene. Alternatively, H_1 may be a tetrazine, or a trans-cyclooctene.

[0558] In specific embodiments, D_1 may include any one selected from a covalent bond, a C_{1-4} alkylene, a C_{2-4} alkenylene, a C_{2-4} alkynylene, and a C_{3-8} cycloalkylene. Also, D_1 may be $-CH_2OCH_2-$. In addition, D_1 may be a covalent bond.

[0559] In one specific embodiment, X_4 may be NH.

[0560] In specific embodiments, Ab may be a human antibody. In other specific embodiments, Ab may be a non-human animal antibody. In specific embodiments, Ab may be immunoglobulin G (IgG). In specific embodiments, Ab may be a whole antibody. In other specific embodiments, Ab may be a fragment of the antibody. In specific embodiments, Ab may be a wild-type antibody. In other specific embodiments, Ab may be a manipulated antibody.

[0561] In specific embodiments, X_4 and Ab may be connected via an Fab domain of Ab.

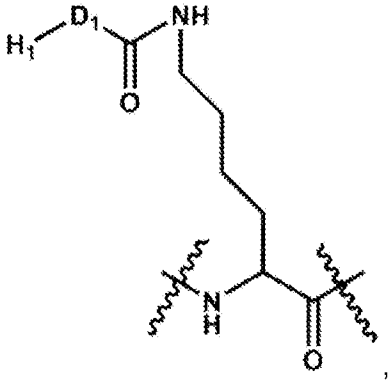
In other specific embodiments, X_4 and Ab may be connected via an Fc domain of Ab. Also, X_4 and Ab may be connected via lysine 246 or 248 in the Fc domain of Ab. Furthermore, X_4 and Ab may be connected via lysine 246 in the Fc domain of Ab. Further, X_4 and Ab may be connected via lysine 248 in the Fc domain of Ab. Alternatively, X_4 and Ab may be connected via lysine 246 and 248 in the Fc domain of Ab. In specific embodiments, X_4 and Ab may be connected via only one of two Fc domains of Ab. In other specific embodiments, X_4 and Ab may be connected via both of the two Fc domains of Ab.

[0562] The present invention provides an antibody or a fragment thereof, which includes an amino acid sequence of the following Formula 8-1:

[0563] [Formula 8-1]

G—P—S—V—F—L—F—P—P—(K)'—P—K—D—T—L—M—I (SEQ ID NO: 20)

[0564] wherein G is glycine, P is proline, S is serine, V is valine, F is phenylalanine, L is leucine, K is lysine, D is aspartic acid, T is threonine, M is methionine, I is isoleucine, and

[0565] (K)' is 

,

[0566] wherein H_1 is a first click-chemistry functional group, and

[0567] D_1 is any alkylene, alkenylene, or alkynylene. The antibody or fragment thereof has H_1 connected via lysine 246 in an Fc domain thereof, or a site corresponding to the lysine 246.

[0568] In specific embodiments, H_1 may include any one selected from an alkyne, an azide, a strained alkyne, a diene, a dienophile, an alkene, a thiol, and a tetrazine. Furthermore, H_1 may be an azide, or a strained alkyne. Further, H_1 may be an azide, or dibenzocyclooctyne-amine. Additionally, H_1 may be a diene, or a dienophile. Further, H_1 may be a tetrazine, or a norbornene. Alternatively, H_1 may be a tetrazine, or a trans-cyclooctene.

[0569] In specific embodiments, D_1 may include any one selected from a covalent bond, a C_{1-4} alkylene, a C_{2-4} alkenylene, a C_{2-4} alkynylene, and a C_{3-8} cycloalkylene. Also, D_1 may be $-CH_2OCH_2-$. In addition, D_1 may be a covalent bond.

[0570] In specific embodiments, the antibody may be a human antibody. In other specific embodiments, the antibody may be a non-human animal antibody. In specific embodiments, the antibody may be immunoglobulin G (IgG). In specific embodiments, the antibody may be a whole antibody. In other specific embodiments, the antibody may be a fragment of the antibody. In specific embodiments, the antibody may be a wild-type antibody. In other specific embodiments, the antibody may be a manipulated antibody.

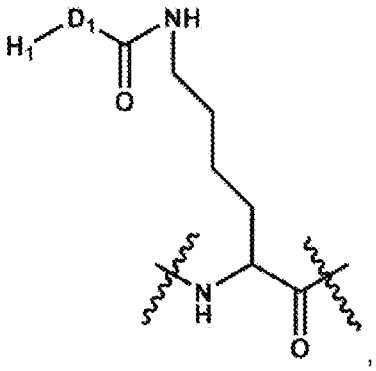
[0571] In specific embodiments, the antibody may include the amino acid sequence of Formula 8-1 in only one of the two Fc domains thereof. In other specific embodiments, the antibody may include the amino acid sequence of Formula 8-1 in both of the two Fc domains thereof.

[0572] The present invention provides an antibody or a fragment thereof, which includes an amino acid sequence of the following Formula 8-2:

[0573] [Formula 8-2]

[0574] G—P—S—V—F—L—F—P—P—K—P-(K)'-D—T—L—M—I (SEQ ID NO: 21)

[0575] wherein G is glycine, P is proline, S is serine, V is valine, F is phenylalanine, L is leucine, K is lysine, D is aspartic acid, T is threonine, M is methionine, I is isoleucine, and

[0576] (K)' is 

,

wherein H_1 is a first click-chemistry functional group, and

[0577] D_1 is any alkylene, alkenylene, or alkynylene. The antibody or fragment thereof has H_1 connected via lysine 248 in an Fc domain thereof, or a site corresponding to the lysine 248.

[0578] In specific embodiments, H_1 may include any one selected from an alkyne, an azide, a strained alkyne, a diene, a dienophile, an alkene, a thiol, and a tetrazine. Furthermore, H_1 may be an azide, or a strained alkyne. Further, H_1 may be an azide, or dibenzocyclooctyne-amine. Additionally, H_1 may be a diene, or a dienophile. Further, H_1 may be a tetrazine, or a norbornene. Alternatively, H_1 may be a tetrazine, or a trans-cyclooctene.

[0579] In specific embodiments, D_1 may include any one selected from a covalent bond, a C_{1-4} alkylene, a C_{2-4} alkenylene, a C_{2-4} alkynylene, and a C_{3-8} cycloalkylene. Also, D_1 may be $-CH_2OCH_2-$. In addition, D_1 may be a covalent bond.

[0580] In specific embodiments, the antibody may be a human antibody. In other specific embodiments, the antibody may be a non-human animal antibody. In specific embodiments, the antibody may be immunoglobulin G (IgG). In specific embodiments, the antibody may be a whole antibody. In other specific embodiments, the antibody may be a fragment of the antibody. In specific embodiments, the antibody may be a wild-type antibody. In other specific embodiments, the antibody may be a manipulated antibody.

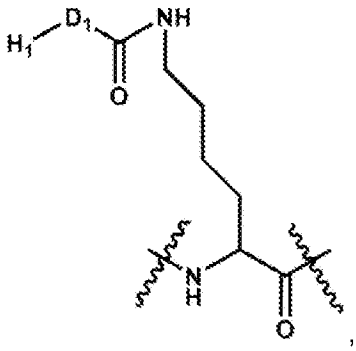
[0581] In specific embodiments, the antibody may include the amino acid sequence of Formula 8-2 in only one of the two Fc domains thereof. In other specific embodiments, the antibody may include the amino acid sequence of Formula 8-2 in both of the two Fc domains thereof.

[0582] The present invention provides an antibody or a fragment thereof, which includes an amino acid sequence of the following Formula 8-3:

[0583] [Formula 8-3]

G—P—S—V—F—L—F—P—P–(K)'–P–(K)'–D—T—L—M—I (SEQ ID NO: 22)

[0584] wherein G is glycine, P is proline, S is serine, V is valine, F is phenylalanine, L is leucine, K is lysine, D is aspartic acid, T is threonine, M is methionine, I is isoleucine, and

[0585] (K)' is 

,

[0586] wherein H_1 is a first click-chemistry functional group, and

[0587] D_1 is any alkylene, alkenylene, or alkynylene. The antibody or fragment thereof has H_1 connected via lysine 246 and 248 in an Fc domain thereof, or sites corresponding to the lysine 246 and 248.

[0588] In specific embodiments, H_1 may include any one selected from an alkyne, an azide, a strained alkyne, a diene, a dienophile, an alkene, a thiol, and a tetrazine. Furthermore, H_1 may be an azide, or a strained alkyne. Further, H_1 may be an azide, or dibenzocyclooctyne-

amine. Additionally, H_1 may be a diene, or a dienophile. Further, H_1 may be a tetrazine, or a norbornene. Alternatively, H_1 may be a tetrazine, or a trans-cyclooctene.

[0589] In specific embodiments, D_1 may include any one selected from a covalent bond, a C_{1-4} alkylene, a C_{2-4} alkenylene, a C_{2-4} alkynylene, and a C_{3-8} cycloalkylene. Also, D_1 may be $-CH_2OCH_2-$. In addition, D_1 may be a covalent bond.

[0590] In specific embodiments, the antibody may be a human antibody. In other specific embodiments, the antibody may be a non-human animal antibody. In specific embodiments, the antibody may be immunoglobulin G (IgG). In specific embodiments, the antibody may be a whole antibody. In other specific embodiments, the antibody may be a fragment of the antibody. In specific embodiments, the antibody may be a wild-type antibody. In other specific embodiments, the antibody may be a manipulated antibody.

[0591] In specific embodiments, the antibody may include the amino acid sequence of Formula 8-3 in only one of the two Fc domains thereof. In other specific embodiments, the antibody may include the amino acid sequence of Formula 8-3 in both of the two Fc domains thereof.

[0592] The present invention provides an antibody or a fragment thereof, which includes one or more amino acid sequences selected from Formulas 8-1, 8-2, and 8-3. In this case, the contents of the sequences of Formulas 8-1 to 8-3 are as described above.

[0593] In specific embodiments, D_1 may be a covalent bond.

[0594] In specific embodiments, the antibody or fragment thereof may include only the amino acid sequence of Formula 8-1 and may not include the amino acid sequences of Formulas 8-2 and 8-3. Also, the antibody or fragment thereof may include the amino acid sequence of Formula 8-1 in only one of the two Fc domains thereof. In addition, the antibody may include the amino acid sequence of Formula 8-1 in both of the two Fc domains thereof.

[0595] In other specific embodiments, the antibody or fragment thereof may include only the amino acid sequence of Formula 8-2 and may not include the amino acid sequences of Formulas 8-1 and 8-3. Also, the antibody or fragment thereof may include the amino acid sequence of Formula 8-2 in only one of the two Fc domains thereof. In addition, the antibody may include the amino acid sequence of Formula 8-2 in both of the two Fc domains thereof.

[0596] In specific embodiments, the antibody or fragment thereof may include only the amino acid sequence of Formula 8-3 and may not include the amino acid sequences of Formulas 8-1 and 8-2. Also, the antibody or fragment thereof may include the amino acid sequence of Formula 8-3 in only one of the two Fc domains thereof. In addition, the antibody may include the amino acid sequence of Formula 8-3 in both of the two Fc domains thereof.

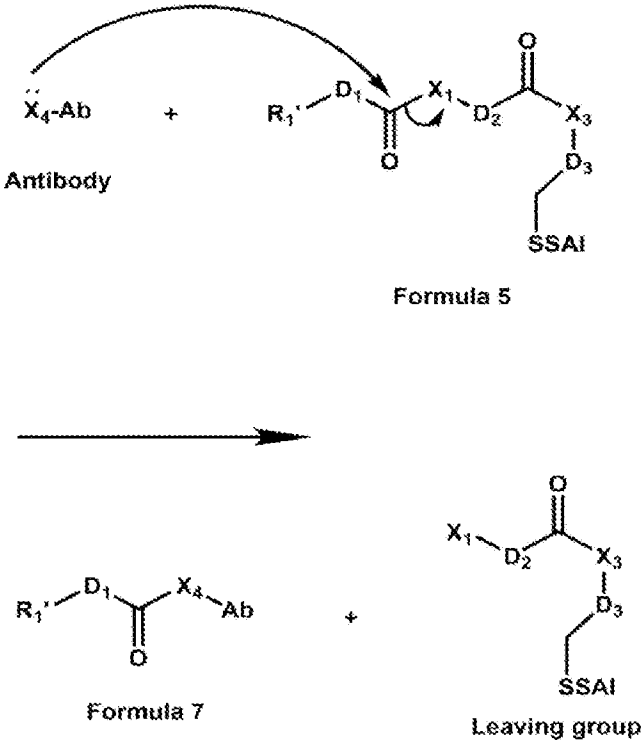
[0597] In specific embodiments, the antibody may be a human antibody. In other specific embodiments, the antibody may be a non-human animal antibody. In specific embodiments, the antibody may be immunoglobulin G (IgG). In specific embodiments, the antibody may be a whole antibody. In other specific embodiments, the antibody may be a fragment of the antibody. In specific embodiments, the antibody may be a wild-type antibody. In other specific embodiments, the antibody may be a manipulated antibody.

[0598] 5.2. Method of preparing antibody containing first chemical functional group

[0599] According to the present invention, there is disclosed a method of preparing the R_1'-Ab, and H_1-Ab (hereinafter generally referred to as "R_1'-Ab"). It will be noted that a description of the following preparation methods is provided to aid in understanding the present invention.

[0600] For example, a method of preparing a compound of Formula 7 will be described. In specific embodiments, the compound of Formula 7 may be prepared through a reaction of the following Scheme 4.

[0601] [Scheme 4]



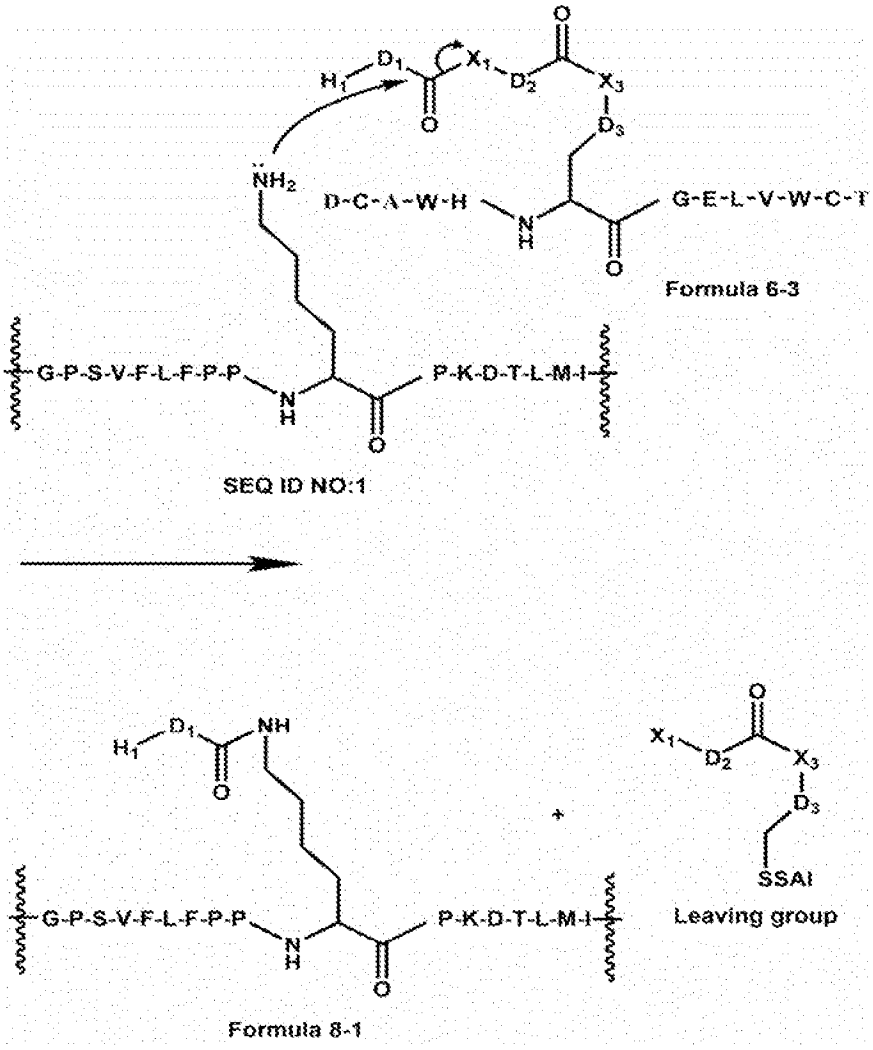
[0602] Because a first carbonyl group of the linker according to the present invention has mild reactivity, cross-linking may be realized when the first carbonyl group of the linker has a close positional relationship with an amine group of an antibody. First, the R_1'-L_2-SSAI represented by

Formula 5 is brought into close contact with a certain site of the antibody to create an environment in which a reaction may occur. Because the R_1'-L_2-SSAI according to the present invention has an activated carbonyl group (i.e., a carbonyl group connected to X_1) corresponding to the first carbonyl group of the linker, the R_1'-L_2-SSAI of the present invention may trigger a nucleophilic substitution reaction. In this case, the compound of Formula 7 may be prepared since an atom (X_4) having a free electron pair present in the antibody serves as a nucleophile to attack the carbonyl group. In this case, by design of the linker, a site-specific antibody interactome (SSAI) leaves while being included in a leaving group and the SSAI is removed from the final product. These characteristics have a positive effect on physical properties of an antibody product, as shown in the section 5.8 below.

[0603] In specific embodiments, the X_4 may be NH_2. Also, the X_4 may be NH_2 of a lysine residue. In other specific embodiments, the X_4 may be SH. Also, the X_4 may be SH of a cysteine residue. In other specific embodiments, the X_4 may be OH.

[0604] As a more specific example, a method of preparing an antibody or a fragment thereof, which includes the amino acid sequence of Formula 8-3, will be described. In specific embodiments, the compound including the amino acid sequence of Formula 8-3 may be prepared through a reaction of the following Scheme 5.

[0605] [Scheme 5]



[0606] The compound having a structure of Formula 6-3 (a structure in which two cysteine residues are optionally connected) is directed towards the Fc domain of the antibody because the compound has an SSFI sequence (see section 3.2). In this case, the antibody including an amino acid sequence of SEQ ID NO: 1 includes lysine residues in an Fc domain thereof, and such a lysine residue may serve as a nucleophile. The exemplary embodiment shows a case where lysine 246 in the Fc domain or a residue corresponding to the lysine 246 (hereinafter referred to as "lysine 246") serves as a nucleophile. In this case, an amine group of the lysine 246 attacks a first carbonyl group of Formula 6-3 to produce a compound of Formula 8-1. In this way, H_1 or R_1 may be transferred to the lysine residue of the Fc domain of the antibody. In this case, to which certain lysine residue the chemical functional group is transferred may depend on the design of the linker, the SSAI, and the R_1'-L_2-SSAI, as described in the sections 5.3 and 5.4 below.

[0607] According to the present invention, there is disclosed a method of preparing an antibody containing a first chemical functional group.

[0608] The present invention provides a method of preparing an R_1'-Ab, which includes:

[0609] reacting an agent for transferring a first chemical functional group to an antibody according to the present invention with an antibody or a fragment thereof.

[0610] In specific embodiments, the agent for transferring a first chemical functional group to an antibody may be any one selected from Formulas 5, 5-1 to 5-3, and 6-1 to 6-3.

[0611] In specific embodiments, the antibody or fragment thereof may be a human antibody. In other specific embodiments, the antibody or fragment thereof may be a non-human animal antibody. In specific embodiments, the antibody or fragment thereof may be immunoglobulin G (IgG). In specific embodiments, the antibody or fragment thereof may be a whole antibody. In other specific embodiments, the antibody or fragment thereof may be a fragment of the antibody. In specific embodiments, the antibody or fragment thereof may be a wild-type antibody. In other specific embodiments, the antibody or fragment thereof may be a manipulated antibody.

[0612] In specific embodiments, the present invention provides a method of preparing an antibody having a first chemical functional group transferred to a specific lysine residue of an Fc domain thereof. In this case, those of aspects as described in the section 5.4 below may be used as the agent for transferring a first chemical functional group to an antibody.

[0613] According to the present invention, there is disclosed a method of preparing an antibody containing a first click-chemistry functional group.

[0614] The present invention provides a method of preparing an H_1-Ab, which includes:

[0615] reacting an agent for transferring a first click-chemistry functional group to an antibody according to the present invention with an antibody or a fragment thereof.

[0616] In specific embodiments, the agent for transferring a first click-chemistry functional group to an antibody may be any one selected from Formulas 6-1 to 6-3. Also, the agent for transferring a first click-chemistry functional group to an antibody may have the structure of Formula 6-3.

[0617] In specific embodiments, the antibody or fragment thereof may be a human antibody. In other specific embodiments, the antibody or fragment thereof may be a non-human animal antibody. In specific embodiments, the antibody or fragment thereof may be immunoglobulin G (IgG). In specific embodiments, the antibody or fragment thereof may be a whole antibody. In other specific embodiments, the antibody or fragment thereof may be a fragment of the antibody. In specific embodiments, the antibody or fragment thereof may be a wild-type antibody. In other specific embodiments, the antibody or fragment thereof may be a

manipulated antibody.

[0618] In specific embodiments, the present invention provides a method of preparing an antibody having a first click-chemistry functional group transferred to a specific lysine residue of an Fc domain thereof. In this case, those of aspects as described in the section 5.4 below may be used as the agent for transferring a first click-chemistry functional group to an antibody.

[0619] According to the present invention, there is disclosed a kit for preparing an antibody or a fragment thereof containing a first chemical functional group.

[0620] The present invention provides a kit for preparing an antibody or a fragment thereof containing a first chemical functional group, which includes an agent for transferring a first chemical functional group to an antibody according to the present invention, and an antibody or a fragment thereof.

[0621] In specific embodiments, the agent for transferring a first chemical functional group to an antibody may be any one selected from Formulas 5, 5-1 to 5-3, and 6-1 to 6-3.

[0622] In specific embodiments, the antibody or fragment thereof may be a human antibody. In other specific embodiments, the antibody or fragment thereof may be a non-human animal antibody. In specific embodiments, the antibody or fragment thereof may be immunoglobulin G (IgG). In specific embodiments, the antibody or fragment thereof may be a whole antibody. In other specific embodiments, the antibody or fragment thereof may be a fragment of the antibody. In specific embodiments, the antibody or fragment thereof may be a wild-type antibody. In other specific embodiments, the antibody or fragment thereof may be a manipulated antibody.

[0623] Also, the present invention provides a kit for preparing an antibody or a fragment thereof containing a first chemical functional group, which includes:

[0624] a linker (R_1'-L_1) according to the present invention;

[0625] a site-specific antibody interactome according to the present invention; and

[0626] an antibody or a fragment thereof.

[0627] In specific embodiments, the linker may be any one selected from Formulas 1, 2, and 2-1 to 2-3.

[0628] In specific embodiments, the site-specific antibody interactome may be any one selected from Formulas 3, 3-1, and 4-1 to 4-6.

[0629] In specific embodiments, the antibody or fragment thereof may be a human antibody. In other specific embodiments, the antibody or fragment thereof may be a non-human animal antibody. In specific embodiments, the antibody or fragment thereof may be

immunoglobulin G (IgG). In specific embodiments, the antibody or fragment thereof may be a whole antibody. In other specific embodiments, the antibody or fragment thereof may be a fragment of the antibody. In specific embodiments, the antibody or fragment thereof may be a wild-type antibody. In other specific embodiments, the antibody or fragment thereof may be a manipulated antibody.

[0630] The present invention provides a kit for preparing an antibody having a first chemical functional group transferred to a specific lysine residue of an Fc domain thereof. In this case, those of aspects as described in the section 5.4 below may be used as the agent for transferring a first chemical functional group to an antibody, the linker, and the site-specific antibody interactome.

[0631] According to the present invention, there is disclosed a kit for preparing an antibody or a fragment thereof containing a first click-chemistry functional group.

[0632] The present invention provides a kit for preparing an antibody or a fragment thereof containing a first click-chemistry functional group, which includes an agent for transferring a first click-chemistry functional group to an antibody according to the present invention, and an antibody or a fragment thereof.

[0633] In specific embodiments, the agent for transferring a first click-chemistry functional group to an antibody may be any one selected from Formulas 6-1 to 6-3. Also, the agent for transferring a first click-chemistry functional group to an antibody may have the structure of Formula 6-3.

[0634] In specific embodiments, the antibody or fragment thereof may be a human antibody. In other specific embodiments, the antibody or fragment thereof may be a non-human animal antibody. In specific embodiments, the antibody or fragment thereof may be immunoglobulin G (IgG). In specific embodiments, the antibody or fragment thereof may be a whole antibody. In other specific embodiments, the antibody or fragment thereof may be a fragment of the antibody. In specific embodiments, the antibody or fragment thereof may be a wild-type antibody. In other specific embodiments, the antibody or fragment thereof may be a manipulated antibody.

[0635] Also, the present invention provides a kit for preparing an antibody or a fragment thereof containing a first click-chemistry functional group, which includes:

[0636] a linker (H_1-L_1) according to the present invention;

[0637] a site-specific antibody interactome according to the present invention; and

[0638] an antibody or a fragment thereof.

[0639] In specific embodiments, the linker may be any one selected from Formulas 2, and 2-1 to 2-3.

[0640] In specific embodiments, the site-specific antibody interactome may be any one selected from Formulas 3, 3-1, and 4-1 to 4-6.

[0641] In specific embodiments, the antibody or fragment thereof may be a human antibody. In other specific embodiments, the antibody or fragment thereof may be a non-human animal antibody. In specific embodiments, the antibody or fragment thereof may be immunoglobulin G (IgG). In specific embodiments, the antibody or fragment thereof may be a whole antibody. In other specific embodiments, the antibody or fragment thereof may be a fragment of the antibody. In specific embodiments, the antibody or fragment thereof may be a wild-type antibody. In other specific embodiments, the antibody or fragment thereof may be a manipulated antibody.

[0642] The present invention provides a kit for preparing an antibody having a first click-chemistry functional group transferred to a specific lysine residue of an Fc domain thereof. In this case, those of aspects as described in the section 5.4 below may be used as the agent for transferring a first click-chemistry functional group to an antibody, the linker, and the site-specific antibody interactome.

[0643] 5.3. Function of $(Xa_1)'$ and design principle of location of $(Xa_1)'$ on R_1'-L_2-SSFI

[0644] In the present content, the compound of Formula 6-3 is provided as one example to aid in understanding the present invention, but the scope of the present invention is not limited thereto. It will be noted that the following description also applies to the compounds of Formulas 5, 5-1 to 5-3, and 6-1 to 6-3, and the compound of Formula 6-3 is merely provided as one example for the sake of convenience.

[0645] As discussed in the section 5.2, $(Xa_1)'$ of the R_1'-L_2-SSFI functions to transfer R_1' to an antibody by means of a nucleophilic substitution reaction. According to the present invention, it is assumed that the conditions for facilitating a nucleophilic substitution reaction satisfy the following requirements: (1) $(Xa_1)'$ is adjacent to a lysine residue of an Fc domain, and (2) a side chain to which R_1' is bound is directed towards the lysine residue. It was expected that the yield and uniformity of a process would drop as the position of $(Xa_1)'$ and the direction of the side chain become farther from the lysine residue of the Fc domain. Also, (3) it will be preferred that a substitution position of $(Xa_1)'$ does not have a great influence on the interaction between the SSFI and the Fc domain (see section 3.2).

[0646] As indirectly seen in FIGS. 3 and 5, it was confirmed that the positions in the SSFI that satisfy the requirement of (1) in relation to lysine 246 and 248 in the Fc domain are positions

5, 6, 7, and 8 based on the following Formula 6-3.

[0647] [Formula 6-3]

D-C-A-W-H-(Xa$_1$)'-G-E-L-V-W-C-T (SEQ ID NO: 16)

[0648] In this case, as histidine corresponding to position 5 forms a salt linkage with glutamic acid 380 in the Fc domain, replacement of the histidine may affect the interaction between the SSFI and the Fc domain. Therefore, this does not satisfy the requirement of (3). Also, because the direction of this side chain is not close to the lysine 246 and 248, this does not satisfy the requirement of (2). Because glycine corresponding to position 7 helps to form a bent structure of the SSFI, it is not desirable to replace the glycine residue with a large (Xa$_1$)' residue. As glutamic acid corresponding to position 8 forms a salt linkage with arginine 255 in the Fc domain, replacement of the glutamic acid may affect the interaction between the SSFI and the Fc domain. Therefore, this does not satisfy the requirement of (3) (see section 3.2). It was judged that position 6 is most suitable for the position of (Xa$_1$)' because position 6 satisfies all the requirements of (1), (2), and (3). Therefore, the R$_1$'-L$_2$-SSFI according to the present invention has been completed based on these facts.

[0649] 5.4. The position of R$_1$' transferred to an antibody may vary depending on the length of D$_2$ in R$_1$'-L$_1$ and D$_3$ in SSFI.

[0650] The present content is intended to explain a preferred design principle of the R$_1$'-L$_2$-SSFI according to the present invention. According to the design of the R1'-L$_2$-SSFI, it is possible to specifically transfer R$_1$' to a specific lysine residue of the Fc domain. Also, the present content is intended to explain a design principle of the R$_1$'-L$_1$ and SSFI to prepare a preferred R$_1$'-L$_2$-SSFI.

[0651] A person of ordinary skill in the art who reads the section 5.2 as described above may recognize that a nucleophilic substitution reaction may occur when the first carbonyl carbon of (Xa$_1$)' of the R$_1$'-L$_2$-SSFI is located adjacent to an amine group of lysine of the Fc domain. Based on the description of the section 3.2 as described above, a person of ordinary skill in the art may also recognize that the R$_1$'-L$_2$-SSFI is arranged with the Fc domain with a specific topology, wherein amine groups of lysine 246 and 248 in the Fc domain are spaced apart a certain distance from the beta carbon of (Xa$_1$)' (FIG. 5). By using this combination to design the R$_1$'-L$_2$-SSFI, it was expected that it is possible to specifically label a desired lysine residue when (1) a distance (hereinafter generally referred to as "L$_c$") between the beta carbon of (Xa$_1$)' and the first carbonyl carbon is the same as or similar to (2) a distance between the beta carbon of (Xa$_1$)' and the amine groups of lysine 246 and 248 in the Fc domain in the specific topology.

[0652] The structure of the R_1'-L_2-SSFI according to the present invention and the L_c are shown in FIG. 8. As shown in FIG. 8, D_3, X_3, carbon atoms, D_2, and X_1 are located between the beta carbon of (Xa_1)' and the first carbonyl carbon. Among these, D_3 and X_3 are associated with the design of SSFI, and D_2 and X_1 are associated with the design of L_1-R_1'.

[0653] For the sake of convenience, the invention was embodied on the assumption that D_3 is a C_x alkylene, X_3 is N, D_2 is a C_y alkylene, a C_y alkenylene, or a C_y alkynylene, and X_1 is S, wherein y is an integer greater than or equal to 1. In this case, it was assumed that the alkylene, the alkenylene, and the alkynylene have the same length. The structure of the linker shown in FIG. 8 was modeled using the software Discovery Studio to calculate L_c values. As a result, the L_c values determined with respect to the x + y value are listed in Table 3. In the calculation process, the chain was modeled when it had the longest length.

<Table 3> Lc values with respect to x + y value

x+y	Lc (Å)
1 (x=0, y=1)	6.616
2	7.784
3	9.102
4	10.299
5	11.599
6	12.816
7	14.100
8	15.334
9	16.601
10	17.848
11	19.099
12	20.357
13	21.592
14	22.857

[0654] Hereinafter, the accompanying drawings provided to show a topology between the R_1'-L_2-SSFI and a lysine residue of the Fc domain are provided based on FIG. 9. FIG. 9 shows a topology between an R_1'-L_2-SSFI and an Fc domain so that a direction (a dotted arrow) of a side chain of (Xa_1)' is parallel with the x axis in the drawing. As shown in FIG. 9, it can be seen that the side chain of (Xa_1)' is directed towards the amine groups of the lysine 246 and 248 (see section 5.3). Also, it can be seen from the drawing that the side chain of (Xa_1)' may specifically react with the lysine 246 or 248 depending on the length of L_c. FIGS. 10 to 12 are diagrams

viewing a diagram of FIG. 9 in a direction (a thick solid line arrow) parallel with the y-axis in the drawing along the exemplary length of L_c.

[0655] The distances ($D_{246,min}$, $D_{246,max}$, $D_{248,min}$, and $D_{248,max}$) between the beta carbon of $(Xa_1)'$ and the amine groups of lysine 246 and 248 in the Fc domain are described in the section 3.2. The beta carbon of $(Xa_1)'$ is closer to the lysine 248 in the Fc domain than the lysine residue at position 246. Therefore, it was expected that the first carbonyl carbon would react well with the lysine 248 when the L_c value is shorter than $D_{246,min}$ (FIG. 10), the first carbonyl carbon would react well with the lysine 246 when the L_c value is longer than $D_{248,max}$ (FIG. 11), and the first carbonyl carbon would selectively react with the lysine 246 and 248 when the L_c value is longer than or equal to $D_{246,min}$ and shorter than or equal to $D_{248,max}$ (FIG. 12).

[0656] According to the present invention, there is disclosed an R_1'-L_2-SSFI for specifically transferring a first chemical functional group to lysine 248 in an Fc domain of an antibody (see FIG. 11).

[0657] The present invention provides an agent for transferring a first chemical functional group to an antibody, characterized in that a distance (L_c) between the beta carbon of $(Xa_1)'$ and the first carbonyl carbon is shorter than $D_{246,min}$ (approximately 11.668 Å). In this case, L_c may have a value of approximately 6.5 Å, approximately 7 Å, approximately 8 Å, approximately 9 Å, approximately 10 Å, approximately 11 Å, or approximately 11.5 Å.

[0658] In specific embodiments, when the R_1'-L_2-SSFI has any one structure selected from Formulas 5, 5-1 to 5-3, and 6-1 to 6-3, D_3 is a C_x alkylene, X_3 is N, D_2 is a C_y alkylene, a C_y alkenylene, or a C_y alkynylene, X_1 is S, wherein y may be an integer greater than or equal to 1, and the sum of x and y may be $1 \leq x + y \leq 5$. Also, the sum of x and y may be 1, 2, 3, 4, or 5. For example, x may be 0, and y may be $1 \leq y \leq 5$. In another exemplary embodiment, x may be 1, and y may be $1 \leq y \leq 4$. In still another exemplary embodiment, x may be 2, and y may be $1 \leq y \leq 3$. In yet another exemplary embodiment, x may be 3, and y may be $1 \leq y \leq 2$. The corresponding numerical range is determined based on the value listed in Table 3.

[0659] According to the present invention, there is disclosed an R_1'-L_2-SSFI for specifically transferring a first chemical functional group to lysine 246 in an Fc domain of an antibody.

[0660] According to one aspect of the present invention, the present invention provides an agent for transferring a first chemical functional group to an antibody, characterized in that a distance (L_c) between the beta carbon of $(Xa_1)'$ and the first carbonyl carbon is longer than $D_{248,max}$ (approximately 16.208 Å). For example, L_c may have a value of approximately 16.5 Å, approximately 17 Å, approximately 18 Å, approximately 19 Å, approximately 20 Å, or approximately 20.5 Å.

[0661] In specific embodiments, when the R_1'-L_2-SSFI has any one structure selected from Formulas 5, 5-1 to 5-3, and 6-1 to 6-3, D_3 is a C_x alkylene, X_3 is N, D_2 is a C_y alkylene, a C_y alkenylene, or a C_y alkynylene, X_1 is S, wherein y may be an integer greater than or equal to 1, and the sum of x and y may be greater than or equal to 9. Also, the sum of x and y may be 9, 10, 11, or 12. For example, x may be 0, and y may be $9 \leq y \leq 12$. In another exemplary embodiment, x may be 1, and y may be $8 \leq y \leq 11$. In still another exemplary embodiment, x may be 2, and y may be $7 \leq y \leq 10$. In yet another exemplary embodiment, x may be 3, and y may be $6 \leq y \leq 9$. Optionally, D_2 may be an alkynylene. When D_2 is an alkynylene, the side chain of (Xa_1)' may become stereoscopically rigid, thereby preventing bending of the side chain.

[0662] According to the present invention, there is disclosed an R_1'-L_2-SSFI for selectively transferring a first chemical functional group to lysine 246 or 248 in an Fc domain of an antibody.

[0663] According to one aspect of the present invention, the present invention provides an agent for transferring a first chemical functional group to an antibody, characterized in that a distance (L_c) between the beta carbon of (Xa_1)' and the first carbonyl carbon has a value longer than or equal to $D_{246,min}$ (approximately 11.668 Å) and shorter than or equal to $D_{248,max}$ (approximately 16.208 Å). In this case, L_c may have a value of approximately 11.668 Å, approximately 12 Å, approximately 13 Å, approximately 14 Å, approximately 15 Å, approximately 15.5 Å, approximately 16 Å, or approximately 16.208 Å.

[0664] In specific embodiments, when the R_1'-L_2-SSFI has any one structure selected from Formulas 5, 5-1 to 5-3, and 6-1 to 6-3, D_3 is a C_x alkylene, X_3 is N, D_2 is a C_y alkylene, a C_y alkenylene, or a C_y alkynylene, X_1 is S, wherein y may be an integer greater than or equal to 1, and the sum of x and y may be $6 \leq x + y \leq 8$. In this case, the sum of x and y may be 6, 7, or 8. For example, x may be 0, and y may be $6 \leq y \leq 8$. In another exemplary embodiment, x may be 1, and y may be $5 \leq y \leq 7$. In still another exemplary embodiment, x may be 2, and y may be $4 \leq y \leq 6$. In yet another exemplary embodiment, x may be 3, and y may be $3 \leq y \leq 5$.

[0665] According to the present invention, there is disclosed a method of preparing an R_1'-L_2-SSFI for specifically transferring a first chemical functional group to lysine 248 in an Fc domain of an antibody.

[0666] As one example of the method of preparing an agent for transferring first chemical functional group to an antibody as described in the section 4.2, the present invention provides a method of preparing an R_1'-L_2-SSAI, which is characterized by including:

[0667] reacting a linker according to the present invention with a site-specific antibody interactome according to the present invention,

[0668] wherein D_2 in the linker is a C_y alkylene, a C_y alkenylene, or a C_y alkynylene, and

[0669] D_3 in the site-specific antibody interactome is a C_x alkylene,

[0670] wherein y is an integer greater than or equal to 1, and the sum of x and y is $1 \leq x + y \leq 5$. Also, the R_1'-L_2-SSAI prepared by the method may react with an antibody to specifically transfer a first chemical functional group to lysine 248 in an Fc domain of the antibody.

[0671] According to the present invention, there is disclosed a kit for preparing an R_1'-L_2-SSFI for specifically transferring a first chemical functional group to lysine 248 in an Fc domain of an antibody.

[0672] As one example of the method of preparing an agent for transferring first chemical functional group to an antibody as described in the section 4.2, the present invention provides a kit for preparing an R_1'-L_2-SSAI, which is characterized by including:

[0673] a linker according to the present invention; and

[0674] a site-specific antibody interactome according to the present invention,

[0675] wherein D_2 in the linker is a C_y alkylene, a C_y alkenylene, or a C_y alkynylene, and

[0676] D_3 in the site-specific antibody interactome is a C_x alkylene,

[0677] wherein y is an integer greater than or equal to 1, and the sum of x and y is $1 \leq x + y \leq 5$.

[0678] According to the present invention, there is disclosed a method of preparing an R_1'-L_2-SSFI for specifically transferring a first chemical functional group to lysine 246 in an Fc domain of an antibody.

[0679] As one example of the method of preparing an agent for transferring first chemical functional group to an antibody as described in the section 4.2, the present invention provides a method of preparing an R_1'-L_2-SSAI, which is characterized by including:

[0680] reacting a linker according to the present invention with a site-specific antibody interactome according to the present invention,

[0681] wherein D_2 in the linker is a C_y alkylene, a C_y alkenylene, or a C_y alkynylene, and

[0682] D_3 in the site-specific antibody interactome is a C_x alkylene,

[0683] wherein y is an integer greater than or equal to 1, and the sum of x and y is $9 \leq x + y \leq 12$. Also, the R_1'-L_2-SSAI prepared by the method may react with an antibody to specifically transfer a first chemical functional group to lysine 246 in an Fc domain of the antibody.

[0684] According to the present invention, there is disclosed a kit for preparing an R_1'-L_2-SSFI for specifically transferring a first chemical functional group to lysine 246 in an Fc domain of an antibody.

[0685] As one example of the method of preparing an agent for transferring first chemical

functional group to an antibody as described in the section 4.2, the present invention provides a kit for preparing an R_1'-L_2-SSAI, which is characterized by including:

[0686] a linker according to the present invention; and

[0687] a site-specific antibody interactome according to the present invention,

[0688] wherein D_2 in the linker is a C_y alkylene, a C_y alkenylene, or a C_y alkynylene, and

[0689] D_3 in the site-specific antibody interactome is a C_x alkylene,

[0690] wherein y is an integer greater than or equal to 1, and the sum of x and y is $9 \leq x + y \leq 12$.

[0691] According to the present invention, there is disclosed a method of preparing an R_1'-L_2-SSFI for selectively transferring a first chemical functional group to lysine 246 or 248 in an Fc domain of an antibody.

[0692] As one example of the method of preparing an agent for transferring first chemical functional group to an antibody as described in the section 4.2, the present invention provides a method of preparing an R_1'-L_2-SSAI, which is characterized by including:

[0693] reacting a linker according to the present invention with a site-specific antibody interactome according to the present invention,

[0694] wherein D_2 in the linker is a C_y alkylene, a C_y alkenylene, or a C_y alkynylene, and

[0695] D_3 in the site-specific antibody interactome is a C_x alkylene,

[0696] wherein y is an integer greater than or equal to 1, and the sum of x and y is $6 \leq x + y \leq 8$. Also, the R_1'-L_2-SSAI prepared by the method may react with an antibody to selectively transfer a first chemical functional group to lysine 246 or 248 in an Fc domain of the antibody.

[0697] According to the present invention, there is disclosed a kit for preparing an R_1'-L_2-SSFI for selectively transferring a first chemical functional group to lysine 246 or 248 in an Fc domain of an antibody.

[0698] As one example of the method of preparing an agent for transferring first chemical functional group to an antibody as described in the section 4.2, the present invention provides a kit for preparing an R_1'-L_2-SSAI, which is characterized by including:

[0699] a linker according to the present invention; and

[0700] a site-specific antibody interactome according to the present invention,

[0701] wherein D_2 in the linker is a C_y alkylene, a C_y alkenylene, or a C_y alkynylene, and

[0702] D_3 in the site-specific antibody interactome is a C_x alkylene,

[0703] wherein y is an integer greater than or equal to 1, and the sum of x and y is $6 \leq x + y \leq 8$.

[0704] According to the present invention, there is disclosed a method of preparing an antibody having a first chemical functional group specifically transferred to lysine 248 in an Fc domain thereof.

[0705] As one example of the method of preparing an antibody containing a first chemical functional group as described in the section 5.2, the present invention provides a method of preparing an R_1'-Ab, which is characterized by including:

[0706] reacting an agent for transferring a first chemical functional group to an antibody according to the present invention with an antibody or a fragment thereof,

[0707] wherein, in the agent for transferring a first chemical functional group to an antibody, a distance (L_c) between the beta carbon of (Xa_1)' and the first carbonyl carbon is shorter than approximately 11.668 Å.

[0708] In specific embodiments, in the agent for transferring a first chemical functional group to an antibody, D_3 is a C_x alkylene, and D_2 is a C_y alkylene, a C_y alkenylene, or a C_y alkynylene,

[0709] wherein y may be an integer greater than or equal to 1, and the sum of x and y may be $1 \leq x + y \leq 5$.

[0710] According to the present invention, there is disclosed a kit for preparing an antibody having a first chemical functional group specifically transferred to lysine 248 in an Fc domain thereof.

[0711] As one example of the method of preparing an antibody containing a first chemical functional group as described in the section 5.2, the present invention provides a kit for preparing an R_1'-Ab, which is characterized by including:

[0712] an agent for transferring a first chemical functional group to an antibody according to the present invention; and

[0713] an antibody or a fragment thereof,

[0714] wherein, in the agent for transferring a first chemical functional group to an antibody, a distance (L_c) between the beta carbon of (Xa_1)' and the first carbonyl carbon is shorter than approximately 11.668 Å.

[0715] In specific embodiments, in the agent for transferring a first chemical functional group to an antibody, D_3 is a C_x alkylene, and D_2 is a C_y alkylene, a C_y alkenylene, or a C_y alkynylene,

[0716] wherein y may be an integer greater than or equal to 1, and the sum of x and y may be $1 \leq x + y \leq 5$.

[0717] Optionally, as one example of the method of preparing an antibody containing a first chemical functional group as described in the section 5.2, the present invention provides a kit for preparing an R_1'-Ab, which is characterized by including:

[0718] a linker according to the present invention;

[0719] a site-specific antibody interactome according to the present invention; and

[0720] an antibody or a fragment thereof,

[0721] wherein D_2 in the linker is a C_y alkylene, a C_y alkenylene, or a C_y alkynylene, and

[0722] D_3 in the site-specific antibody interactome is a C_x alkylene,

[0723] wherein y is an integer greater than or equal to 1, and the sum of x and y is $1 \leq x + y \leq 5$.

[0724] According to the present invention, there is disclosed a method of preparing an antibody having a first chemical functional group specifically transferred to lysine 246 in an Fc domain thereof.

[0725] As one example of the method of preparing an antibody containing a first chemical functional group as described in the section 5.2, the present invention provides a method of preparing an R_1'-Ab, which is characterized by including:

[0726] allowing an antibody or a fragment thereof to react with an agent for transferring a first chemical functional group to an antibody according to the present invention,

[0727] wherein, in the agent for transferring a first chemical functional group to an antibody, a distance (L_c) between the beta carbon of (Xa_1)' and the first carbonyl carbon is longer than approximately 16.208 Å.

[0728] In specific embodiments, in the agent for transferring a first chemical functional group to an antibody, D_3 is a C_x alkylene, and D_2 is a C_y alkylene, a C_y alkenylene, or a C_y alkynylene,

[0729] wherein y may be an integer greater than or equal to 1, and the sum of x and y may be $9 \leq x + y \leq 12$.

[0730] According to the present invention, there is disclosed a kit for preparing an antibody having a first chemical functional group specifically transferred to lysine 246 in an Fc domain thereof.

[0731] As one example of the method of preparing an antibody containing a first chemical functional group as described in the section 5.2, the present invention provides a kit for preparing an R_1'-Ab, which is characterized by including:

[0732] an agent for transferring a first chemical functional group to an antibody according

to the present invention; and

[0733] an antibody or a fragment thereof,

[0734] wherein, in the agent for transferring a first chemical functional group to an antibody, a distance (L_c) between the beta carbon of (Xa_1)' and the first carbonyl carbon is longer than approximately 16.208 Å.

[0735] In specific embodiments, in the agent for transferring a first chemical functional group to an antibody, D_3 is a C_x alkylene, and D_2 is a C_y alkylene, a C_y alkenylene, or a C_y alkynylene,

[0736] wherein y may be an integer greater than or equal to 1, and the sum of x and y may be $9 \leq x + y \leq 12$.

[0737] Optionally, as one example of the method of preparing an antibody containing a first chemical functional group as described in the section 5.2, the present invention provides a kit for preparing an R_1'-Ab, which is characterized by including:

[0738] a linker according to the present invention;

[0739] a site-specific antibody interactome according to the present invention; and

[0740] an antibody or a fragment thereof,

[0741] wherein D_2 in the linker is a C_y alkylene, a C_y alkenylene, or a C_y alkynylene, and

[0742] D_3 in the site-specific antibody interactome is a C_x alkylene,

[0743] wherein y is an integer greater than or equal to 1, and the sum of x and y is $9 \leq x + y \leq 12$.

[0744] According to the present invention, there is disclosed a method of preparing an antibody having a first chemical functional group selectively transferred to lysine 246 or 248 in an Fc domain thereof.

[0745] As one example of the method of preparing an antibody containing a first chemical functional group as described in the section 5.2, the present invention provides a method of preparing an R_1'-Ab, which is characterized by including:

[0746] reacting an agent for transferring a first chemical functional group to an antibody according to the present invention with an antibody or a fragment thereof,

[0747] wherein, in the agent for transferring a first chemical functional group to an antibody, a distance (L_c) between the beta carbon of (Xa_1)' and the first carbonyl carbon is longer than or equal to approximately 11.668 Å and shorter than or equal to approximately 16.208 Å.

[0748] In specific embodiments, in the agent for transferring a first chemical functional group to an antibody, D_3 is a C_x alkylene, and D_2 is a C_y alkylene, a C_y alkenylene, or a C_y

alkynylene,

[0749] wherein y may be an integer greater than or equal to 1, and the sum of x and y may be $6 \leq x + y \leq 8$.

[0750] According to the present invention, there is disclosed a kit for preparing an antibody having a first chemical functional group selectively transferred to lysine 246 or 248 in an Fc domain thereof.

[0751] As one example of the method of preparing an antibody containing a first chemical functional group as described in the section 5.2, the present invention provides a kit for preparing an R_1'-Ab, which is characterized by including:

[0752] an agent for transferring a first chemical functional group to an antibody according to the present invention; and

[0753] an antibody or a fragment thereof,

[0754] wherein, in the agent for transferring a first chemical functional group to an antibody, a distance (L_c) between the beta carbon of (Xa_1)' and the first carbonyl carbon is longer than or equal to approximately 11.668 Å and shorter than or equal to approximately 16.208 Å.

[0755] In specific embodiments, in the agent for transferring a first chemical functional group to an antibody, D_3 is a C_x alkylene, and D_2 is a C_y alkylene, a C_y alkenylene, or a C_y alkynylene,

[0756] wherein y may be an integer greater than or equal to 1, and the sum of x and y may be $6 \leq x + y \leq 8$.

[0757] Optionally, as one example of the method of preparing an antibody containing a first chemical functional group as described in the section 5.2, the present invention provides a kit for preparing an R_1'-Ab, which is characterized by including:

[0758] a linker according to the present invention;

[0759] a site-specific antibody interactome according to the present invention; and

[0760] an antibody or a fragment thereof,

[0761] wherein D_2 in the linker is a C_y alkylene, a C_y alkenylene, or a C_y alkynylene, and

[0762] D_3 in the site-specific antibody interactome is a C_x alkylene,

[0763] wherein y is an integer greater than or equal to 1, and the sum of x and y is $6 \leq x + y \leq 8$.

[0764] According to the present invention, there is disclosed a method of preparing an antibody having a first chemical functional group transferred to both lysine 246 and 248 in an Fc domain thereof.

[0765] The present invention provides a method of preparing an R_1'-Ab, which includes:

[0766] reacting a first agent for transferring a first chemical functional group to an antibody with an antibody or a fragment thereof; and

[0767] reacting a second agent for transferring a first chemical functional group to an antibody with the antibody or fragment thereof.

[0768] In specific embodiments, the first agent for transferring a first chemical functional group to an antibody may be an R_1'-L_2-SSFI for specifically transferring a first chemical functional group to lysine 248 in the Fc domain as described above, and the second agent for transferring a first chemical functional group to an antibody may be an R_1'-L_2-SSFI for specifically transferring a first chemical functional group to lysine 246 in the Fc domain as described above.

[0769] In specific embodiments, the first agent for transferring a first chemical functional group to an antibody may be an R_1'-L_2-SSFI for specifically transferring a first chemical functional group to lysine 246 in the Fc domain as described above, and the second agent for transferring a first chemical functional group to an antibody may be an R_1'-L_2-SSFI for specifically transferring a first chemical functional group to lysine 248 in the Fc domain as described above.

[0770] In specific embodiments, the reacting of the first agent for transferring a first chemical functional group to an antibody with the antibody or fragment thereof; and the reacting of the second agent for transferring a first chemical functional group to an antibody with the antibody or fragment thereof may be sequentially performed.

[0771] According to the present invention, there is disclosed a kit for preparing an antibody having a first chemical functional group transferred to both lysine 246 and 248 in an Fc domain thereof.

[0772] The present invention provides a kit for preparing an R_1'-Ab, which includes:

[0773] a first agent for transferring a first chemical functional group to an antibody;

[0774] a second agent for transferring a first chemical functional group to an antibody; and

[0775] an antibody or a fragment thereof.

[0776] In specific embodiments, the first agent for transferring a first chemical functional group to an antibody may be an R_1'-L_2-SSFI for specifically transferring a first chemical functional group to lysine 248 in the Fc domain as described above, and the second agent for transferring a first chemical functional group to an antibody may be an R_1'-L_2-SSFI for specifically transferring a first chemical functional group to lysine 246 in the Fc domain as described above.

[0777] In specific embodiments, the first agent for transferring a first chemical functional

group to an antibody may be an R_1'-L_2-SSFI for specifically transferring a first chemical functional group to lysine 246 in the Fc domain as described above, and the second agent for transferring a first chemical functional group to an antibody may be an R_1'-L_2-SSFI for specifically transferring a first chemical functional group to lysine 248 in the Fc domain as described above.

[0778] 5.5. It is possible to transfer first chemical functional group more site-specifically by modulating the reactivity of the first carbonyl group.

[0779] As described above in the section 5.2, an antibody containing a first chemical functional group may be prepared by a nucleophile of the antibody attacking a first carbonyl group of the R_1'-L_2-SSFI. The first carbonyl group is characterized by having milder reactivity than the second carbonyl group of the linker (see section 2.2). The reactivity of the first carbonyl group may be modulated so that a certain amine group of the antibody can react with the first carbonyl group only when the certain amine group is in the vicinity of the first carbonyl group. In this way, the present invention allows the first carbonyl group not to react well with any amine group through the vicinity conditions for reaction, and enables high positional specificity of the reaction.

[0780] The prior art disclosed in Publication Nos. US 2018/0141976 A1 and WO 2018/199337 A1 aims to site-selectively modulate lysine 246 or 248 in an Fc domain using an analogue of Fc-III. Because the prior art uses a disuccinimidyl cross-linker, the first carbonyl group and the second carbonyl group are equally highly reactive (see section 2.2). Therefore, because the first carbonyl group has high reactivity and has no vicinity conditions for reaction, any lysine residue is highly likely to be labeled.

[0781] 5.6. The antibody containing a first chemical functional group prepared according to the present invention has high uniformity and yield.

[0782] The technical problem and the technical solution to specifically transfer (1) “a desired number” of first chemical functional groups to (2) “certain sites of an antibody” have been described in detail with reference to the sections 5.3 and 5.4. As can be seen from Experimental Examples below, it can be seen that the chemical functional group of the antibody containing a first chemical functional group prepared by means of the technical solution is transferred to a certain lysine residue in the Fc domain thereof with high specificity and yield (see Experimental Example 3.3). Therefore, the present invention has been completed based on these facts.

[0783] As shown in the background art, the conjugate product having high uniformity due to antibody labeling has advantages in that (1) the functions of the antibody conjugate are uniformly guaranteed, (2) the antibody conjugate is safe due to its predictable effects, and (3) a drop in function of an antibody may be avoided because it is possible to label an antibody while

avoiding a functional region of the antibody.

[0784] The prior art disclosed in Publication Nos. US 2018/0141976 A1 and WO 2018/199337 A1 aims to site-selectively modulate lysine 246 or 248 in an Fc domain using an analogue of Fc-III. However, according to the prior art, the uniformity and yield of the antibody may be inevitably low due to two reasons. According to the prior art, first, because a distance (L_c) between the beta carbon of $(Xa_1)'$ and the first carbonyl carbon is approximately 15 Å, it is possible to selectively label lysine at either position 246 or 248, but it is impossible to specifically select one of the lysine 246 and 248 to be labeled. Second, a first carbonyl group of a cross-linker according to the prior art has no vicinity conditions for reaction due to high reactivity (see section 5.5). As a result, the first carbonyl group is highly likely to react with any lysine residue. Therefore, the prior art has a drawback in that the uniformity and yield of an antibody-labeled product are inferior to those of the present invention.

[0785] 5.7. No functions of the antibody are lowered because the antibody containing a first chemical functional group according to the present invention has no FcRn binding site blocked therein.

[0786] The design principle for the antibody labeling site being (1) spaced apart from the paratope; and (2) spaced apart from the recognition site of FcR including FcRn has been described above in the section 1.1. Because the lysine 246 and 248 are included in the Fc domain, the lysine 246 and 248 are spaced apart from the paratope, but the corresponding site may overlap the recognition site of FcRn, which may be problematic.

[0787] It is known that FcRn has various functions, and particularly plays an important role in extending the half-life of the antibody by participating in IgG recycling. When the antibody is used *in vivo*, that is, when the antibody is used as a therapeutic agent, a contrast medium, and the like, the interaction between FcRn and the antibody may not occur smoothly, thereby making it impossible for the antibody to function normally due to the short half-life of the antibody.

[0788] 5.7.1. Lysine 246 and lysine 248 are spaced apart from the FcRn binding site of the antibody

[0789] The spacing between the lysine residues in the Fc domain and the FcRn binding site of Fc was an important consideration to choose the lysine residue in the Fc domain. FIG. 13 shows a binding structure between an Fc domain and FcRn and positions of the FcRn binding site and lysine 246 and 248 in the Fc domain. Papers and computer modeling were employed to show a binding structure between the Fc domain and FcRn (Ying T, Ju TW, Wang Y, Prabakaran P, Dimitrov DS., Interactions of IgG1 CH2 and CH3 Domains with FcRn; Front Immunol. 2014; 5:146., Monnet C, Jorieux S, Urbain R, *et al.*, Selection of IgG Variants with Increased FcRn

Binding Using Random and Directed Mutagenesis: Impact on Effector Functions. Front Immunol. 2015; 6:39.) (see the left image of FIG. 13). The FcRn binding site and the lysine 246 and 248 disclosed in the same article data are indicated in the Fc domain (see the right image of FIG. 13). Referring to the two images, it can be seen that side chains of the lysine 246 and 248 are directed in a direction opposite the FcRn binding site.

[0790] From these facts, it was contemplated that, when an antibody labeling site is chosen, the antibody labeling site does not affect the interaction between the FcRn and Fc domain. In effect, it was confirmed that the half-life of the prepared antibody is not reduced so that the antibody labeling site does not influence binding of FcRn as intended (see Experimental Example 5).

[0791] 5.8. A process of preparing the antibody containing a first chemical functional group according to the present invention is characterized by SSAI leaving,

[0792] As seen from Schemes 4 and 5 in the section 5.2, a process of preparing the R_1'-Ab according to the present invention is characterized in that SSAI leaves the final product R_1'-Ab because the SSAI is included in a leaving group of a nucleophilic substitution reaction. The binding structure between the Fc domain and the SSFI according to the present invention (see section 3.2) was analyzed in comparison with the binding site between Fc and FcRn. As a result, it was confirmed that a portion of the FcRn binding site is covered with the SSFI (FIG. 14). In this regard, it can be expected that, when the SSFI does not leave during the process of preparing the R_1'-Ab, the SSFI has a negative influence on the physical properties (e.g., half-life, and the like) of the antibody.

[0793] The prior art disclosed in Publication No. US 2018/0141976 A1 aims to site-selectively modulate lysine 246 or 248 in an Fc domain using an analogue of Fc-III. However, the corresponding prior art is different from the present invention in that SSFI is included in the final antibody-labeled product because the SSFI does not leave during the preparation process.

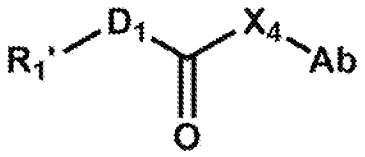
[0794] The prior art disclosed in Publication No. WO 2018/199337 A1 aims to site-selectively modulate lysine 246 or 248 in an Fc domain using an analogue of Fc-III, wherein SSFI is not included in the final antibody-labeled product. However, the corresponding prior art further includes cleaving a "cleavable linker that is a divalent group" in order to remove the SSFI during the preparation process, but the present invention has an advantage in that the SSFI leaves during a conjugation reaction without any additional processes. Also, the cleaving of the cleavable linker that is a divalent group according to the corresponding invention has a drawback in that a disulfide bond forming a structure of the antibody may be cleaved. Because the present invention uses a nucleophilic substitution reaction which occurs easily without any special

conditions, this problem may be dramatically improved.

[0795] 5.9. No side reactions other than a conjugate formation reaction occurs because the antibody containing a first click-chemistry functional group according to the present invention does not contain a highly bioreactive chemical functional group.

[0796] The antibody prepared by the method of preparing an R_1'-Ab according to the present invention has a structure represented by Formula 7:

[0797] [Formula 7]



[0798] When R_1' is transferred to lysine 246 or 248, X_4 may be NH, and D_1 may be any alkylene, alkenylene, or alkynylene. In this case, -NH-(CO)- connected to the antibody is an amide bond that is generally stable *in vivo*. Also, D_1 generally has a structure which is not highly bioreactive. Therefore, it is expected that the R_1'-Ab prepared according to the present invention has high safety because a highly bioreactive structure is not added to molecules other than the labeled molecule (R_1').

[0799] In particular, when the R_1'-Ab is an H_1-Ab having a click-chemistry residue transferred thereto, a highly bioreactive structure is not added to the R_1'-Ab. Therefore, the yield of the click-chemistry reaction may be improved because no secondary reactions other than the click-chemistry reaction occur.

[0800] The prior art disclosed Publication No. WO 2018/199337 A1 aims to site-selectively modulate lysine 246 or 248 in an Fc domain using an analogue of Fc-III and use a biorthogonal functional group. However, the corresponding prior art is different from the present invention in that the antibody product according to the corresponding prior art is allowed to have an additional chemical functional group such as thiol, hydroxy, carboxylic acid, phosphoric acid, amine, and the like during the cleaving of the "cleavable linker that is a divalent group." Such an additional chemical functional group is a bioreactive functional group, and thus may affect the safety of the antibody product.

[0801] 6. Payload (C_m-H_2)

[0802] According to the present invention, there is disclosed a payload. Such a compound is herein indicated by the symbol "C_m-H_2."

[0803] The present invention provides a C_m-H_2 represented by Formula 9:

[0804] [Formula 9]



[0805] wherein C_m is a cargo moiety,

[0806] H_2 is a second click-chemistry functional group.

[0807] In specific embodiments, C_m may include a carrier moiety, a fluorescent moiety, a drug moiety, or a radioactive moiety. Also, R_1' may include a drug moiety. In addition, R_1' may include a VC linker. In other specific embodiments, R_1' may include an antibody or an analogue thereof, which includes a paratope.

[0808] In specific embodiments, when C_m includes a drug moiety, the drug moiety may be an anti-cancer drug. Also, the anti-cancer drug may include one or more selected from DM1, DM3, DM4, Abrin, Ricin A, a *Pseudomonas* exotoxin, a *Cholera* toxin, a *Diphtheria* toxin, a tumor necrosis factor, α interferon, β interferon, a nerve growth factor, a platelet-derived growth factor, a tissue plasminogen activator, a cytokine, an apoptosis-inducing agent, an anti-angiogenic agent, a lymphokine, taxane, a DNA-alkylating agent, anthracyclin, a Tubulysin analogue, a duocarmycin analogue, auristatin E, auristatin F, a maytansinoid, a cytotoxic agent including a reactive polyethylene glycol residue, taxon, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, T. Colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dihydrotestosterone, a glucocorticoid, procaine, tetracaine, lidocaine, propranolol, puromycin, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine, mechlorethamine, thiotepa, chlorambucil, Meiphalan, carmustine, lomustine, cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, cisplatin, dactinomycin, bleomycin, anthramycin, calicheamicin, abiraterone, bendamustine, bortezomib, carboplatin, cabazitaxel, dasatinib, doxetaxel, epirubicin, erlotinib, everolimus, gemcitabine, gefitinib, idarubicin, imatinib, hydroxyurea, lapatinib, leuprorelin, melphalan, nedaplatin, nilotinib, oxaliplatin, pazopanib, pemetrexed, picoplatin, romidepsin, satraplatin, sorafenib, vemurafenib, sunitinib, teniposide, triplatin, and vinorelbine.

[0809] In specific embodiments, C_m may include a plurality of carrier moieties, fluorescent moieties, drug moieties, or radioactive moieties. Also, C_m may include two or more drug moieties. In addition, C_m may include a VC linker.

[0810] In specific embodiments, H_2 may include any one selected from an alkyne, an azide, a strained alkyne, a diene, a dienophile, an alkene, a thiol, and a tetrazine. Furthermore, H_2 may be an azide, or a strained alkyne. Further, H_2 may be an azide, or dibenzocyclooctyne-amine. Additionally, H_2 may be a diene, or a dienophile. Further, H_2 may be a tetrazine, or a

norbornene. Alternatively, H_2 may be a tetrazine, or a trans-cyclooctene.

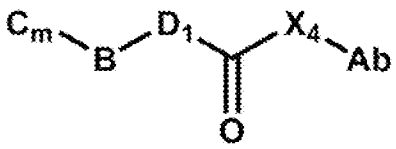
[0811] In specific embodiments, when H_2 is to react with the H_1-Ab according to the present invention, H_2 may be complementary to the first click-chemistry functional group (H_1).

[0812] 7. Antibody-payload conjugate (C_m-Ab)

[0813] According to the present invention, there is disclosed a conjugate of an antibody and a payload (i.e., an antibody-payload conjugate). Such a compound is herein indicated by the symbol "C_m-Ab."

[0814] The present invention provides a C_m-Ab represented by Formula 10:

[0815] [Formula 10]



[0816] wherein C_m is a cargo moiety,

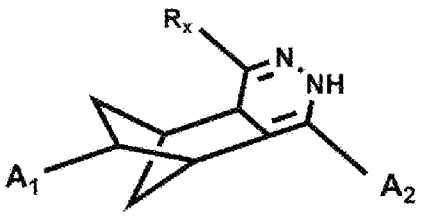
[0817] B is a structure formed by a click-chemistry reaction between a first click-chemistry functional group and a second click-chemistry functional group,

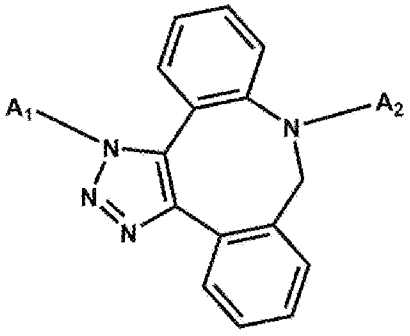
[0818] D_1 is any alkylene, alkenylene, or alkynylene,

[0819] X_4 is NH, O, or S, and

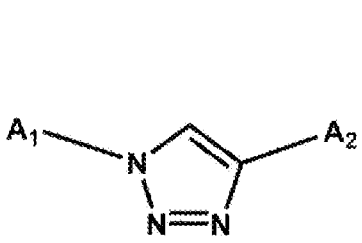
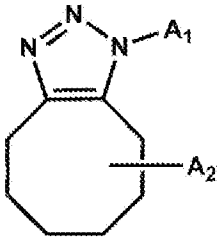
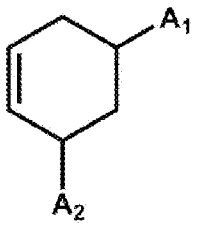
[0820] Ab is an antibody.

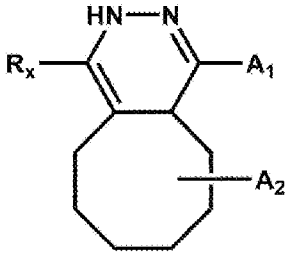
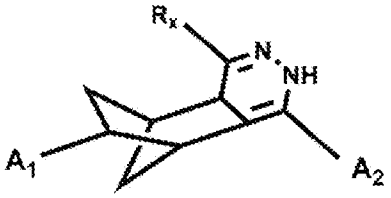
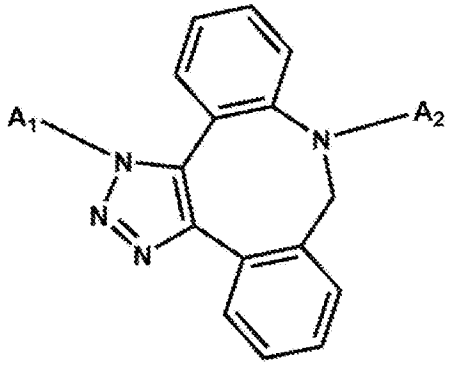
[0821] The contents of the cargo moiety apply to the contents disclosed in the section '6. Payload.'

[0822] In specific embodiments, B may be a structure formed by a click-chemistry reaction between any one selected from an alkyne, an azide, a strained alkyne, a diene, a dienophile, an alkene, a thiol, and a tetrazine and its partner "click-chemistry functional group." Also, B may be any one selected from 

, 

,

, and

, wherein both A_1 and A_2 are connected to the cargo moiety or D_1 so that the A_1 and A_2 cannot be connected to the same moiety, and R_x may be selected from H, a halogen, and a C_{1-3} alkyl. Furthermore, B may be

. Additionally, B may be

.

[0823] In specific embodiments, D_1 may include any one selected from a covalent bond, a C_{1-4} alkylene, a C_{2-4} alkenylene, a C_{2-4} alkynylene, and a C_{3-8} cycloalkylene. Also, D_1 may be $-CH_2OCH_2-$. In addition, D_1 may be a covalent bond.

[0824] In one specific embodiment, X_4 may be NH.

[0825] In specific embodiments, Ab may be a human antibody. In other specific embodiments, Ab may be a non-human animal antibody. In specific embodiments, Ab may be immunoglobulin G (IgG). In specific embodiments, Ab may be a whole antibody. In other specific embodiments, Ab may be a fragment of the antibody.

[0826] In specific embodiments, X_4 and Ab may be connected via an Fab domain of Ab. In other specific embodiments, X_4 and Ab may be connected via an Fc domain of Ab. Also, X_4

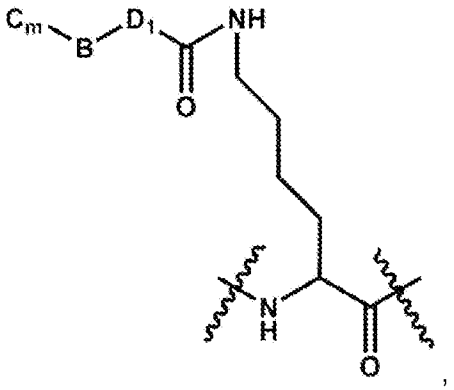
and Ab may be connected via lysine 246 or 248 in the Fc domain of Ab. Furthermore, X_4 and Ab may be connected via lysine 246 in the Fc domain of Ab. Further, X_4 and Ab may be connected via lysine 248 in the Fc domain of Ab. Alternatively, X_4 and Ab may be connected via lysine 246 and 248 in the Fc domain of Ab. In specific embodiments, X_4 and Ab may be connected via only one of two Fc domains of Ab. In other specific embodiments, X_4 and Ab may be connected via both of the two Fc domains of Ab.

[0827] The present invention provides an antibody or a fragment thereof including an amino acid sequence of the following Formula 10-1:

[0828] [Formula 10-1]

[0829] G—P—S—V—F—L—F—P—P-(K)"-P—K—D—T—L—M—I (SEQ ID NO: 23)

[0830] wherein G is glycine, P is proline, S is serine, V is valine, F is phenylalanine, L is leucine, K is lysine, D is aspartic acid, T is threonine, M is methionine, I is isoleucine, and

[0831] (K)" is 

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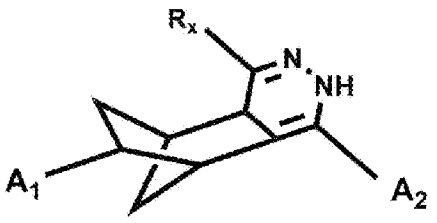
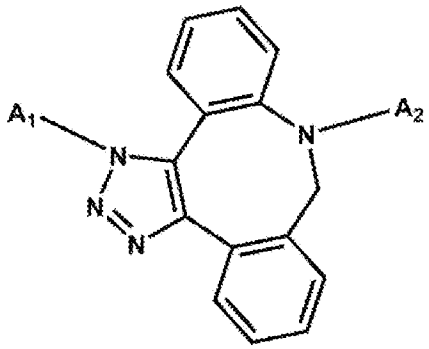
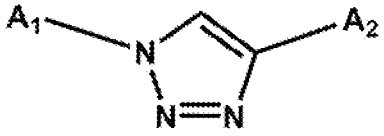
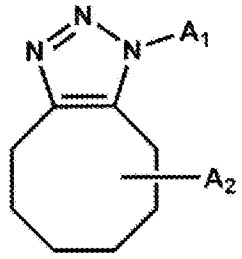
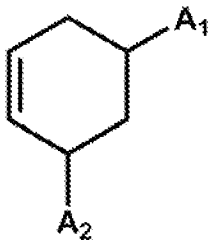
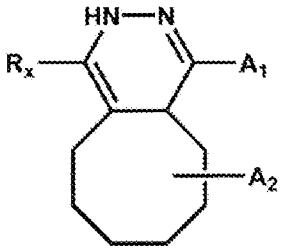
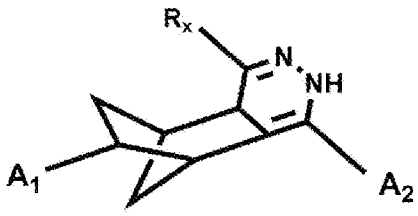
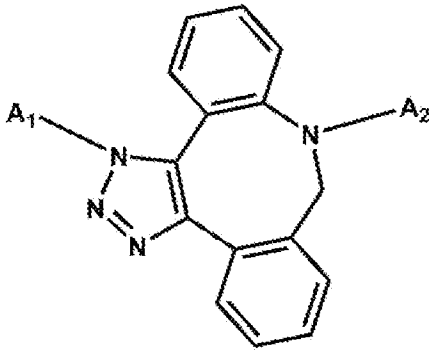
[0832] wherein C_m is a cargo moiety,

[0833] B is a structure formed by a click-chemistry reaction between a first click-chemistry functional group and a second click-chemistry functional group, and

[0834] D_1 is any alkylene, alkenylene, or alkynylene. The antibody or fragment thereof has a cargo moiety conjugated to lysine 246 in an Fc domain thereof, or a site corresponding to the lysine 246.

[0835] The contents of the cargo moiety apply to the contents disclosed in the section '6. Payload.'

[0836] In specific embodiments, B may be a structure formed by a click-chemistry reaction between any one selected from an alkyne, an azide, a strained alkyne, a diene, a dienophile, an alkene, a thiol, and a tetrazine and its partner "click-chemistry functional group." Also, B may be

any one selected from  ,  ,  ,  ,  ,  ,  , and , wherein both A_1 and A_2 are connected to the cargo moiety or D_1 so that the A_1 and A_2 cannot be connected to the same moiety, and R_x may be selected from H, a halogen, and a C_{1-3} alkyl. Furthermore, B may be  . Additionally, B may be  .

[0837] In specific embodiments, D_1 may include any one selected from a covalent bond, a C_{1-4} alkylene, a C_{2-4} alkenylene, a C_{2-4} alkynylene, and a C_{3-8} cycloalkylene. Also, D_1 may be $-CH_2OCH_2-$. In addition, D_1 may be a covalent bond.

[0838] In specific embodiments, the antibody may be a human antibody. In other specific embodiments, the antibody may be a non-human animal antibody. In specific embodiments, the antibody may be immunoglobulin G (IgG). In specific embodiments, the antibody may be a whole antibody. In other specific embodiments, the antibody may be a fragment of the antibody.

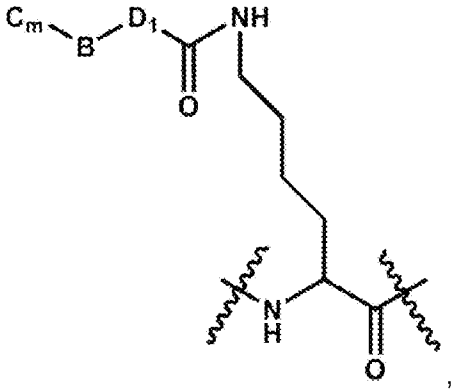
[0839] In specific embodiments, the antibody may include the amino acid sequence of Formula 10-1 in only one of the two Fc domains thereof. In other specific embodiments, the antibody may include the amino acid sequence of Formula 10-1 in both of the two Fc domains thereof.

[0840] The present invention provides an antibody or a fragment thereof including an amino acid sequence of the following Formula 10-2:

[0841] [Formula 10-2]

[0842] G—P—S—V—F—L—F—P—P—K—P-(K)"-D—T—L—M—I (SEQ ID NO: 24)

[0843] wherein G is glycine, P is proline, S is serine, V is valine, F is phenylalanine, L is leucine, K is lysine, D is aspartic acid, T is threonine, M is methionine, I is isoleucine, and

[0844] (K)" is 

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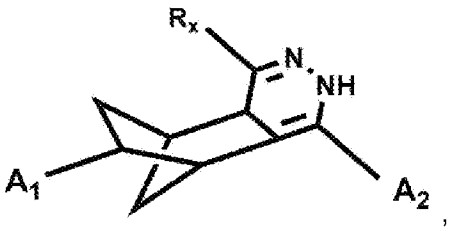
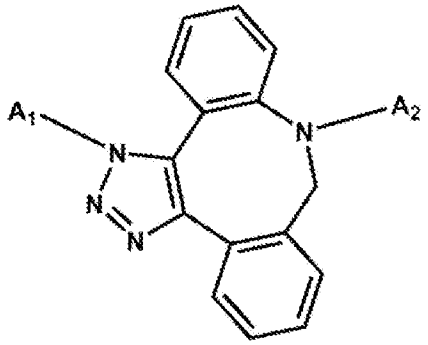
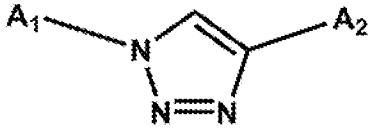
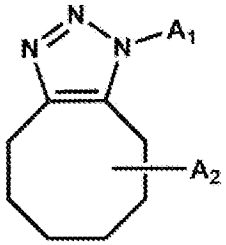
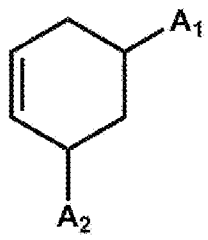
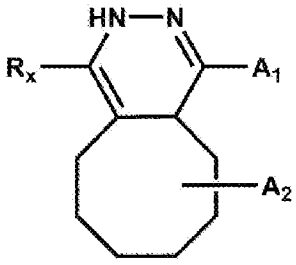
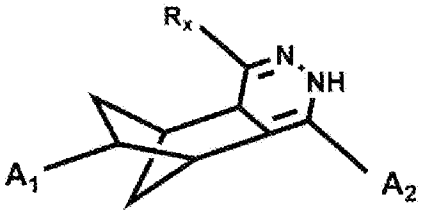
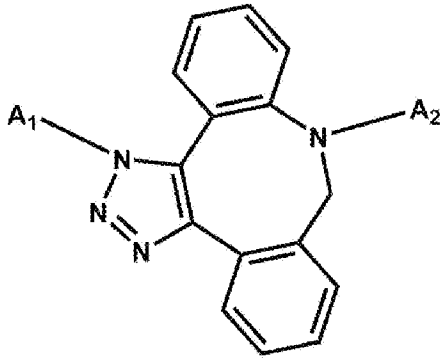
[0845] wherein C_m is a cargo moiety,

[0846] B is a structure formed by a click-chemistry reaction between a first click-chemistry functional group and a second click-chemistry functional group, and

[0847] D_1 is any alkylene, alkenylene, or alkynylene. The antibody or fragment thereof has a cargo moiety conjugated to lysine 246 in an Fc domain thereof, or a site corresponding to the lysine 246.

[0848] The contents of the cargo moiety apply to the contents disclosed in the section '6. Payload.'

[0849] In specific embodiments, B may be a structure formed by a click-chemistry reaction between any one selected from an alkyne, an azide, a strained alkyne, a diene, a dienophile, an

alkene, a thiol, and a tetrazine and its partner "click-chemistry functional group." Also, B may be any one selected from  ,  ,  ,  ,  ,  , and  , wherein both A_1 and A_2 are connected to the cargo moiety or D_1 so that the A_1 and A_2 cannot be connected to the same moiety, and R_x may be selected from H, a halogen, and a $C_{1\text{-}3}$ alkyl. Furthermore, B may be  . Additionally, B may be  .

[0850] In specific embodiments, D_1 may include any one selected from a covalent bond,

a C_{1-4} alkylene, a C_{2-4} alkenylene, a C_{2-4} alkynylene, and a C_{3-8} cycloalkylene. Also, D_1 may be $-CH_2OCH_2-$. In addition, D_1 may be a covalent bond.

[0851] In specific embodiments, the antibody may be a human antibody. In other specific embodiments, the antibody may be a non-human animal antibody. In specific embodiments, the antibody may be immunoglobulin G (IgG). In specific embodiments, the antibody may be a whole antibody. In other specific embodiments, the antibody may be a fragment of the antibody.

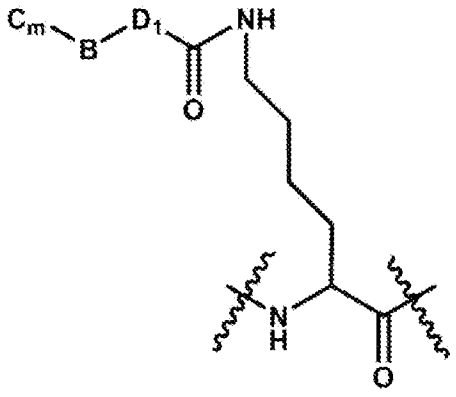
[0852] In specific embodiments, the antibody may include the amino acid sequence of Formula 10-2 in only one of the two Fc domains thereof. In other specific embodiments, the antibody may include the amino acid sequence of Formula 10-2 in both of the two Fc domains thereof.

[0853] The present invention provides an antibody or a fragment thereof including an amino acid sequence of the following Formula 10-3:

[0854] [Formula 10-3]

G—P—S—V—F—L—F—P—P-(K)"-P-(K)"-D—T—L—M—I (SEQ ID NO: 25)

[0855] wherein G is glycine, P is proline, S is serine, V is valine, F is phenylalanine, L is leucine, K is lysine, D is aspartic acid, T is threonine, M is methionine, I is isoleucine, and

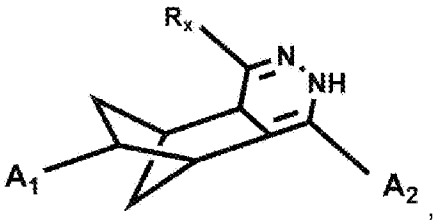
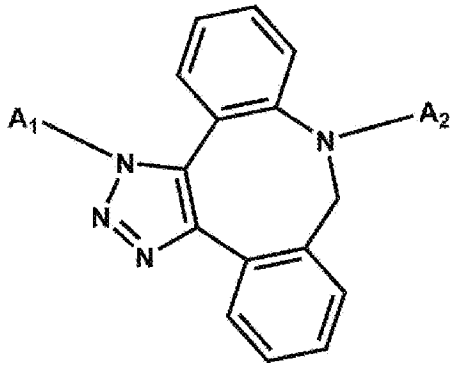
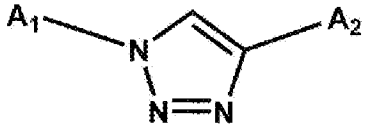
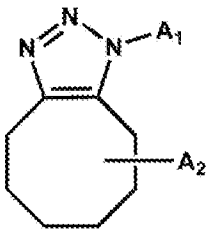
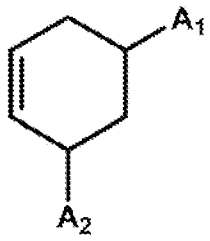
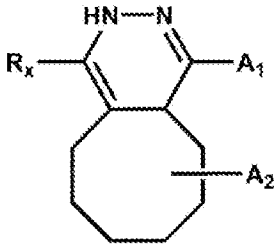
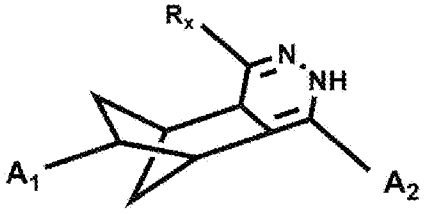
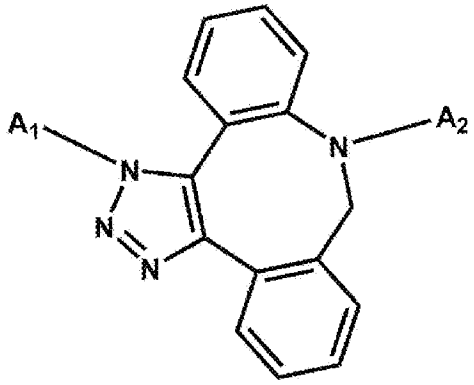
[0856] (K)" is each independently  , wherein C_m is a cargo moiety,

[0857] B is a structure formed by a click-chemistry reaction between a first click-chemistry functional group and a second click-chemistry functional group, and

[0858] D_1 is any alkylene, alkenylene, or alkynylene. The antibody or fragment thereof has a cargo moiety conjugated to lysine 246 in an Fc domain thereof, or a site corresponding to the lysine 246.

[0859] The contents of the cargo moiety apply to the contents disclosed in the section "6. Payload."

[0860] In specific embodiments, B may be a structure formed by a click-chemistry reaction

between any one selected from an alkyne, an azide, a strained alkyne, a diene, a dienophile, an alkene, a thiol, and a tetrazine and its partner "click-chemistry functional group." Also, B may be any one selected from  ,  ,  ,  ,  ,  , and  , wherein both A_1 and A_2 are connected to the cargo moiety or D_1 so that the A_1 and A_2 cannot be connected to the same moiety, and R_x may be selected from H, a halogen, and a $C_{1\text{-}3}$ alkyl. Furthermore, B may be  . Additionally, B may be  .

[0861] In specific embodiments, D_1 may include any one selected from a covalent bond, a C_{1-4} alkylene, a C_{2-4} alkenylene, a C_{2-4} alkynylene, and a C_{3-8} cycloalkylene. Also, D_1 may be $-CH_2OCH_2-$. In addition, D_1 may be a covalent bond.

[0862] In specific embodiments, the antibody may be a human antibody. In other specific embodiments, the antibody may be a non-human animal antibody. In specific embodiments, the antibody may be immunoglobulin G (IgG). In specific embodiments, the antibody may be a whole antibody. In other specific embodiments, the antibody may be a fragment of the antibody.

[0863] In specific embodiments, the antibody may include the amino acid sequence of Formula 10-3 in only one of the two Fc domains thereof. In other specific embodiments, the antibody may include the amino acid sequence of Formula 10-3 in both of the two Fc domains thereof.

[0864] The present invention provides an antibody or a fragment thereof including one or more amino acid sequences selected from Formulas 10-1, 10-2, and 10-3. In this case, the contents of Formulas 10-1 to 10-3 are as described above.

[0865] In specific embodiments, D_1 may be a covalent bond.

[0866] In specific embodiments, the antibody or fragment thereof may include only the amino acid sequence of Formula 10-1 and may not include the amino acid sequences of Formulas 10-2 and 10-3. Also, the antibody or fragment thereof may include the amino acid sequence of Formula 10-1 in only one of the two Fc domains thereof. In addition, the antibody may include the amino acid sequence of Formula 10-1 in both of the two Fc domains thereof.

[0867] In other specific embodiments, the antibody or fragment thereof may include only the amino acid sequence of Formula 10-2 and may not include the amino acid sequences of Formulas 10-1 and 10-3. Also, the antibody or fragment thereof may include the amino acid sequence of Formula 10-2 in only one of the two Fc domains thereof. In addition, the antibody may include the amino acid sequence of Formula 10-2 in both of the two Fc domains thereof.

[0868] In specific embodiments, the antibody or fragment thereof may include only the amino acid sequence of Formula 10-3 and may not include the amino acid sequences of Formulas 10-1 and 10-2. Also, the antibody or fragment thereof may include the amino acid sequence of Formula 10-3 in only one of the two Fc domains thereof. In addition, the antibody may include the amino acid sequence of Formula 10-3 in both of the two Fc domains thereof.

[0869] 7.1. Antibody-drug conjugate (ADC)

[0870] According to the present invention, there is disclosed a novel antibody-drug conjugate (ADC). The ADC according to the present invention means that a payload in the

antibody-payload conjugate includes a drug moiety.

[0871] The present invention provides a C_m-Ab according to the present invention in which a cargo moiety includes a drug moiety.

[0872] In specific embodiments, the cargo moiety may include two or more drug moieties.

[0873] In specific embodiments, the drug moiety may be an anti-cancer drug. Also, the anti-cancer drug may include one or more selected from DM1, DM3, DM4, Abrin, Ricin A, a *Pseudomonas* exotoxin, a *Cholera* toxin, a *Diphtheria* toxin, a tumor necrosis factor, α interferon, β interferon, a nerve growth factor, a platelet-derived growth factor, a tissue plasminogen activator, a cytokine, an apoptosis-inducing agent, an anti-angiogenic agent, a lymphokine, taxane, a DNA-alkylating agent, anthracyclin, a Tubulysin analogue, a duocarmycin analogue, auristatin E, auristatin F, a maytansinoid, a cytotoxic agent including a reactive polyethylene glycol residue, taxon, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, T. Colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dihydrotestosterone, a glucocorticoid, procaine, tetracaine, lidocaine, propranolol, puromycin, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine, mechlorethamine, thiotepa, chlorambucil, Meiphalan, carmustine, lomustine, cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, cisplatin, dactinomycin, bleomycin, anthramycin, calicheamicin, abiraterone, bendamustine, bortezomib, carboplatin, cabazitaxel, dasatinib, doxetaxel, epirubicin, erlotinib, everolimus, gemcitabine, gefitinib, idarubicin, imatinib, hydroxyurea, lapatinib, leuprorelin, melphalan, nedaplatin, nilotinib, oxaliplatin, pazopanib, pemetrexed, picoplatin, romidepsin, satraplatin, sorafenib, vemurafenib, sunitinib, teniposide, triplatin, and vinorelbine.

[0874] In specific embodiments, the cargo moiety may include a VC linker.

[0875] 7.2. Method of preparing antibody-payload conjugate

[0876] According to the present invention, there is disclosed a method of preparing an antibody-payload conjugate.

[0877] The antibody-payload conjugate of the present invention may be prepared by allowing a C_m-H_2 to react with an H_1-Ab. In this case, the contents of the H_1-Ab apply to the contents disclosed in the section 5.1, and the contents disclosed in the section 6 apply for the C_m-H_2. When the first click-chemistry functional group of H_1-Ab and the second click-chemistry functional group of C_m-H_2 are complementary to each other, that is, when the first click-chemistry functional group of H_1-Ab and the second click-chemistry functional group of C_m-H_2 function as partner click-chemistry functional groups, a click-chemistry reaction may occur to prepare an antibody-payload conjugate according to the present invention. The contents of the click-

chemistry reaction were sufficiently described above in the section "Definition." The click-chemistry reaction is a biorthogonal reaction, and has an advantage in that the reaction occurs at a very rapid reaction rate and is used to form a strong binding structure. Therefore, the click-chemistry reaction has the same advantages as in the following sections 7.3 and 7.4.

[0878] The present invention provides a method of preparing an antibody-payload conjugate, characterized in that the method includes reacting an antibody containing a first click-chemistry functional group according to the present invention with a payload according to the present invention, wherein H_2 of the payload is a second click-chemistry functional group complementary to the first click-chemistry functional group.

[0879] In this case, the antibody provided in the section 5.1 applies to the antibody containing the first click-chemistry functional group, and the payload provided in the section 6 applies to the payload. Because all the antibodies containing a first click-chemistry functional group in the lysine 246 and/or the lysine 248 are disclosed in the section 5.1, it is desirable that the antibody-payload conjugates in which a cargo moiety is conjugated to the lysine 246 and/or the lysine 248 are disclosed to a fully reproducible extent (see Formulas 10 and 10-1 to 10-3 in the section 7).

[0880] The present invention provides a kit for preparing an antibody-payload conjugate, characterized in that the kit includes an antibody containing a first click-chemistry functional group according to the present invention; and a payload according to the present invention, wherein H_2 of the payload is a second click-chemistry functional group complementary to the first click-chemistry functional group.

[0881] In this case, the antibody provided in the section 5.1 applies to the antibody containing the first click-chemistry functional group, and the payload provided in the section 6 applies to the payload.

[0882] Optionally, the present invention provides a kit for preparing an antibody-payload conjugate, which includes:

[0883] an antibody;

[0884] an agent for transferring a first click-chemistry functional group to an antibody according to the present invention;

[0885] a payload according to the present invention.

[0886] In specific embodiments, the agent for transferring a first click-chemistry functional group to an antibody may be an H_1-L_2-SSFI for specifically transferring a first click-chemistry functional group to lysine 246 or 248 in an Fc domain of an antibody according to the present

invention. In other specific embodiments, the agent for transferring a first click-chemistry functional group to an antibody may be an H_1-L_2-SSFI for specifically transferring a first click-chemistry functional group to lysine 246 in an Fc domain of an antibody according to the present invention. In still another specific embodiment, the agent for transferring a first click-chemistry functional group to an antibody may be an H_1-L_2-SSFI for specifically transferring a first click-chemistry functional group to lysine 248 in an Fc domain of an antibody according to the present invention.

[0887] Optionally, the present invention provides a kit for preparing an antibody-payload conjugate, which includes:

[0888] an antibody;

[0889] a linker according to the present invention;

[0890] a site-specific antibody interactome according to the present invention; and

[0891] a payload according to the present invention.

[0892] In specific embodiments, D_2 in the linker is a C_y alkylene, a C_y alkenylene, or a C_y alkynylene, and D_3 in the site-specific antibody interactome is a C_x alkylene, wherein y may be an integer greater than or equal to 1. Also, the sum of x and y may be $1 \leq x + y \leq 5$. In addition, the sum of x and y may be $6 \leq x + y \leq 8$. Further, the sum of x and y may be $9 \leq x + y \leq 12$.

[0893] 7.3. The antibody-payload conjugate according to the present invention uses a biorthogonal reaction to form a stable bond.

[0894] The antibody-payload conjugate according to the present invention is formed through a click-chemistry reaction. The click-chemistry reaction is a biorthogonal reaction that does not influence biochemical phenomena naturally occurring *in vivo*. Also, a bond formed by the biorthogonal reaction is not recognized by *in vivo* lyases. Therefore, the antibody-payload conjugate according to the present invention has an advantage in that the antibody and the cargo moiety form a very stable bond *in vivo*.

[0895] 7.4. The antibody-payload conjugate prepared according to the present invention has high uniformity and yield.

[0896] As described above in the section 5.6, the antibody containing a first click-chemistry functional group according to the present invention has high uniformity and yield. Therefore, the antibody-payload conjugate prepared from the antibody according to the present invention has an advantage in that it also has high uniformity and yield. The uniformity of C_m-Ab has to be high in order that the antibody conjugate has uniform performance.

[0897] In the case of the antibody conjugates prepared by the prior art disclosed in

Publication Nos. US 2018/0141976 A1 and WO 2018/199337 A1, since the antibody conjugates have poor yield and uniformity, as described above in the section 5.6, there may be a negative effect on the uniformity of performance.

[0898] 7.5. No functions of the antibody are lowered because the antibody-payload conjugate according to the present invention has no FcRn binding site blocked therein.

[0899] The C_m-Ab according to present invention has the same advantages as described in the sections 5.7 and 5.8. In particular, pharmacokinetic (PK) characteristics of the ADC used *in vivo* may be greatly improved because the ADC has an increased half-life.

[0900] An FcRn binding site of the ADC prepared by the prior art disclosed in Publication No. US 2018/0141976 A1 is blocked because SSFI is included in the final antibody-labeled product. Therefore, the ADC according to the present invention has superior PK characteristics, compared to the ADC according to the corresponding prior art.

[0901] 7.6. The antibody-drug conjugate according to the present invention is stable because the antibody-drug conjugate does not contain a highly bioreactive chemical functional group.

[0902] The C_m-Ab according to the present invention has the same advantages as described in the section 5.9. In particular, pharmacodynamic (PD) characteristics of the ADC used *in vivo* may be greatly improved because the ADC enables a smooth antibody-drug action.

[0903] In the case of the ADC prepared by the prior art disclosed in Publication No. WO 2018/199337 A1, an additional chemical functional group such as thiol, hydroxy, a carboxylic acid, phosphoric acid, an amine, and the like are included in the final antibody-labeled product. Therefore, the ADC according to the present invention has superior PD characteristics, compared to the ADC according to the corresponding prior art.

[0904] 8. Composition

[0905] According to the present invention, there is disclosed a composition including an antibody. In this case, the antibody may be an antibody containing a first chemical functional group according to the present invention. Alternatively, the antibody may be an antibody-payload conjugate according to the present invention. Also, the antibody may be an antibody-drug conjugate according to the present invention.

[0906] The composition according to the present invention may be used for various applications depending on the function of an antibody included in the composition. For example, when a first chemical functional group or a cargo moiety includes a radioactive moiety, the corresponding composition may be used as a radioactive contrast medium, and the like.

Alternatively, when the first chemical functional group or the cargo moiety includes a fluorescent moiety, the corresponding composition may be used as a label used in an enzyme-linked immunosorbent assay (ELISA), and the like. Alternatively, when the first chemical functional group or the cargo moiety includes a drug moiety, the corresponding composition may be used as a pharmaceutical composition. In this case, components of the composition generally used in the related art fall within the scope of the present invention. Also, compositional ratios of the corresponding components generally used in the art also fall within the scope of the present invention.

[0907] The present invention provides a composition including an antibody containing a first chemical functional group according to the present invention. In specific embodiments, the antibody containing the first chemical functional group may be at least one selected from Formulas 7, 7-1 to 7-3, 8, and 8-1 to 8-3.

[0908] Also, the present invention provides a composition including an antibody containing a first click-chemistry functional group according to the present invention. In specific embodiments, the antibody containing the first click-chemistry functional group may be at least one selected from Formulas 8, and 8-1 to 8-3.

[0909] In addition, the present invention provides a composition including an antibody-payload conjugate according to the present invention. In this case, the contents described in the section 7 apply to the contents of the antibody-payload conjugate.

[0910] Optionally, the present invention provides a composition including an antibody-drug conjugate according to the present invention. In this case, the contents described in the section 7.1 apply to the contents of the antibody-drug conjugate.

[0911] Pharmaceutical composition

[0912] The following content relates to the where the composition is a pharmaceutical composition used for diagnostic, prophylactic and/or therapeutic purposes. Only within the content "pharmaceutical composition" here, all the R_1'-Ab, H_1-Ab, C_m-Ab, and ADC according to the present invention are used interchangeably with the term "antibody or fragment thereof."

[0913] The present invention provides a pharmaceutical composition including an antibody or a fragment thereof according to the present invention. Also, the pharmaceutical composition may be a composition for treating cancer. Furthermore, the cancer may include any one selected from bladder cancer, bone cancer, brain cancer, breast cancer, heart cancer, cervical cancer, colon cancer, rectal cancer, esophageal cancer, fibrosarcoma, gastric cancer, stomach cancer, head and neck cancer, Kaposi's sarcoma, renal cancer, leukemia, liver cancer, lung cancer, lymphoma, melanoma, myeloma, ovarian cancer, pancreatic cancer, penile cancer,

prostate cancer, testicular germ cell cancer, thymoma, and thymic carcinoma. Further, the cancer may be breast cancer.

[0914] The present invention provides a therapeutic method which includes administering a pharmaceutical composition including an antibody or a fragment thereof according to the present invention into a subject. Also, the therapeutic method may be a method of treating cancer. Furthermore, the cancer may include any one selected from bladder cancer, bone cancer, brain cancer, breast cancer, heart cancer, cervical cancer, colon cancer, rectal cancer, esophageal cancer, fibrosarcoma, gastric cancer, stomach cancer, head and neck cancer, Kaposi's sarcoma, renal cancer, leukemia, liver cancer, lung cancer, lymphoma, melanoma, myeloma, ovarian cancer, pancreatic cancer, penile cancer, prostate cancer, testicular germ cell cancer, thymoma, and thymic carcinoma. Further, the cancer may be breast cancer.

[0915] To prepare a pharmaceutical or sterilized composition including an antibody or a fragment thereof, the antibody or fragment thereof according to the present invention may be mixed with a pharmaceutically acceptable carrier or excipient. The composition may further include one or more other therapeutic agents which are suitable for treating or preventing cancer (e.g., breast cancer, colorectal cancer, lung cancer, multiple myeloma, ovarian cancer, liver cancer, gastric cancer, pancreatic cancer, acute myelogenous leukemia, chronic myelogenous leukemia, osteosarcoma, squamous cell carcinoma, peripheral nerve sheath tumors, schwannoma, head and neck cancer, bladder cancer, esophageal cancer, Barrett's esophageal cancer, glioblastoma, clear cell sarcoma of soft tissue, malignant mesothelioma, neurofibromatosis, kidney cancer, melanoma, prostate cancer, benign prostate hypertrophy (BPH), gynecomastia, rhabdomyosarcoma, and endometriosis).

[0916] A formulation of therapeutic and diagnostic agents may be prepared by mixing with a physiologically acceptable carrier, an excipient, or a stabilizing agent, for example, in the form of a freeze-dried powder, slurry, an aqueous solution, a lotion, or a suspension (see, for example, Hardman *et al.*, Goodman and Gilman's The Pharmacological Basis of Therapeutics, McGraw-Hill, New York, N.Y., 2001; Gennaro, Remington: The Science and Practice of Pharmacy, Lippincott, Williams, and Wilkins, New York, N.Y., 2000; Avis, *et al*. (eds.), Pharmaceutical Dosage Forms: Parenteral Medications, Marcel Dekker, NY, 1993; Lieberman, *et al*. (eds.), Pharmaceutical Dosage Forms: Tablets, Marcel Dekker, NY, 1990; Lieberman, *et al*. (eds.) Pharmaceutical Dosage Forms: Disperse Systems, Marcel Dekker, NY, 1990; Weiner and Kotkoskie, Excipient Toxicity and Safety, Marcel Dekker, Inc., New York, N.Y., 2000).

[0917] In specific embodiments, the clinical service form (CSF) of the antibody-drug conjugate according to the present invention is a lyophilisate present in a vial containing ADC,

sodium succinate, and polysorbate 20. The lyophilisate may be reconstituted with water for injection, and the solution includes ADC, sodium succinate, sucrose and polysorbate 20 at approximately pH 5.0. For sequential intravenous administration, the resulting solution will be usually further diluted into a carrier solution.

[0918] The choice of a therapeutic dosing regimen depends on various factors including a rate of serum or tissue replacement of a substance, a level of symptoms, the immunogenicity of the substance, and the accessibility of target cells in a biological matrix. In specific embodiments, the dosing regimens maximize an amount of a therapeutic agent to be delivered to a patient so as to satisfy acceptable levels of side effects. Therefore, an amount of a biological agent to be administered depends in part on the certain substance and the severity of the condition being treated. Guidance is available for selection of the appropriate doses of antibodies, cytokines, and small molecules (see, for example, Wawrzynczak, Antibody Therapy, Bios Scientific Pub. Ltd, Oxfordshire, UK, 1996; Kresina (ed.), Monoclonal Antibodies, Cytokines and Arthritis, Marcel Dekker, New York, N.Y., 1991; Bach (ed.), Monoclonal Antibodies and Peptide Therapy in Autoimmune Diseases, Marcel Dekker, New York, N.Y., 1993; Baert *et al.*, New Engl. J. Med. 348:601-608, 2003; Milgrom *et al.*, New Engl. J. Med. 341:1966-1973, 1999; Slamon *et al.*, New Engl. J. Med. 344:783-792, 2001; Beniaminovitz *et al.*, New Engl. J. Med. 342:613-619, 2000; Ghosh *et al.*, New Engl. J. Med. 348:24-32, 2003; Lipsky *et al.*, New Engl. J. Med. 343:1594-1602, 2000).

[0919] The appropriate dose is, for example, determined by a clinical practitioner using parameters or factors known in the related art or suspected to affect treatment, or using parameters or factors expected to affect treatment. In general, the dose begins with an amount somewhat smaller than the optimal dose and then increases in small increments until the desirable or optimal effects are achieved relative to any negative side effects. Important diagnostic measures, for example, include symptoms of inflammation or levels of inflammatory cytokines produced.

[0920] An actual dose level of the active ingredient in the pharmaceutical composition according to the present invention may vary in order to achieve an effective amount of the active ingredient to realize desired therapeutic responses in a certain patient, a composition, and a mode of administration without causing any toxicity in patients. The chosen dose level may be determined depending on various pharmacokinetic factors including the activities of a certain composition of the present invention used, or esters, salts or amides thereof, a route of administration, an administration time, a secretion rate of a certain compound used, a duration of treatment, other drugs, compounds and/or substances used in combination with the certain

compound used, the age, sex, weight, condition, overall health, and previous medical history of a patient to be treated, and other factors known in the medical field.

[0921] The composition including an antibody or a fragment thereof according to the present invention may be given by continuous infusion, or given in a single dose, for example, daily, weekly, 1 to 7 times per week, bi-weekly, once every three weeks, once every four weeks, once every five weeks, once every six weeks, once every seven weeks, or once every eight weeks. The dose may be given intravenously, subcutaneously, topically, orally, intranasally, intrarectally, intramuscularly, intracerebrally, or by inhalation. A certain dosing protocol involves the maximum dose or administration frequency that avoids significant unwanted side effects.

[0922] For the antibody or fragment thereof according to the present invention, the dose to be administered to a patient may be in a range of 0.0001 mg/kg to 100 mg/kg (patient weight). The dose may be in a range of 0.0001 mg/kg to 20 mg/kg, 0.0001 mg/kg to 10 mg/kg, 0.0001 mg/kg to 5 mg/kg, 0.0001 to 2 mg/kg, 0.0001 to 1 mg/kg, 0.0001 mg/kg to 0.75 mg/kg, 0.0001 mg/kg to 0.5 mg/kg, 0.0001 mg/kg to 0.25 mg/kg, 0.0001 to 0.15 mg/kg, 0.0001 to 0.10 mg/kg, 0.001 to 0.5 mg/kg, 0.01 to 0.25 mg/kg, or 0.01 to 0.10 mg/kg (patient weight). The dose of the antibody or fragment thereof according to the present invention may be calculated by multiplying the weight (kilogram (kg)) of a patient by the dose (mg/kg) to be administered.

[0923] The antibody or fragment thereof according to the present invention may be repeatedly administered, and the administration may be performed at intervals of at least a day, two days, three days, five days, 10 days, 15 days, 30 days, 45 days, 2 months, 75 days, 3 months, or at least 6 months. In specific embodiments, the antibody or fragment thereof according to the present invention may be repeatedly administered every three weeks.

[0924] An effective amount to be administered to a certain patient may vary depending on factors such as the condition to be treated, the overall health of a patient, the mode, route and dosage of administration, and the severity of side effects (see, for example, Maynard *et al.*, A Handbook of SOPs for Good Clinical Practice, Interpharm Press, Boca Raton, Fla., 1996; Dent, Good Laboratory and Good Clinical Practice, Urch Publ., London, UK, 2001).

[0925] The route of administration may be, for example, by topical or skin application, by intravenous, intraperitoneal, intracerebral, intramuscular, intraocular, intra-arterial, intramedullary, intralesional injection or infusion, or by a sustained release system or an implant (see, for example, Sidman *et al.*, Biopolymers 22:547-556, 1983; Langer *et al.*, J. Biomed. Mater. Res. 15:167-277, 1981; Langer, Chem. Tech. 12:98-105, 1982; Epstein *et al.*, Proc. Natl. Acad. Sci. USA 82:3688-3692, 1985; Hwang *et al.*, Proc. Natl. Acad. Sci. USA 77:4030-4034, 1980; US Patent Nos. 6,350,466 and 6,316,024). When necessary, the composition may also include a solubilizing

agent and a topical anesthetic (for example, lidocaine) for relieving pain at a site of injection. For example, the composition may also be formulated using an inhaler or a nebulizer, or may be used for pulmonary administration by formulation using an aerosolizing agent. See, for example, US Patent Nos. 6,019,968, 5,985,320, 5,985,309, 5,934,272, 5,874,064, 5,855,913, 5,290,540, and 4,880,078; and PCT Publication Nos. WO 92/19244, WO 97/32572, WO 97/44013, WO 98/31346, and WO 99/66903, the entire contents of which are hereby incorporated by reference.

[0926] Also, the composition of the present invention may be administered through one or more routes of administration using one or more various methods known in the related art. As recognized by a person of ordinary skill in the art, the route and/or mode of administration will depend on the desired results. The route of administration selected for the antibody or fragment thereof according to the present invention includes intravenous, intramuscular, intradermal, intraperitoneal, subcutaneous, intraspinal, or other parenteral routes of administration, for example, routes of administration by injection or infusion. Parenteral administration generally represents a mode of administration by injection other than the enteral and topical administration, and includes intravenous, intramuscular, intra-arterial, intrathecal, intracapsular, intraorbital, intracardial, intradermal, intraperitoneal, transtracheal, subcutaneous, subepidermal, intra-articular, subcapsular, subarachnoid, intraspinal, epidural, and intrasternal injections and infusions, but the present invention is not limited thereto. Alternatively, the composition of the present invention may be administered through a non-parenteral route of administration, for example, a topical, intraepidermal or intramucosal route of administration, and may be, for example, administered intranasally, orally, vaginally, intrarectally, sublingually, or topically. In one exemplary embodiment, the antibody or fragment thereof according to the present invention may be administered by infusion. In another exemplary embodiment, the antibody or fragment thereof according to the present invention may be subcutaneously administered.

[0927] When the antibody or fragment thereof according to the present invention is administered using a controlled or delayed release system, a pump may be used to achieve the controlled or delayed release (see Langer, supra; Sefton, CRC Crit. Ref Biomed. Eng. 14:20, 1987; Buchwald *et al.*, Surgery 88:507, 1980; Saudek *et al.*, N. Engl. J. Med. 321:574, 1989). A polymeric material may be used to achieve the controlled or delayed release in the therapy of the present invention (see, for example, Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Fla., 1974; Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York, 1984; Ranger and Peppas, J. Macromol. Sci. Rev. Macromol. Chem. 23:61, 1983; also see Levy *et al.*, Science 228:190, 1985; During *et al.*, Ann. Neurol. 25:351, 1989; Howard *et al.*, J. Neurosurg. 7 1:105, 1989; US Patent

No. 5,679,377; US Patent No. 5,916,597; US Patent No. 5,912,015; US Patent No. 5,989,463; US Patent No. 5,128,326; PCT Publication No. WO 99/15154; and PCT Publication No. WO 99/20253). Examples of polymers used for a sustained release formulation include poly(2-hydroxyethyl methacrylate), poly(methyl methacrylate), poly(acrylic acid), poly(ethylene-co-vinyl acetate), poly(methacrylic acid), polyglycolide (PLG), a polyanhydride, poly(N-vinyl pyrrolidone), poly(vinyl alcohol), polyacrylamide, poly(ethylene glycol), polylactide (PLA), poly(lactide-co-glycolide) (PLGA), and a polyorthoester, but the present invention is not limited thereto. In one exemplary embodiment, the polymer used for a sustained release formulation is inert, has no filterable impurities, and is stable upon storage, sterilization, and biodegradability. The controlled or delayed release system may be located adjacent to a prophylactic or therapeutic target, and thus requires a portion of the systemic dose (see, for example, Goodson, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138, 1984).

[0928] The controlled release system is discussed in the review of the article "Langer, Science 249:1527-1533, 1990." Any prior art known to a person of ordinary skill in the art may be used to produce a sustained release formulation including one or more antibodies or a fragments thereof according to the present invention. See, for example, US Patent No. 4,526,938, PCT Publication No. WO 91/05548, PCT Publication No. WO 96/20698, Ning *et al.*, Radiotherapy & Oncology 39:179-189, 1996; Song *et al.*, PDA Journal of Pharmaceutical Science & Technology 50:372-397, 1995; Cleek *et al.*, Pro. Int'l. Symp. Control. Rel. Bioact. Mater. 24:853-854, 1997; and Lam *et al.*, Proc. Int'l. Symp. Control Rel. Bioact. Mater. 24:759-760, 1997, the entire contents of which are hereby incorporated by reference.

[0929] When the antibody or fragment thereof according to the present invention is topically administered, the antibody or fragment thereof may be formulated into forms of an ointment, a cream, a transdermal patch, a lotion, a gel, a shampoo, a spray, an aerosol, a solution, or an emulsion, or other forms well known to a person of ordinary skill in the art. See, for example, Remington's Pharmaceutical Sciences and Introduction to Pharmaceutical Dosage Forms, 19th ed., Mack Pub. Co., Easton, Pa. (1995). A non-sprayable topical dosage form includes a carrier or one or more excipients suitable for topical application. In some cases, viscous, semisolid, or solid forms having a higher kinematic viscosity than water may be generally used as the topical dosage form. Suitable preparations include a solution, a suspension, an emulsion, a cream, an ointment, a powder, a liniment, a salve, and the like, all of which are sterilized, or mixed with an adjuvant (for example, a preservative, a stabilizing agent, a wetting agent, a buffer, or a salt) influencing various characteristics, for example, such as osmotic pressure, when necessary, but the present invention is not limited thereto. In some cases, other suitable topical dosage forms

include a sprayable aerosol formulation filled in a mixture of compressed volatile substances (for example, a gaseous propellant such as freon) or a squeeze bottle after the active ingredient is combined with a solid or liquid inert carrier. When necessary, a moisturizing agent or a humectant may be also added to the pharmaceutical composition and the dosage form. Examples of such an additional component are known in the related art.

[0930] When the composition including an antibody or a fragment thereof is intranasally administered, the composition may be formulated into forms of an aerosol, a spray, mist, or drops. In particular, a prophylactic or therapeutic agent for use in the present invention may be conveniently delivered in an aerosol spray dosage form from a pressurized pack or an atomizer using a suitable propellant (for example, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide, or other suitable gases). In the case of the pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. A capsule and cartridge (for example, consisting of gelatin) for use in an inhaler or an insufflator may be formulated to include a powder mixture of compounds, and a suitable powder base, for example, lactose or starch.

[0931] Methods for co-administration or treatment with a second therapeutic agent, for example, a cytokine, a steroid, a chemotherapeutic agent, an antibiotic, or radiation are known in the related art (see, for example, Hardman *et al.*, (eds.) (2001) Goodman and Gilman's The Pharmacological Basis of Therapeutics, 10th ed., McGraw-Hill, New York, N.Y.; Poole and Peterson (eds.) (2001) Pharmacotherapeutics for Advanced Practice:A Practical Approach, Lippincott, Williams & Wilkins, Phila., Pa.; Chabner and Longo (eds.) (2001) Cancer Chemotherapy and Biotherapy, Lippincott, Williams & Wilkins, Phila., Pa.). An effective amount of the therapeutic agent may reduce symptoms by at least 10%; at least 20%; at least approximately 30%; at least 40%, or at least 50%.

[0932] An additional therapy (for example, a prophylactic or therapeutic agent) that may be administered in combination with the antibody or fragment thereof according to the present invention may be administered together with the antibody or fragment thereof according to the present invention at intervals of less than 5 minutes, intervals of less than 30 minutes, intervals of an hour, intervals of approximately an hour, intervals of approximately 1 to approximately 2 hours, intervals of approximately 2 hours to approximately 3 hours, intervals of approximately 3 hours to approximately 4 hours, intervals of approximately 4 hours to approximately 5 hours, intervals of approximately 5 hours to approximately 6 hours, intervals of approximately 6 hours to approximately 7 hours, intervals of approximately 7 hours to approximately 8 hours, intervals of approximately 8 hours to approximately 9 hours, intervals of approximately 9 hours to

approximately 10 hours, intervals of approximately 10 hours to approximately 11 hour, intervals of approximately 11 hour to approximately 12 hours, intervals of approximately 12 hours to 18 hours, intervals of 18 hours to 24 hours, intervals of 24 hours to 36 hours, intervals of 36 hours to 48 hours, intervals of 48 hours to 52 hours, intervals of 52 hours to 60 hours, intervals of 60 hours to 72 hours, intervals of 72 hours to 84 hours, intervals of 84 hours to 96 hours, or intervals of 96 hours to 120 hours. Two or more therapeutic agents may be administered during the same visit by a patient.

[0933] In specific embodiments, the antibody or fragment thereof according to the present invention may be formulated to insure a proper distribution *in vivo*. For example, the blood-brain barrier (BBB) excludes a number of highly hydrophilic compounds. To ensure therapeutic compounds of the invention cross the BBB (when necessary), they can be, for example, formulated in liposomes. For a method of preparing liposomes, for example, see US Patent Nos. 4,522,811; 5,374,548; and 5,399,331. The liposomes may include one or more moieties that are selectively transported into specific cells or organs, thereby enhancing targeted drug delivery (see, for example, Ranade, (1989) J. Clin. Pharmacol. 29:685). Exemplary targeting moieties include folate or biotin (see, for example, US Patent No. 5,416,016 (to Low *et al.*)); mannosides (see Umezawa *et al.*, (1988) Biochem. Biophys. Res. Commun. 153:1038); antibodies (see Bloeman *et al.*, (1995) FEBS Lett. 357:140; Owais *et al.*, (1995) Antimicrob. Agents Chemother. 39:180); a surfactant protein A receptor (see Briscoe *et al.*, (1995) Am. J. Physiol. 1233:134); p 120 (see Schreier *et al.*, (1994) J. Biol. Chem. 269:9090), and the like. Also, see K. Keinanen; M. L. Laukkanen (1994) FEBS Lett. 346:123; J. J. Killion; I. J. Fidler (1994) Immunomethods 4:273.

[0934] The present invention provides a protocol for administering a pharmaceutical composition, which includes the antibody or fragment thereof according to the present invention, to a subject in need thereof alone or in combination with other therapies. The therapeutic agent (for example, a prophylactic or therapeutic agent) for combination therapy of the present invention may be simultaneously or sequentially administered to the subject. The therapeutic agent (for example, a prophylactic or therapeutic agent) for combination therapy of the present invention may also be administered periodically. A periodic therapy includes administering a first therapeutic agent (for example, a first prophylactic or therapeutic agent) for a predetermined period of time, administering a second therapeutic agent (for example, a second prophylactic or therapeutic agent) for a predetermined period of time, and then sequentially repeating the steps of administration, that is, a predetermined cycle for reducing the occurrence of resistance to one of the therapeutic agents (for example, agonists), and/or preventing or reducing side effects of one of the therapeutic agents (for example, agonists), and/or improving efficacy of the therapeutic

agents.

[0935] The therapeutic agent (for example, a prophylactic or therapeutic agent) for combination therapy of the present invention may be simultaneously administered to a subject.

[0936] The term "simultaneously" means that therapies (for example, prophylactic or therapeutic agents) need not be administered at exactly the same time without limitation, but are rather administered to a subject sequentially with the pharmaceutical composition including an antibody or a fragment thereof according to the present invention, and are administered at time intervals that may serve to provide higher benefits compared to when the antibody of the present invention is administered at a different time from other therapy(ies). For example, the respective therapeutic agents may be sequentially administered to a subject at the same time or different points of time in any order; but should be administered at sufficiently short time intervals to provide a desire therapeutic or prophylactic effect when they are not administered at the same time. The respective therapeutic agents may be administered to a subject in any adequate form through any suitable route of administration. In various exemplary embodiments, the therapeutic agent (for example, a prophylactic or therapeutic agent) may be administered to a subject at intervals of less than 15 minutes, intervals of less than 30 minutes, intervals of less than an hour, intervals of approximately an hour, intervals of approximately 1 to approximately 2 hours, intervals of approximately 2 hours to approximately 3 hours, intervals of approximately 3 hours to approximately 4 hours, intervals of approximately 4 hours to approximately 5 hours, intervals of approximately 5 hours to approximately 6 hours, intervals of approximately 6 hours to approximately 7 hours, intervals of approximately 7 hours to approximately 8 hours, intervals of approximately 8 hours to approximately 9 hours, intervals of approximately 9 hours to approximately 10 hours, intervals of approximately 10 hours to approximately 11 hour, intervals of approximately 11 hour to approximately 12 hours, intervals of 24 hours, 48 hours, 72 hours, or intervals of a week. In other exemplary embodiments, two or more therapeutic agents (for example, prophylactic or therapeutic agents) may be administered during the same visit by a patient.

[0937] In the same pharmaceutical composition, the prophylactic or therapeutic agent for combination therapy may be administered to a subject. Alternatively, in the individual pharmaceutical compositions, the prophylactic or therapeutic agent for combination therapy may be simultaneously administered to a subject. The prophylactic or therapeutic agent may be administered to a subject through the same or different routes of administration.

Experimental Examples

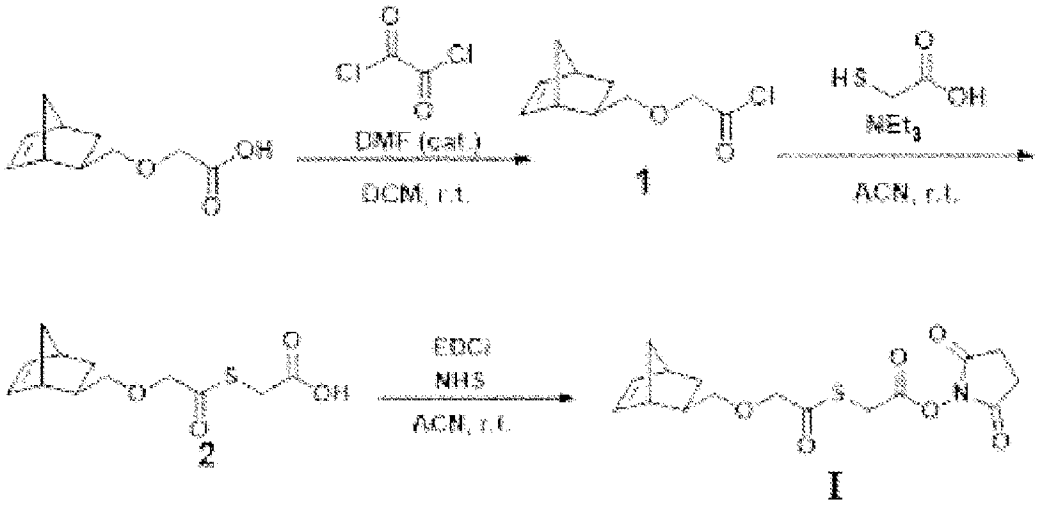
[0938] 1. Preparation Examples of compounds for preparing an agent for transferring a

site-specific first click-chemistry functional group to an antibody

[0939] 1.1. Synthesis and confirmation of structure of linker (H_1-L_1)

[0940] 1.1.1.: Compound I (SO1 Linker: NHS & Norbornene)

[0941] [Scheme 6]



[0942] Synthesis of Compound I (Scheme 6, FIG. 15)

[0943] Synthesis of Compound 1

[0944] 2 g (10.98 mmol, 1.0 eq.) of 2-(bicyclo[2.2.1]hept-5-ene-2-ylmethoxy)acetic acid was dissolved in 50 mL of DCM, and 0.085 mL (1.098 mmol, 0.1 eq.) of DMF and 1.91 mL (21.96 mmol, 2.0 eq.) of oxalyl chloride were then added dropwise while stirring at room temperature. The reaction solution was stirred for 3 hours, and then concentrated under reduced pressure to obtain 1.97 g of a target compound (yield: 90%).

[0945] Synthesis of Compound 2

[0946] 1.97 g (9.88 mmol, 1.0 eq.) of Compound 1 was dissolved in 20 mL of acetonitrile (ACN), and 0.68 mL (9.88 mmol, 1.0 eq.) of thioglycolic acid and 2.06 mL (14.82 mmol, 1.5 eq.) of triethylamine were then added dropwise while stirring at room temperature. The reaction solution was stirred for 18 hours, and then concentrated under reduced pressure. Subsequently, water was added to the reaction solution, and the reaction solution was extracted three times with ethyl acetate (EA). An organic layer was dried over magnesium sulfate, concentrated under reduced pressure, and then purified by column chromatography (DCM:MeOH = 10:1) to obtain 2.05 g (yield: 79%) of a target compound.

[0947] ^{1}H NMR (500 MHz, $CDCl_3$) δ ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.15 - 6.01 (m, 2H), 4.20 (t, J = 5.0 Hz, 1H), 3.77 - 3.71 (m, 2H), 3.68 - 3.57 (m, 1H), 3.56 - 3.45 (m, 1H), 2.84 (s, 2H),

1.81 - 1.69 (m, 2H), 1.30 (ddd, J = 19.8, 14.5, 8.6 Hz, 4H), 1.15 (ddd, J = 16.0, 7.6, 3.3 Hz, 1H).

[0948] Synthesis of Compound I

[0949] 2.05 g (7.81 mmol, 1.0 eq.) of Compound 2 was dissolved in 50 mL of ACN, and 2.76 g (14.44 mmol, 1.8 eq.) of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCi) and 2.21 g (19.25 mmol, 2.46 eq.) of N-hydroxysuccinimide (NHS) were then added while stirring at room temperature. The reaction solution was stirred for 18 hours, and then concentrated under reduced pressure. Subsequently, water was added to the reaction solution, and the reaction solution was extracted three times with EA. An organic layer was recovered, dried over magnesium sulfate, and then concentrated under reduced pressure. Then, the residue was purified on a silica gel column using chromatography (EA:Hex = 2:1) to obtain 2.7 g (yield: 98%) of a target compound.

[0950] ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.15 - 6.03 (m, 2H), 4.45 (s, 1H), 4.21 (d, J = 5.0 Hz, 1H), 3.97 (s, 1H), 3.65 (dd, J = 12.0, 7.3 Hz, 1H), 3.52 (t, J = 8.9 Hz, 1H), 2.83 (dd, J = 24.4, 11.8 Hz, 6H), 1.81 - 1.67 (m, 1H), 1.30 (dq, J = 27.0, 9.5 Hz, 4H), 1.16 (ddd, J = 15.3, 7.4, 3.8 Hz, 1H).

[0951] Confirmation of structure of Compound I

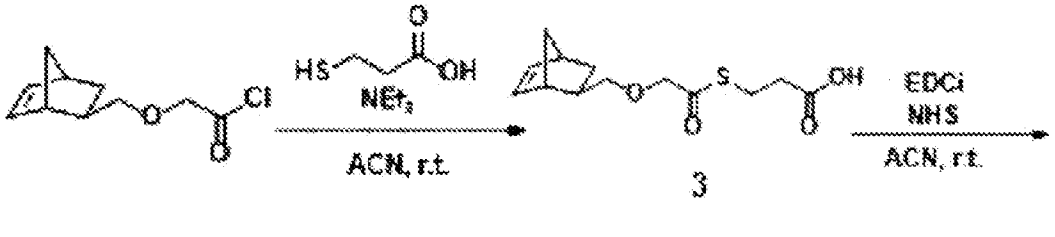
[0952] ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.15 - 6.03 (m, 2H), 4.45 (s, 1H), 4.21 (d, J = 5.0 Hz, 1H), 3.97 (s, 1H), 3.65 (dd, J = 12.0, 7.3 Hz, 1H), 3.52 (t, J = 8.9 Hz, 1H), 2.83 (dd, J = 24.4, 11.8 Hz, 6H), 1.81 - 1.67 (m, 1H), 1.30 (dq, J = 27.0, 9.5 Hz, 4H), 1.16 (ddd, J = 15.3, 7.4, 3.8 Hz, 1H).

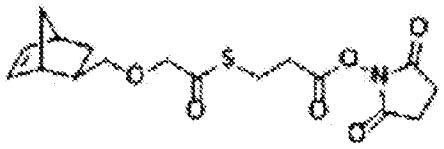
[0953] LRMS (ESI): m/z 371.2 [M+ NH_4^+]

[0954] The structure of Compound I was confirmed by mass spectrometry. The results are shown in FIG. 16.

[0955] 1.1.2. Compound II (SO2 Linker: NHS & Norbornene)

[0956] [Scheme 7]





II

[0957] Synthesis of Compound II (Scheme 7, FIG. 17)

[0958] Synthesis of Compound 3

[0959] 0.55 g (2.33 mmol, 1.0 eq.) of Compound 1 was dissolved in 5 mL of acetonitrile (ACN), and 0.2 mL (2.33 mmol, 1.0 eq.) of 3-mercaptopropionic acid and 0.49 mL (3.49 mmol, 1.5 eq.) of triethylamine were then added dropwise while stirring at room temperature. The reaction solution was stirred for 11 hours, and then concentrated under reduced pressure. Subsequently, water was added to the reaction solution, and the reaction solution was extracted three times with ethyl acetate (EA). An organic layer was dried over magnesium sulfate, concentrated under reduced pressure, and then purified by silica gel column chromatography (DCM:MeOH = 10:1) to obtain 0.56 g (yield: 89%) of a target compound.

[0960] Synthesis of Compound II

[0961] 0.56 g (2.07 mmol, 1.0 eq.) of Compound 3 was dissolved in 10 mL of acetonitrile (ACN), and 0.81 g (4.21 mmol, 2.0 eq.) of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCi) and 0.65 g (5.61 mmol, 2.7 eq.) of N-hydroxysuccinimide (NHS) were added while stirring at room temperature. The reaction solution was stirred for 12 hours, and then concentrated under reduced pressure. Subsequently, water was added to the reaction solution, and the reaction solution was extracted three times with EA. An organic layer was dried over magnesium sulfate, and concentrated under reduced pressure. Then, the residue was purified by silica gel column chromatography (EA:Hex = 2:1) to obtain 0.63 g (yield: 83%) of a target compound.

[0962] Confirmation of structure of Compound II

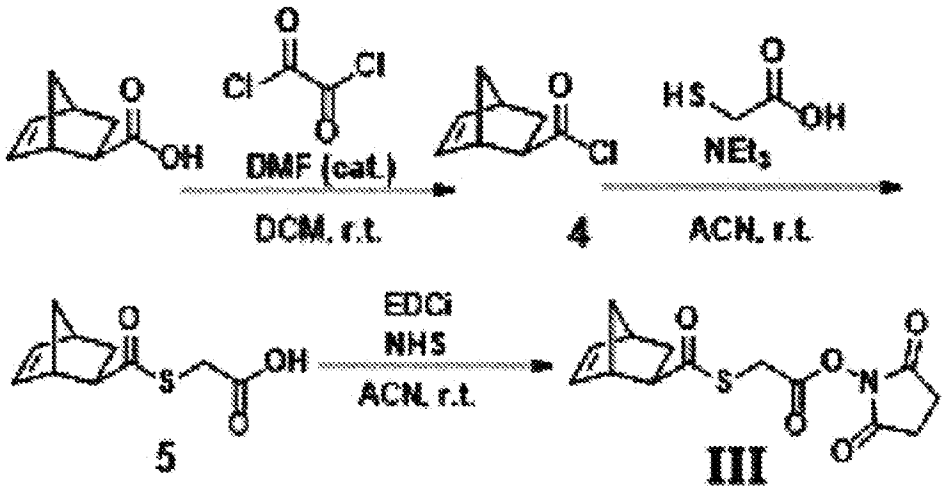
[0963] ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.16-6.14 (m, 2H), 4.45 (s, 1H), 4.21 - 4.08 (m, 1H), 3.69 - 3.58 (m, 1H), 3.51 (dt, J = 14.5, 8.9 Hz, 1H), 3.22 (t, J = 7.1 Hz, 2H), 2.96 (t, J = 7.1 Hz, 2H), 2.79-2.83 (m, 6H), 1.81 - 1.67 (m, 1H), 1.37 - 1.21 (m, 4H), 1.13-1.18 (m, 1H).

[0964] LRMS (ESI): m/z 385.1 [M+ NH_4^+]

[0965] The structure of Compound II was confirmed by mass spectrometry. The results are shown in FIG. 18.

[0966] 1.1.3. Compound III (SO3 Linker: NHS & Norbornene)

[0967] [Scheme 8]



[0968] Synthesis of Compound III (Scheme 8, FIG. 19)

[0969] Synthesis of Compound 4

[0970] 0.5 g (3.62 mmol, 1.0 eq.) of exo-5-norbornenecarboxylic acid was dissolved in 20 mL of DCM, and 0.028 mL (0.37 mmol, 0.94 eq.) of DMF and 0.63 mL (7.24 mmol, 2.0 eq.) of oxalyl chloride were then added dropwise while stirring at room temperature. The reaction solution was stirred for 3 hours, and then concentrated under reduced pressure to obtain 0.47 g (yield: 83%) of a target compound.

[0971] Synthesis of Compound 5

[0972] 0.47 g (3.0 mmol, 1.0 eq.) of Compound 4 was dissolved in 15 mL of acetonitrile (ACN), and 0.21 mL (3.0 mmol, 1.0 eq.) of thioglycolic acid and 0.63 mL (4.5 mmol, 1.5 eq.) of triethylamine were then added dropwise while stirring at room temperature. The reaction solution was stirred for 18 hours, and then concentrated under reduced pressure. Subsequently, water was added to the reaction solution, and the reaction solution was extracted three times with ethyl acetate (EA). An organic layer was dried over magnesium sulfate, concentrated under reduced pressure, and then purified by column chromatography (DCM:MeOH = 10:1) to obtain 0.3 g (yield: 47%) of a target compound.

[0973] ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.82 (brs, 1H), 6.24 - 6.07 (m, 2H), 3.76 (s, 2H), 3.12 (s, 1H), 2.97 (s, 1H), 2.54 (dd, J = 9.0, 4.7 Hz, 1H), 2.04 - 1.87 (m, 1H), 1.57 (d, J = 8.5 Hz, 1H), 1.48 - 1.35 (m, 2H).

[0974] Synthesis of Compound III

[0975] 0.26 g (1.22 mmol, 1.0 eq.) of Compound 5 was dissolved in 15 mL of acetonitrile (ACN), and 0.35 g (1.83 mmol, 1.5 eq.) of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCi) and 0.28g (2.44 mmol, 2.0 eq.) of N-hydroxysuccinimide (NHS) were added while stirring at room temperature. The reaction solution was stirred for 3 hours, and then concentrated under reduced

pressure. Subsequently, water was added to the reaction solution, and the reaction solution was extracted three times with EA. An organic layer was recovered, dried over magnesium sulfate, and then concentrated under reduced pressure. Then, the residue was purified on a silica gel column using chromatography (EA:Hex = 2:1) to obtain 0.32 g (yield: 85%) of a target compound.

[0976] Confirmation of structure of Compound III

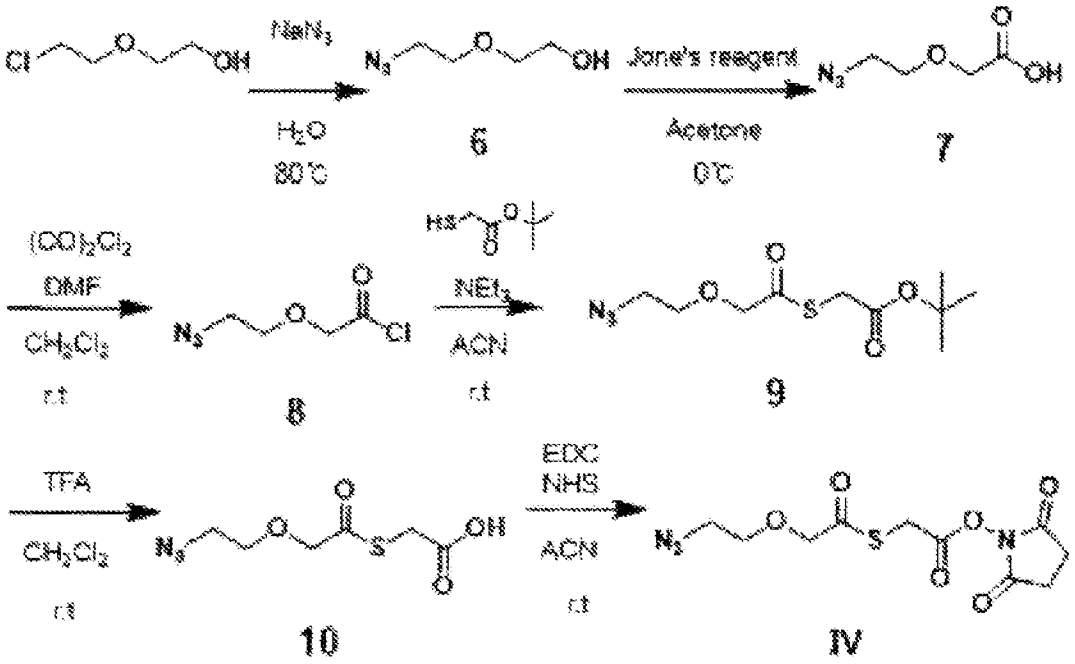
[0977] ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.21 (dd, J = 5.5, 3.0 Hz, 1H), 6.15 (dd, J = 5.5, 3.1 Hz, 1H), 4.01 (s, 2H), 3.15 (s, 1H), 2.98 (s, 1H), 2.86 (s, 4H), 2.54 (dd, J = 9.2, 4.6 Hz, 1H), 2.02 (dt, J = 11.9, 4.0 Hz, 1H), 1.59 (d, J = 8.6 Hz, 1H), 1.46 - 1.36 (m, 2H).

[0978] LCMS (ESI): m/z 332.16 [M+Na$^+$]

[0979] The structure of Compound III was confirmed by mass spectrometry. The results are shown in FIG. 20.

[0980] 1.1.4. Compound IV (SO4 Linker: NHS & azide)

[0981] [Scheme 9]



[0982] Synthesis of Compound IV (Scheme 9, FIGS. 21 and 22)

[0983] Synthesis of Compound 6

[0984] 2-(2-Chloroethoxy)ethanol (2 mL, 18.94 mmol) was dissolved in distilled water (12 mL), and NaN_3 (3.08 g, 47.35 mmol, 2.5 eq.) was added thereto. The resulting mixture was stirred at 80°C for 16 hours. The reaction mixture was cooled to room temperature, and poured into 5% NaOH (aq.) (20 mL), and then stirred for approximately 10 minutes. The reaction mixture was extracted three times with diethyl ether, and an organic layer was dried over magnesium

sulfate, and filtered. The filtrate was concentrated under reduced pressure to obtain 2.47 g (yield: 99%) of a target compound.

[0985] Synthesis of Compound 7

[0986] 2.47 g (18.84 mmol) of Compound 6 was dissolved in acetone (50 mL), and a 1 M Jone's reagent (75.36 mL, 75.36 mmol, 4 eq.) was then slowly added at 0°C. The reaction mixture was stirred at 0°C for 3 hours, warmed to room temperature, and then stirred for approximately 10 minutes. The reaction mixture was extracted three times with ethyl acetate (EA), and an organic layer was dried over magnesium sulfate, and filtered. The filtrate was concentrated under reduced pressure to obtain 2.69 g (yield: 98%) of a target compound.

[0987] Synthesis of Compound 8

[0988] 2.69 g (18.57 mmol, 1.0 eq.) of Compound 7 was dissolved in 50 mL of DCM, and 0.1 mL (1.29 mmol, 0.07 eq.) of DMF and 2.43 mL (27.86 mmol, 1.5 eq.) of oxalyl chloride were then added dropwise while stirring at room temperature. The reaction solution was stirred for 3 hours, and then concentrated under reduced pressure to obtain 2.42 g (yield: 80%) of a target compound.

[0989] Synthesis of Compound 9

[0990] 0.88 g (5.40 mmol, 1.0 eq.) of Compound 8 was dissolved in 30 mL of DCM, and 0.8 g (5.40 mmol, 1.0 eq.) of tert-butyl 2-mercaptoacetate and 1.41 mL (8.10 mmol, 1.5 eq.) of N,N-diisopropylethylamine were then added dropwise while stirring at room temperature. The reaction mixture was stirred for 2 hours, and then concentrated under reduced pressure. Subsequently, water was added to the reaction solution, and the reaction solution was extracted three times with dichloromethane (DCM). An organic layer was dried over magnesium sulfate, concentrated under reduced pressure, and then purified by column chromatography (Hex:EA = 5:1) to obtain 0.73 g (yield: 49%) of a target compound.

[0991] Synthesis of Compound 10

[0992] 0.73 g (2.66 mmol, 1 eq.) of Compound 9 was dissolved in 10 mL of DCM, and 10 mL (129.8 mmol, 48 eq.) of trifluoroacetic acid was then added dropwise while stirring at room temperature. The reaction solution was stirred for 8 hours, and then concentrated under reduced pressure to obtain 0.40 g (yield: 70%) of a target compound.

[0993] Synthesis of Compound IV

[0994] 1.97 g (9.0 mmol, 1.0 eq.) of Compound 10 was dissolved in 25 mL of acetonitrile (ACN), and 2.58 g (13.5 mmol, 1.5 eq.) of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCi) and 2.07 g (18.0 mmol, 2.0 eq.) of N-hydroxysuccinimide (NHS) were added while stirring at room

temperature. The reaction solution was stirred for 3 hours, and then concentrated under reduced pressure. Subsequently, water was added to the reaction solution, and the reaction solution was extracted three times with EA. An organic layer was recovered, dried over magnesium sulfate, and then concentrated under reduced pressure. Then, the residue was purified on a silica gel column using chromatography (EA:Hex = 2:1) to obtain 1.03 g (yield: 36%) of a target compound.

[0995] Confirmation of structure of Compound IV

[0996] ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.30 (s, 2H), 4.00 (s, 2H), 3.87 - 3.75 (m, 2H), 3.55 - 3.47 (m, 2H), 2.86 (s, 4H).

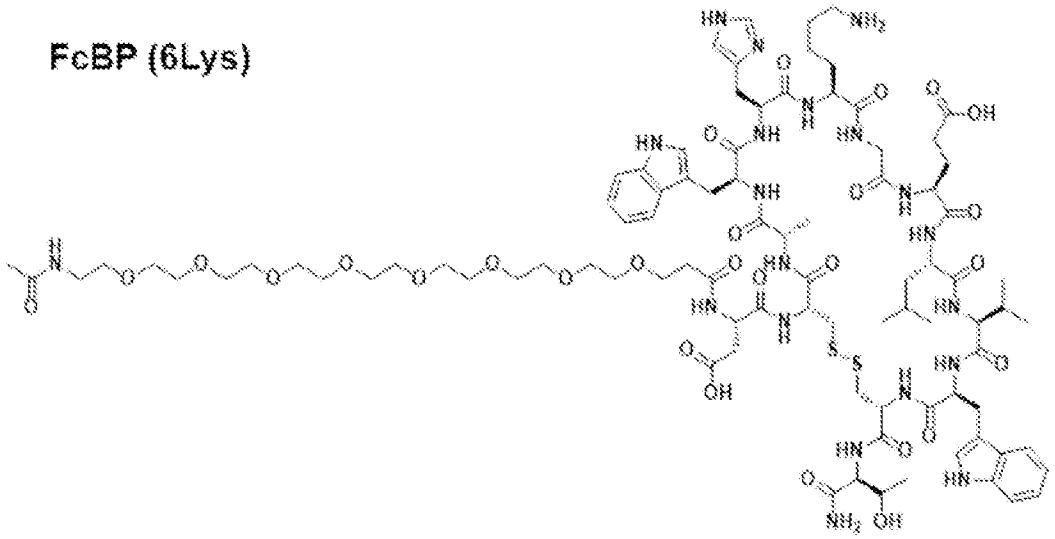
[0997] LRMS (ESI): m/z 334.0 [M+ NH_4^+]

[0998] The structure of Compound IV was confirmed by mass spectrometry. The results are shown in FIG. 23.

[0999] 1.2. Synthesis and confirmation of structure of site-specific Fc interactome (SSFI)

[1000] 1.2.1. Synthesis and confirmation of structure of SSFI (where Xa_1 is lysine)

[1001] Synthesis of SSFI (6Lys)



[1002] List and order of introduction of Fmoc amino acids used

[1003] Fmoc-L-Thr(tBu)-OH, Fmoc-Cys(Trt)-OH, Fmoc-L-Trp(Boc)-OH, Fmoc-L-Val-OH, Fmoc-L-Leu-OH, Fmoc-L-Glu(OtBu)-OH, Fmoc-Gly-OH, Fmoc-Lys(Boc)-OH, Fmoc-L-His(Trt)-OH, Fmoc-L-Trp(Boc)-OH, Fmoc-Ala-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(tBu)-OH.

[1004] Preparation method

[1005] (a) Introduction of amino acids

[1006] Amounts of reagents used in the following process were based on 0.25 mmole. 0.5 g (0.48 mmole/g) of a clear amide resin (Peptide International Inc., USA) was put into a

synthesis reactor, and 1 mmole of each Fmoc-amino acid block was weighed to prepare a peptide amino acid sequence from the C-terminus to the N-terminus in the above order.

[1007] A reaction for activating an Fmoc-amino acid to attach the activated residue to a clear amide resin was performed sequentially from the C-terminal amino acid.

[1008] Removal of Fmoc was performed in 20% piperidine-containing DMF, and activation and introduction of the residue was performed by mixing amino acids prepared to correspond to the sequence with 2 mL of a 0.5 M HOBt-containing DMF solution, 2 mL of a 0.5 M HBTU-containing DMF solution, and 174 μL of DIPEA for 5 minutes, and mixing the resulting mixture for 2 hours in a reactor containing the resin.

[1009] Confirmation of an introduction reaction was carried out using a Kaiser test method. When the introduction reaction was confirmed to be incomplete, the introduction reaction was repeated once more, or capping was performed using a 20% Ac_2O-containing DMF solution. In each of the introduction reaction and the Fmoc removal, the resin was thoroughly washed with DMF and DCM before moving to the next step. This process was repeatedly performed until the targeted peptide sequence was completed.

[1010] (b) Introduction of H-PEG8-OH

[1011] To introduce H-PEG_8-OH to the N-terminus of the sequence after the introduction of the amino acids was completed, 1 mL of a 0.5 M Fmoc-N-amido-dPEG8-acid-in-DMF solution, 1 mL of a 0.5 M HBTU-containing DMF solution, 1 mL of a 0.5 M HOBt-containing DMF solution, and 87 μL of DIPEA were mixed for 5 minutes, and the resulting mixture was mixed for 2 hours in a reactor containing a reactive resin.

[1012] Progression of the reaction was monitored by the Kaiser test method. When the unreacted amine was found to remain, the reaction time was extended for another 1 to 3 hours, or the reaction solution was discarded, and the aforementioned reaction process was repeated again. Removal of the N-terminal Fmoc protective group was performed using 20% piperidine-containing DMF, and the resin to which the peptide was attached was then dried and weighed.

[1013] (c) 250 mg of the resin to which the peptide was attached as prepared in the step (b) was stirred with 2 mL of a mixed solution of TFA, TIS, water and EDT (94:1.0:2.5:2.5) at room temperature for 120 minutes to cleave the peptide from the resin. The cleaved mixture was filtered, and the filtrate was concentrated to about half its volume using nitrogen gas. Thereafter, ether was poured into the mixture to precipitate the peptide. The precipitated peptide was washed three times with ether, and dried under nitrogen gas. The dried precipitate was dissolved in 0.1% TFA-30% ACN-containing water, stirred for 6 hours, and then concentrated.

[1014] The concentrate was dissolved at a concentration of 0.1 mg/mL in a 5%-DMSO-

20%-ACN-containing 0.01 M ammonium acetate buffer (pH 6.5) solution, and then stirred for 3 days while being exposed to air. Progression of a disulfide bond-forming reaction was monitored by HPLC. When the reaction was found not to progress any more, the reaction solution was freeze-dried to obtain a peptide precipitate.

[1015] (d) Purification

[1016] The peptide precipitate obtained by freeze-drying in the step (c) was separated under the prep-LC primary purification conditions listed in the following Table 4, further purified under the prep-LC secondary purification conditions listed in the following Table 5, and then freeze-dried. The resulting peptide was confirmed to have a purity of 90% or more by analytical HPLC, and the molecular weight of the synthesized peptide was confirmed using an LC/MS mass spectrometer.

[1017] H-PEG8-Asp-Cys*-Ala-Trp-His-Lys-Gly-Glu-Leu-Val-Trp-Cys*-Thr-NH_2 (Cys*: disulfide bonding sites)

<Table 4> Prep-LC primary purification condition

Prep-LC primary purification condition			
Equipment name	Waters 2525 pump, Waters 2487 UV detector		
Column	Waters, XBridge Prep C18 5 μm OBD 19 x 250 mm		
Mobile phase A/B	0.1% TFA-20% ACN / 0.1% TFA in 80% ACN		
Gradient	0 min → 30 min : B 0% → B 100%		
Flow rate	17 mL/min	Detection wavelength	UV 280 nm

<Table 5> Prep-LC secondary purification condition

Prep-LC secondary purification condition			
Equipment name	Waters 2525 pump, Waters 2487 UV detector		
Column	Waters, XBridge Prep C18 5 μm OBD 19 x 250 mm		
Mobile phase A/B	0.1% TFA-30% ACN / 0.1% TFA in 70% ACN		
Gradient	0 min → 30 min : B 0% → B 100%		
Flow rate	15 mL/min	Detection wavelength	UV 280 nm

[1018] Confirmation of structure of SSFI (6Lys)

[1019] Synthesis of SSFI (6Lys) was confirmed by molecular weight measurement by matrix-assisted laser desorption/ionization (MALDI) mass spectrometry.

[1020] Measuring equipment: Ultraflextreme (Bruker)

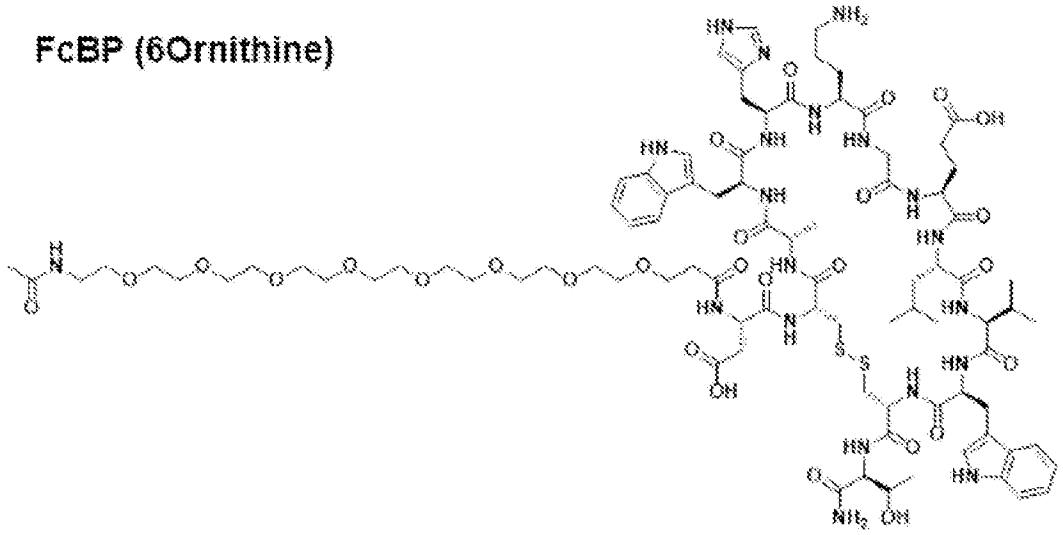
[1021] Measuring matrix: CHCA (α-Cyano-4-hydroxycinnamic acid) & DHB (2,5-Dihydroxybenzoic acid)

[1022] Calculated molecular weight: 2010.29 g/mol

[1023] Measured molecular weight $(M+H)^+$: 2011.89 g/mol

[1024] The mass spectrometry results of SSFI (6Lys) are shown in FIG. 24.

[1025] 1.2.2. Synthesis and confirmation of structure of SSFI (where Xa_1 is ornithine)



[1026] Synthesis of SSFI (6Orn)

[1027] List and order of introduction of Fmoc amino acids used

[1028] Fmoc-L-Thr(tBu)-OH, Fmoc-Cys(Trt)-OH, Fmoc-L-Trp(Boc)-OH, Fmoc-L-Val-OH, Fmoc-L-Leu-OH, Fmoc-L-Glu(OtBu)-OH, Fmoc-Gly-OH, Fmoc-Orn(Boc)-OH, Fmoc-L-His(Trt)-OH, Fmoc-L-Trp(Boc)-OH Fmoc-Ala-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(tBu)-OH.

[1029] Preparation method

[1030] (a) Introduction of amino acids

[1031] Amounts of reagents used in the following process were based on 0.25 mmole. 0.5 g (0.48 mmole/g) of a clear amide resin (Peptide International Inc., USA) was put into a synthesis reactor, and 1 mmole of each Fmoc-amino acid block was weighed to prepare a peptide

amino acid sequence from the C-terminus to the N-terminus in the above.

[1032] A reaction for activating an Fmoc-amino acid to attach the activated residue to a clear amide resin was performed sequentially from the C-terminal amino acid.

[1033] Removal of Fmoc was performed in 20% piperidine-containing DMF, and activation and introduction of the residue was performed by mixing amino acids prepared to correspond to the sequence with 2 mL of a 0.5 M HOBt-containing DMF solution, 2 mL of a 0.5 M HBTU-containing DMF solution, and 174 μL of DIPEA for 5 minutes, and mixing the resulting mixture for 2 hours in a reactor containing the resin.

[1034] Confirmation of an introduction reaction was carried out using a Kaiser test method. When the introduction reaction was confirmed to be incomplete, the introduction reaction was repeated once more, or capping was performed using a 20% Ac_2O-containing DMF solution. In each of the introduction reaction and the Fmoc removal, the resin was thoroughly washed with DMF and DCM before moving to the next step. This process was repeatedly performed until the targeted peptide sequence was completed.

[1035] (b) Introduction of H-PEG8-OH

[1036] To introduce H-PEG_8-OH to the N-terminus of the sequence after the introduction of the amino acids was completed, 1 mL of a 0.5 M Fmoc-N-amido-dPEG8-acid-in-DMF solution, 1 mL of a 0.5 M HBTU-containing DMF solution, 1 mL of a 0.5 M HOBt-containing DMF solution, and 87 μL of DIPEA were mixed for 5 minutes, and the resulting mixture was mixed for 2 hours in a reactor containing a reactive resin.

[1037] Progression of the reaction was monitored by the Kaiser test method. When the unreacted amine was found to remain, the reaction time was extended for another 1 to 3 hours, or the reaction solution was discarded, and the aforementioned reaction process was repeated again. Removal of the N-terminal Fmoc protective group was performed using 20% piperidine-containing DMF, and the resin to which the peptide was attached was then dried and weighed.

[1038] (c) 250 mg of the resin to which the peptide was attached as prepared in the step (b) was stirred with 2 mL of a mixed solution of TFA, TIS, water and EDT (94:1.0:2.5:2.5) at room temperature for 120 minutes to cleave the peptide from the resin. The cleaved mixture was filtered, and the filtrate was concentrated to about half its volume using nitrogen gas. Thereafter, ether was poured into the mixture to precipitate the peptide. The precipitated peptide was washed three times with ether, and dried under nitrogen gas. The dried precipitate was dissolved in 0.1% TFA-30% ACN-containing water, stirred for 6 hours, and then concentrated.

[1039] The concentrate was dissolved at a concentration of 0.1 mg/mL in a 5%-DMSO-20%-ACN-containing 0.01 M ammonium acetate buffer (pH 6.5) solution, and then stirred for 3

days while being exposed to air. Progression of a disulfide bond-forming reaction was monitored by HPLC. When the reaction was found not to progress any more, the reaction solution was freeze-dried to obtain a peptide precipitate.

[1040] (d) Purification

[1041] The peptide precipitate obtained by freeze-drying in the step (c) was separated under the prep-LC primary purification conditions listed in the following Table 6, further purified under the prep-LC secondary purification conditions listed in the following Table 7, and then freeze-dried. The resulting peptide was confirmed to have a purity of 90% or more by analytical HPLC, and the molecular weight of the synthesized peptide was confirmed using an LC/MS mass spectrometer.

[1042] H-PEG8-Asp-Cys*-Ala-Trp-His-Orn-Gly-Glu-Leu-Val-Trp-Cys*-Thr-NH_2 (Cys*: disulfide bonding sites)

<Table 6> Prep-LC primary purification condition

Prep-LC primary purification condition			
Equipment name	Waters 2525 pump, Waters 2487 UV detector		
Column	Waters, XBridge Prep C18 5 μm OBD 19 x 250 mm		
Mobile phase A/B	0.1% TFA-20% ACN / 0.1% TFA in 80% ACN		
Gradient	0 min → 30 min : B 0% → B 100%		
Flow rate	17 mL/min	Detection wavelength	UV 280 nm

<Table 7> Prep-LC secondary purification condition

Prep-LC secondary purification condition			
Equipment name	Waters 2525 pump, Waters 2487 UV detector		
Column	Waters, XBridge Prep C18 5 μm OBD 19 x 250 mm		
Mobile phase A/B	0.1% TFA-30% ACN / 0.1% TFA in 70% ACN		
Gradient	0 min → 30 min : B 0% → B 100%		
Flow rate	15 mL/min	Detection wavelength	UV 280 nm

[1043] Confirmation of structure of SSFI (6Orn)

[1044] Synthesis of SSFI (6Ornithine) was confirmed by molecular weight measurement

by matrix-assisted laser desorption/ionization (MALDI) mass spectrometry.

[1045] Measuring equipment: Ultraflextreme (Bruker)

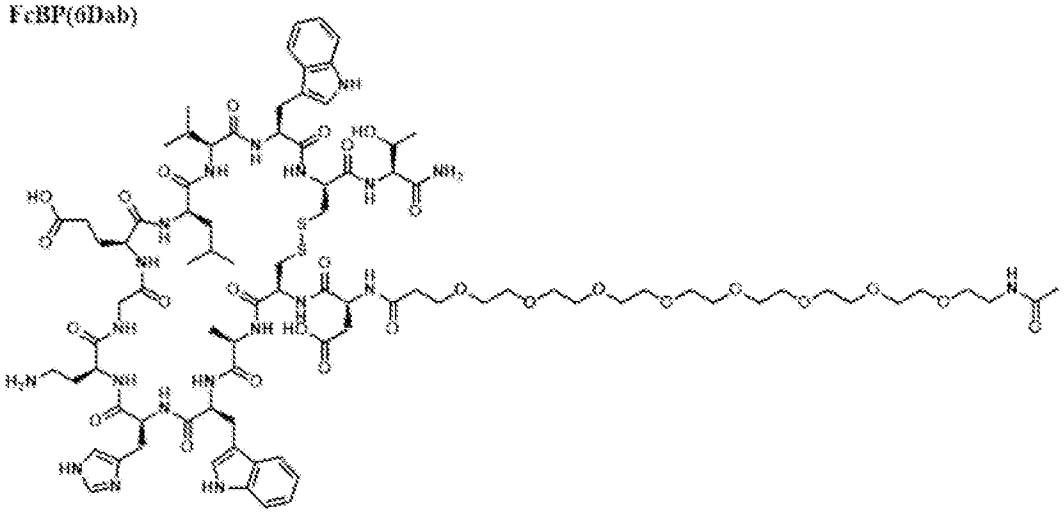
[1046] Measuring matrix: CHCA (α-Cyano-4-hydroxycinnamic acid) & DHB (2,5-Dihydroxybenzoic acid)

[1047] Calculated molecular weight: 1996.26 g/mol

[1048] Measured molecular weight $(M+2H)^{2+}$: 999.13 g/mol

[1049] The mass spectrometry results of SSFI (6Orn) are shown in FIG. 25.

[1050] 1.2.3. Synthesis and confirmation of structure of SSFI (where Xa_1 is diaminobutyric acid (Dab))



[1051] Synthesis of SSFI (6Dab)

[1052] List and order of introduction of Fmoc amino acids used

[1053] Fmoc-L-Thr(tBu)-OH, Fmoc-Cys(Trt)-OH, Fmoc-L-Trp(Boc)-OH, Fmoc-L-Val-OH, Fmoc-L-Leu-OH, Fmoc-L-Glu(OtBu)-OH, Fmoc-Gly-OH, Fmoc-Dab(Boc)-OH, Fmoc-L-His(Trt)-OH, Fmoc-L-Trp(Boc)-OH Fmoc-Ala-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(tBu)-OH.

[1054] Preparation method

[1055] (a) Introduction of amino acids

[1056] Amounts of reagents used in the following process were based on 0.25 mmole. 0.5 g (0.48 mmole/g) of a clear amide resin (Peptide International Inc., USA) was put into a synthesis reactor, and 1 mmole of each Fmoc-amino acid block was weighed to prepare a peptide amino acid sequence from the C-terminus to the N-terminus in the above order.

[1057] A reaction for activating an Fmoc-amino acid to attach the activated residue to a clear amide resin was performed sequentially from the C-terminal amino acid.

[1058] Removal of Fmoc was performed in 20% piperidine-containing DMF, and activation

and introduction of the residue was performed by mixing amino acids prepared to correspond to the sequence with 2 mL of a 0.5 M HOBt-containing DMF solution, 2 mL of a 0.5 M HBTU-containing DMF solution, and 174 μL of DIPEA for 5 minutes, and mixing the resulting mixture for 2 hours in a reactor containing the resin.

[1059] Confirmation of an introduction reaction was carried out using a Kaiser test method. When the introduction reaction was confirmed to be incomplete, the introduction reaction was repeated once more, or capping was performed using a 20% Ac_2O-containing DMF solution. In each of the introduction reaction and the Fmoc removal, the resin was thoroughly washed with DMF and DCM before moving to the next step. This process was repeatedly performed until the targeted peptide sequence was completed.

[1060] (b) Introduction of H-PEG8-OH

[1061] To introduce H-PEG_8-OH to the N-terminus of the sequence after the introduction of the amino acids was completed, 1 mL of a 0.5 M Fmoc-N-amido-dPEG8-acid-in-DMF solution, 1 mL of a 0.5 M HBTU-containing DMF solution, 1 mL of a 0.5 M HOBt-containing DMF solution, and 87 μL of DIPEA were mixed for 5 minutes, and the resulting mixture was mixed for 2 hours in a reactor containing a reactive resin.

[1062] Progression of the reaction was monitored by the Kaiser test method. When the unreacted amine was found to remain, the reaction time was extended for another 1 to 3 hours, or the reaction solution was discarded, and the aforementioned reaction process was repeated again. Removal of the N-terminal Fmoc protective group was performed using 20% piperidine-containing DMF, and the resin to which the peptide was attached was then dried and weighed.

[1063] (c) 250 mg of the resin to which the peptide was attached as prepared in the step (b) was stirred with 2 mL of a mixed solution of TFA, TIS, water and EDT (94:1.0:2.5:2.5) at room temperature for 120 minutes to cleave the peptide from the resin. The cleaved mixture was filtered, and the filtrate was concentrated to about half its volume using nitrogen gas. Thereafter, ether was poured into the mixture to precipitate the peptide. The precipitated peptide was washed three times with ether, and dried under nitrogen gas. The dried precipitate was dissolved in 0.1% TFA-30% ACN-containing water, stirred for 6 hours, and then concentrated.

[1064] The concentrate was dissolved at a concentration of 0.1 mg/mL in a 5%-DMSO-20%-ACN-containing 0.01 M ammonium acetate buffer (pH 6.5) solution, and then stirred for 3 days while being exposed to air. Progression of a disulfide bond-forming reaction was monitored by HPLC. When the reaction was found not to progress any more, the reaction solution was freeze-dried to obtain a peptide precipitate.

[1065] (d) Purification

[1066] The peptide precipitate obtained by freeze-drying in the step (c) was separated under the prep-LC primary purification conditions listed in the following Table 8, further purified under the prep-LC secondary purification conditions listed in the following Table 9, and then freeze-dried. The resulting peptide was confirmed to have a purity of 90% or more by analytical HPLC, and the molecular weight of the synthesized peptide was confirmed using an LC/MS mass spectrometer.

[1067] H-PEG8-Asp-Cys*-Ala-Trp-His-Dab-Gly-Glu-Leu-Val-Trp-Cys*-Thr-NH_2 (Cys*: disulfide bonding sites)

<Table 8> Prep-LC primary purification condition

Prep-LC primary purification condition			
Equipment name	Waters 2525 pump, Waters 2487 UV detector		
Column	Waters, XBridge Prep C18 5 μm OBD 19 x 250 mm		
Mobile phase A/B	0.1% TFA-20% ACN / 0.1% TFA in 80% ACN		
Gradient	0 min → 30 min : B 0% → B 100%		
Flow rate	17 mL/min	Detection wavelength	UV 280 nm

<Table 9> Prep-LC secondary purification condition

Prep-LC secondary purification condition			
Equipment name	Waters 2525 pump, Waters 2487 UV detector		
Column	Waters, XBridge Prep C18 5 μm OBD 19 x 250 mm		
Mobile phase A/B	0.1% TFA-30% ACN / 0.1% TFA in 70% ACN		
Gradient	0 min → 30 min : B 0% → B 100%		
Flow rate	15 mL/min	Detection wavelength	UV 280 nm

[1068] Confirmation of structure of SSFI (6Dab)

[1069] Synthesis of SSFI (6Dab) was confirmed by molecular weight measurement by matrix-assisted laser desorption/ionization (MALDI) mass spectrometry.

[1070] Measuring equipment: Ultraflextreme (Bruker)

[1071] Measuring matrix: CHCA (α-Cyano-4-hydroxycinnamic acid) & DHB (2,5-Dihydroxybenzoic acid)

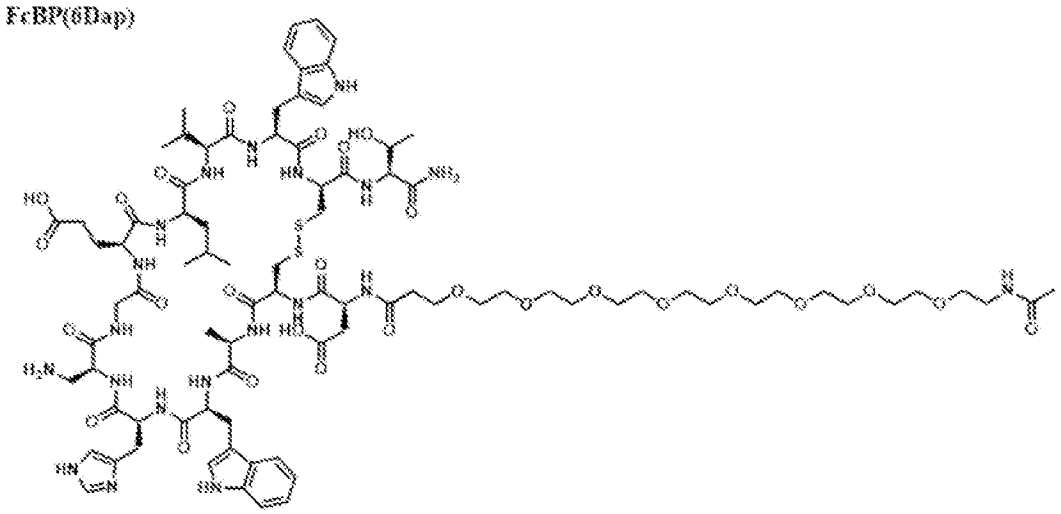
[1072] Calculated molecular weight: 1982.24 g/mol

[1073] Measured molecular weight $(M+2H)^{2+}$: 992.33 g/mol

[1074] The mass spectrometry results of SSFI (6Dab) are shown in FIG. 26.

[1075]

[1076] 1.2.4. Synthesis and confirmation of structure of SSFI (where Xa_1 is diaminopropionic acid (Dap))



[1077] Synthesis of SSFI (6Dap)

[1078] Sequence: Nor.-PEG8-DCAWHA(beta amino alanine, Dap)GELVWCT-$CONH_2$

[1079] List and order of introduction of Fmoc amino acids used

[1080] Fmoc-L-Thr(tBu)-OH, Fmoc-Cys(Trt)-OH, Fmoc-L-Trp(Boc)-OH, Fmoc-L-Val-OH, Fmoc-L-Leu-OH, Fmoc-L-Glu(OtBu)-OH, Fmoc-Gly-OH, Fmoc-Dap(Boc)-OH, Fmoc-L-His(Trt)-OH, Fmoc-L-Trp(Boc)-OH Fmoc-Ala-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(tBu)-OH.

[1081] Preparation method

[1082] (a) Introduction of amino acids

[1083] Amounts of reagents used in the following process were based on 0.25 mmole. 0.5 g (0.48 mmole/g) of a clear amide resin (Peptide International Inc., USA) was put into a synthesis reactor, and 1 mmole of each Fmoc-amino acid block was weighed to prepare a peptide amino acid sequence from the C-terminus to the N-terminus in the above order.

[1084] A reaction for activating an Fmoc-amino acid to attach the activated residue to a clear amide resin was performed sequentially from the C-terminal amino acid.

[1085] Removal of Fmoc was performed in 20% piperidine-containing DMF, and activation and introduction of the residue was performed by mixing amino acids prepared to correspond to the sequence with 2 mL of a 0.5 M HOBt-containing DMF solution, 2 mL of a 0.5 M HBTU-

containing DMF solution, and 174 μL of DIPEA for 5 minutes, and mixing the resulting mixture for 2 hours in a reactor containing the resin.

[1086] Confirmation of an introduction reaction was carried out using a Kaiser test method. When the introduction reaction was confirmed to be incomplete, the introduction reaction was repeated once more, or capping was performed using a 20% Ac_2O-containing DMF solution. In each of the introduction reaction and the Fmoc removal, the resin was thoroughly washed with DMF and DCM before moving to the next step. This process was repeatedly performed until the targeted peptide sequence was completed.

[1087] (b) Introduction of H-PEG8-OH

[1088] To introduce H-PEG_8-OH to the N-terminus of the sequence after the introduction of the amino acids was completed, 1 mL of a 0.5 M Fmoc-N-amido-dPEG8-acid-in-DMF solution, 1 mL of a 0.5 M HBTU-containing DMF solution, 1 mL of a 0.5 M HOBt-containing DMF solution, and 87 μL of DIPEA were mixed for 5 minutes, and the resulting mixture was mixed for 2 hours in a reactor containing a reactive resin.

[1089] Progression of the reaction was monitored by the Kaiser test method. When the unreacted amine was found to remain, the reaction time was extended for another 1 to 3 hours, or the reaction solution was discarded, and the aforementioned reaction process was repeated again. Removal of the N-terminal Fmoc protective group was performed using 20% piperidine-containing DMF, and the resin to which the peptide was attached was then dried and weighed.

[1090] (c) 250 mg of the resin to which the peptide was attached as prepared in the step (b) was stirred with 2 mL of a mixed solution of TFA, TIS, water and EDT (94:1.0:2.5:2.5) at room temperature for 120 minutes to cleave the peptide from the resin. The cleaved mixture was filtered, and the filtrate was concentrated to about half its volume using nitrogen gas. Thereafter, ether was poured into the mixture to precipitate the peptide. The precipitated peptide was washed three times with ether, and dried under nitrogen gas. The dried precipitate was dissolved in 0.1% TFA-30% ACN-containing water, stirred for 6 hours, and then concentrated.

[1091] The concentrate was dissolved at a concentration of 0.1 mg/mL in a 5%-DMSO-20%-ACN-containing 0.01 M ammonium acetate buffer (pH 6.5) solution, and then stirred for 3 days while being exposed to air. Progression of a disulfide bond-forming reaction was monitored by HPLC. When the reaction was found not to progress any more, the reaction solution was freeze-dried to obtain a peptide precipitate.

[1092] (d) Purification

[1093] The peptide precipitate obtained by freeze-drying in the step (c) was separated under the prep-LC primary purification conditions listed in the following Table 10, further purified

under the prep-LC secondary purification conditions listed in the following Table 11, and then freeze-dried. The resulting peptide was confirmed to have a purity of 90% or more by analytical HPLC, and the molecular weight of the synthesized peptide was confirmed using an LC/MS mass spectrometer.

[1094] H-PEG8-Asp-Cys*-Ala-Trp-His-Dap-Gly-Glu-Leu-Val-Trp-Cys*-Thr-NH_2 (Cys*: disulfide bonding sites)

<Table 10> Prep-LC primary purification condition

Prep-LC primary purification condition			
Equipment name	Waters 2525 pump, Waters 2487 UV detector		
Column	Waters, XBridge Prep C18 5 μm OBD 19 x 250 mm		
Mobile phase A/B	0.1% TFA-20% ACN / 0.1% TFA in 80% ACN		
Gradient	0 min → 30 min : B 0% → B 100%		
Flow rate	17 mL/min	Detection wavelength	UV 280 nm

<Table 12> Prep-LC secondary purification condition

Prep-LC secondary purification condition			
Equipment name	Waters 2525 pump, Waters 2487 UV detector		
Column	Waters, XBridge Prep C18 5 μm OBD 19 x 250 mm		
Mobile phase A/B	0.1% TFA-30% ACN / 0.1% TFA in 70% ACN		
Gradient	0 min → 30 min : B 0% → B 100%		
Flow rate	15 mL/min	Detection wavelength	UV 280 nm

[1095] Confirmation of structure of SSFI (6Dap)

[1096] Measuring equipment: Quattro Premier Xe (Waters)

[1097] Calculated molecular weight: 1968.21 g/mol

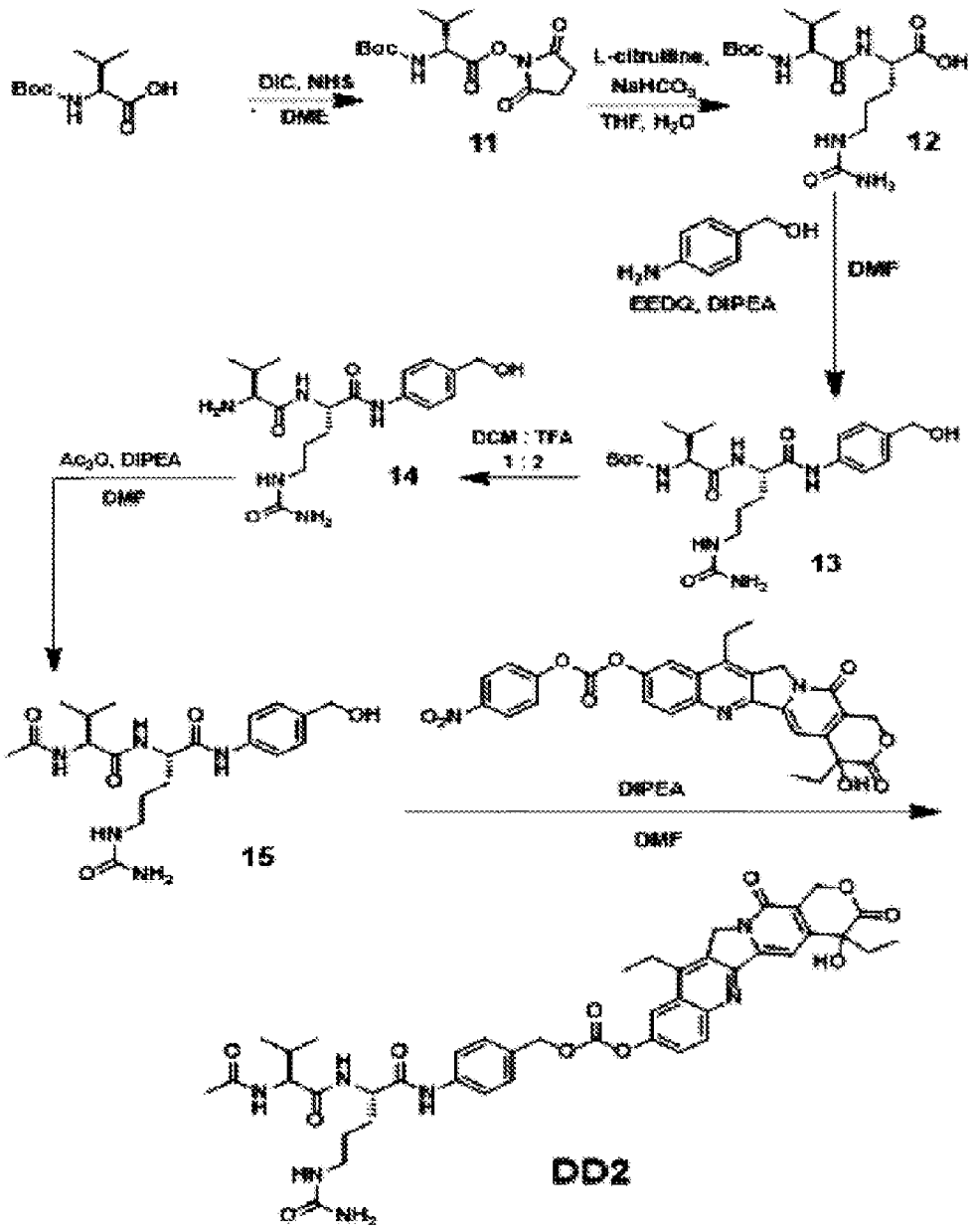
[1098] Measured molecular weight $(M+2H)^{2+}$: 984.71 g/mol

[1099] The mass spectrometry results of SSFI (6Dap) are shown in FIG. 27.

[1100] 1.3. Synthesis and confirmation of structure of VC linker

[1101] 1.3.1. Synthesis and confirmation of structure of VC linker (DD2)

[1102] [Scheme 10]



[1103] Synthesis of VC linker (DD2)

[1104] Synthesis of Compound 11

[1105] 5 g (14.7 mmol, 1.0 eq.) of Fmoc-Val-OH and 1.7 g (14.7 mmol, 1.0 eq.) of N-hydroxysuccinimide (NHS) were dissolved in 140 mL of dimethoxyethane (DME), and stirred. 2.5 mL (16.2 mmol, 1.1 eq.) of N,N'-diisopropylcarbodiimide (DIC) was added dropwise at 0°C, and stirred for 16 hours. The reaction solution was filtered under reduced pressure to remove floating matter, and the filtrate was concentrated under reduced pressure. The residue was dissolved in acetone, and stored at a low temperature for 4 hours in a refrigerator. Thereafter, the resulting solution was filtered under reduced pressure to remove the re-formed floating matter,

and used in the next reaction without any purification (crude yield: 5.5 g, 86%). TLC (EA:Hex = 1:1); R_f = 0.5.

[1106] Synthesis of Compound 12

[1107] 2.0 g (11.5 mmol, 1.0 eq.) of L-citrulline was dissolved in 100 mL of a 1:1 mixed solution of tetrahydrofuran (THF) and water, and stirred. 988 mg of sodium hydrogen carbonate was added thereto, and stirred. Thereafter, 5.0 g (11.5 mmol, 1.0 eq.) of Compound 8 was dissolved in 80 mL of acetone, added dropwise to the reaction solution, and then stirred. The resulting mixture was stirred for 21 hours, and then concentrated under reduced pressure to remove the organic solvent. An aqueous layer was washed with ethyl acetate (EA), and then titrated to pH 3 by slow dropwise addition of 2 N HCl. EA was added thereto so that the precipitate in an organic layer was extracted. Then, the organic layer was dried over a saturated saline solution and sodium sulfate, and used in the next reaction without any purification (crude yield: 5.6 g, 98%). TLC (DCM:MeOH = 10:1, one drop of formic acid); R_f = 0.1.

[1108] Synthesis of Compound 13

[1109] 50 mL of 10% piperidine in N,N-dimethylformamide (DMF) was added dropwise to 2.84 g (5.72 mmol, 1.0 eq.) of Compound 12, and stirred. After 4 hours, the resulting mixture was concentrated under reduced pressure to remove a reaction solution, and the residue was dissolved in water, and filtered under reduced pressure to remove the formed floating matter. An aqueous layer was concentrated to obtain a target compound, which was used in the next reaction without any purification. TLC (DCM:MeOH = 10:1); R_f = 0.01.

[1110] Synthesis of Compound 14

[1111] 54 mg (0.22 mmol, 1.0 eq.) of Compound 10 was dissolved in 2 mL of DMF, and 0.05 mL (0.264 mmol, 1.2 eq.) of N,N-diisopropylethylamine (DIPEA) was added dropwise thereto. Compound 13 was completely dissolved by adding 2 mL of water, and 0.05 mL (0.528 mmol, 2.4 eq.) of an acetic anhydride was added dropwise thereto at room temperature. After 3 hours, the resulting mixture was concentrated under reduced pressure to remove a reaction solution, and the concentrate was purified by reversed-phase column chromatography to obtain a target compound. TLC (DCM:MeOH = 10:1); R_f = 0.05.

[1112] Synthesis of Compound 15

[1113] 1 g (3.16 mmol, 1.0 eq.) of Compound 14 was dissolved in 30 mL of a 2:1 mixed solution of DCM and methanol, and 868 mg (3.48 mmol, 1.1 eq.) of N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) was added thereto, and stirred. 451 mg (3.66 mmol, 1.16 eq.) of 4-aminobenzyl alcohol was added thereto, and stirred for 5 hours. The resulting mixture was concentrated under reduced pressure to remove a reaction solution, and the concentrate was

purified by column chromatography (10% MeOH in DCM) to obtain 419 mg of a target compound (yield: 32%). TLC (DCM:MeOH = 10:1); R_f = 0.1.

[1114] Synthesis of Compound DD2

[1115] 109 mg (0.3 mmol, 1.2 eq.) of phenol-activated SN38 and 0.06 mL (0.33 mmol, 1.3 eq.) of DIPEA were added dropwise to a solution obtained by dissolving 105 mg (0.25 mmol, 1.0 eq.) of Compound 15 in 10 mL of DMF. The resulting mixture was stirred for 20 hours, and then concentrated under reduced pressure to remove a reaction solution. The concentrate was purified by column chromatography (10% MeOH in DCM) to obtain a target compound. TLC (DCM:MeOH = 10:1); R_f = 0.2.

[1116] Confirmation of structure of VC linker (DD2)

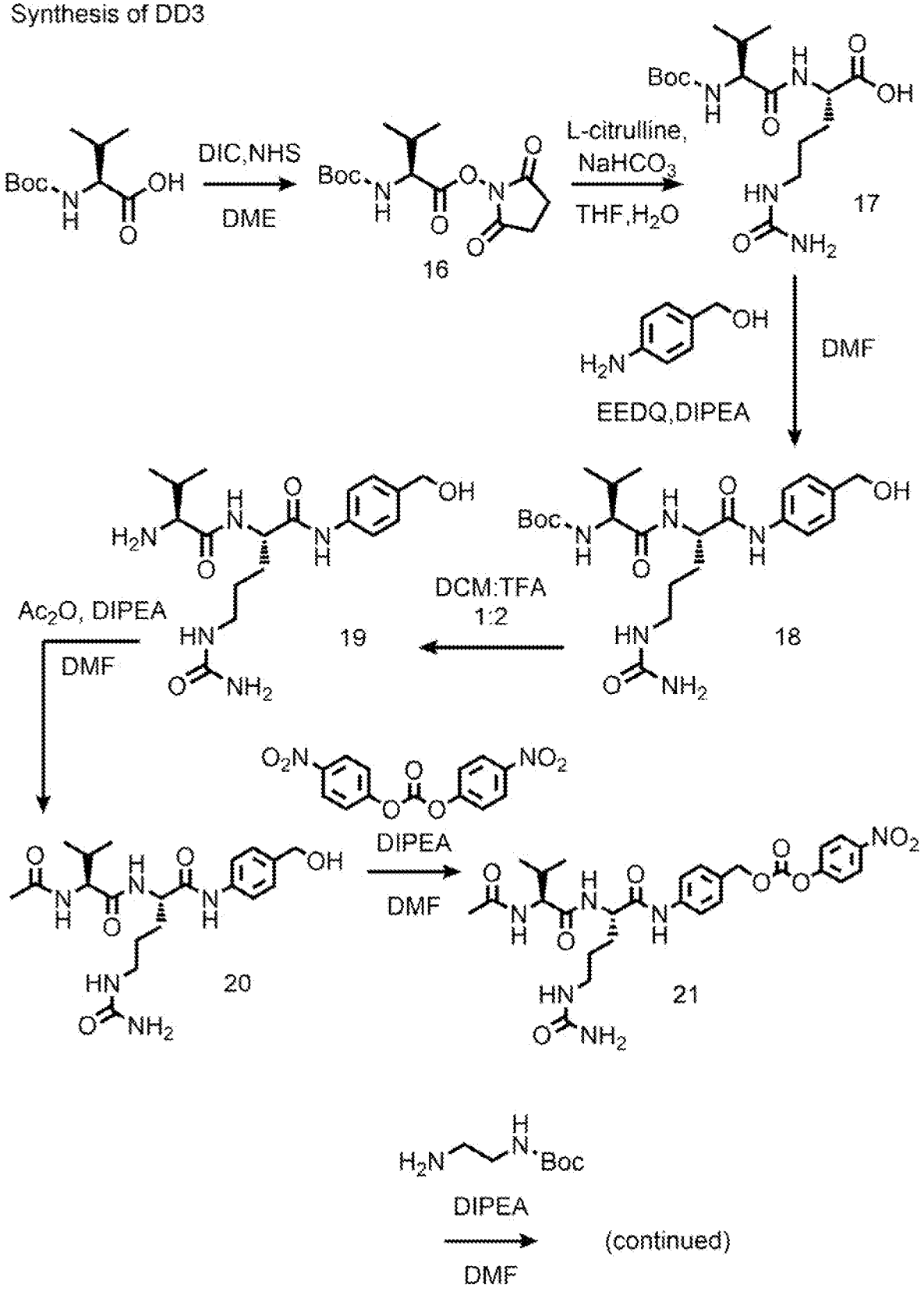
[1117] LRMS (ESI): m/z 840.4 [$M+H^+$]

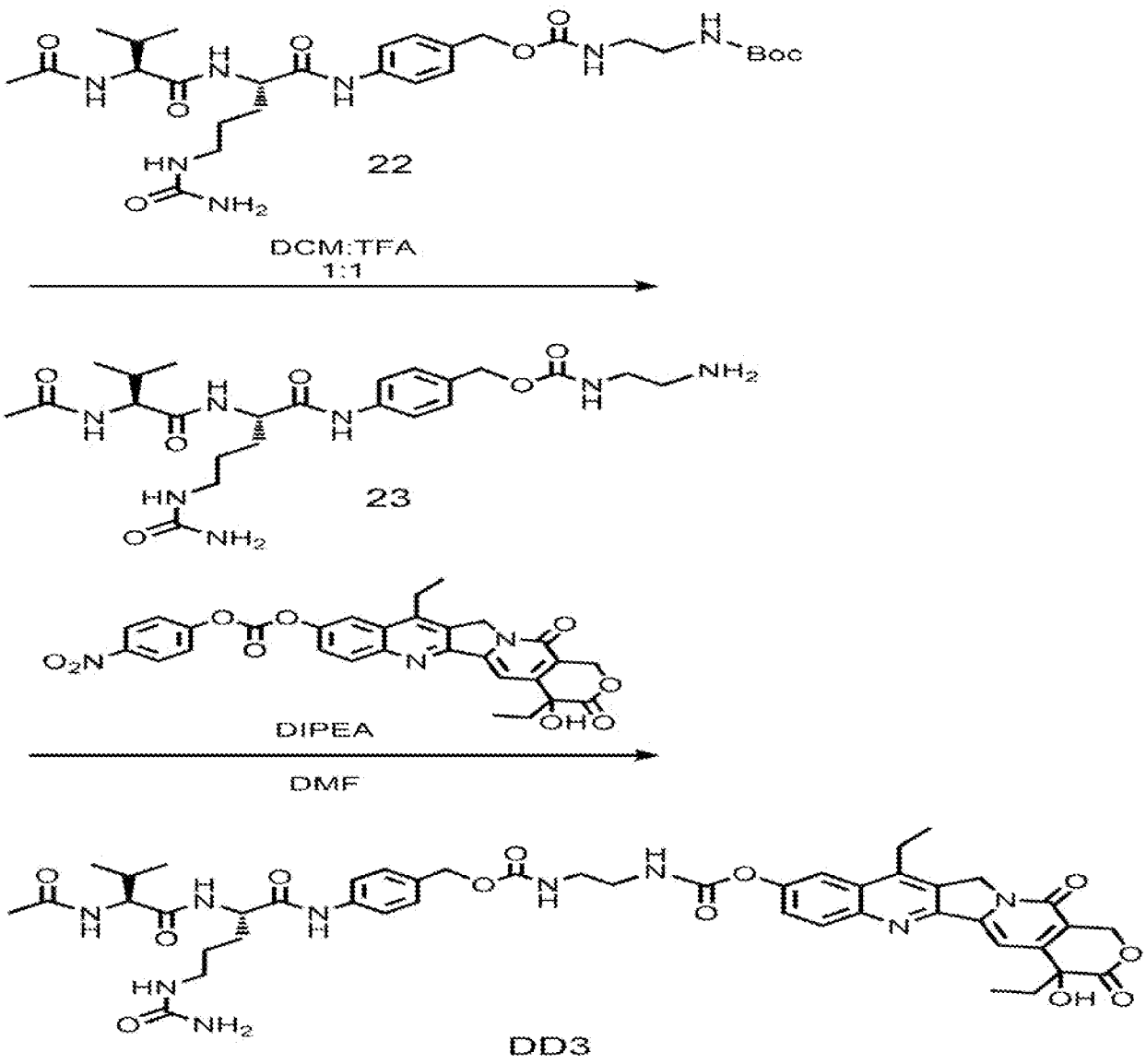
[1118] The results are shown in FIG. 28.

[1119] 1.3.2. Synthesis and confirmation of structure of VC linker (DD3)

[1120] [Scheme 11]

Synthesis of DD3





[1121] Synthesis of VC linker (DD3)

[1122] Synthesis of Compound 16

[1123] 5 g (14.7 mmol, 1.0 eq.) of Fmoc-Val-OH and 1.7 g (14.7 mmol, 1.0 eq.) of N-hydroxysuccinimide (NHS) were dissolved in 140 mL of dimethoxyethane (DME), and stirred. 2.5 mL (16.2 mmol, 1.1 eq.) of N,N'-diisopropylcarbodiimide (DIC) was added dropwise at 0°C, and stirred for 16 hours. The reaction solution was filtered under reduced pressure to remove floating matter, and the filtrate was concentrated under reduced pressure. The residue was dissolved in acetone, and stored at a low temperature for 4 hours in a refrigerator. Thereafter, the resulting solution was filtered under reduced pressure to remove the re-formed floating matter, and used in the next reaction without any purification (crude yield: 5.5 g, 86%). TLC (EA:Hex = 1:1); R_f = 0.5.

[1124] Synthesis of Compound 17

[1125] 2.0 g (11.5 mmol, 1.0 eq.) of L-citrulline was dissolved in 100 mL of a 1:1 mixed solution of tetrahydrofuran (THF) and water, and stirred. 988 mg of sodium hydrogen carbonate was added thereto, and stirred. Thereafter, 5.0 g (11.5 mmol, 1.0 eq.) of Compound 16 was dissolved in 80 mL of acetone, added dropwise to the reaction solution, and then stirred. The resulting mixture was stirred for 21 hours, and then concentrated under reduced pressure to remove the organic solvent. An aqueous layer was washed with ethyl acetate (EA), and then titrated to pH 3 by dropwise addition of 2 N HCl. EA was added thereto so that the precipitate in an organic layer was extracted. Then, the organic layer was dried over a saturated saline solution and sodium sulfate, and used in the next reaction without any purification (crude yield: 5.6 g, 98%). TLC (DCM:MeOH = 10:1, one drop of formic acid); R_f = 0.1.

[1126] Synthesis of Compound 18

[1127] 50 mL of 10% piperidine in N,N-dimethylformamide (DMF) was added dropwise to 2.84 g (5.72 mmol, 1.0 eq.) of Compound 17, and stirred. After 4 hours, the resulting mixture was concentrated under reduced pressure to remove a reaction solution, and the residue was dissolved in water, and filtered under reduced pressure to remove the formed floating matter. An aqueous layer was concentrated to obtain a target compound, which was used in the next reaction without any purification. TLC (DCM:MeOH = 10:1); R_f = 0.01.

[1128] Synthesis of Compound 19

[1129] 54 mg (0.22 mmol, 1.0 eq.) of Compound 18 was dissolved in 2 mL of DMF, and 0.05 mL (0.264 mmol, 1.2 eq.) of N,N-diisopropylethylamine (DIPEA) was added dropwise thereto. Compound 15 was completely dissolved by adding 2 mL of water, and 0.05 mL (0.528 mmol, 2.4 eq.) of an acetic anhydride was added dropwise thereto at room temperature. After 3 hours, the resulting mixture was concentrated under reduced pressure to remove a reaction solution, and the concentrate was purified by reversed-phase column chromatography to obtain a target compound. TLC (DCM:MeOH = 10:1); R_f = 0.05.

[1130] Synthesis of Compound 20

[1131] 1 g (3.16 mmol, 1.0 eq.) of Compound 19 was dissolved in 30 mL of a 2:1 mixed solution of DCM and methanol, and 868 mg (3.48 mmol, 1.1 eq.) of N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) was added thereto, and stirred. 451 mg (3.66 mmol, 1.16 eq.) of 4-aminobenzyl alcohol was added thereto, and stirred for 5 hours. The resulting mixture was concentrated under reduced pressure to remove a reaction solution, and the concentrate was purified by column chromatography (10% MeOH in DCM) to obtain 419 mg of a target compound (yield: 32%). TLC (DCM:MeOH = 10:1); R_f = 0.1.

[1132] Synthesis of Compound 21

[1133] 240 mg (0.55 mmol, 1.0 eq.) of Compound 20 was dissolved in 10 mL of DMF, and 0.3 mL (1.65 mmol, 3.0 eq.) of DIPEA was added dropwise thereto, and stirred. Thereafter, a reaction was carried out by adding 509 mg (1.65 mmol, 3.0 eq.) of bis-(4-aminophenyl) carbonate. After 3 hours, the resulting mixture was concentrated under reduced pressure to remove a reaction solution, and diethyl ether was added thereto to precipitate a target compound, which was filtered under reduced pressure. The obtained target compound was used in the next reaction without any purification.

[1134] Synthesis of Compound 22

[1135] 33 mg (0.06 mmol, 1.0 eq.) of Compound 21 was dissolved in 5 mL of DMF, and 16 mg (0.08 mmol, 1.3 eq.) of 1-[(tert-butoxycarbonyl)amino]-2-aminoethane and 0.02 mL (0.08 mmol, 1.3 eq.) of DIPEA were added dropwise thereto, and stirred. The resulting mixture was stirred for 7 hours, and then concentrated under reduced pressure to remove a reaction solution, and diethyl ether was added thereto to precipitate a target compound, which was filtered under reduced pressure. The obtained target compound was used in the next reaction without any purification. TLC (DCM:MeOH = 10:1); R_f = 0.1.

[1136] Synthesis of Compound 23

[1137] 17 mg (0.03 mmol, 1.0 eq.) of Compound 22 was dissolved in 1 mL of DCM, and 1 mL of trifluoroacetic acid (TFA) was then added dropwise thereto at 0°C. A reaction was carried out by stirring the resulting mixture at 0°C for 30 minutes, followed by stirring at room temperature for 30 minutes. Thereafter, the mixture was concentrated under reduced pressure to remove a reaction solution such as DCM, and this concentration was then repeated three times. The concentrated matter was dried under high vacuum, and purified by reversed-phase column chromatography to obtain 13 mg of a target compound. TLC (DCM:MeOH = 10:1); R_f = 0.01.

[1138] Synthesis of Compound DD3

[1139] 0.01 mL (0.04 mmol, 1.5 eq.) of DIPEA was added dropwise to a solution obtained by dissolving 13 mg (0.03 mmol, 1.0 eq.) of Compound 23 in 3 mL of DMF, and 22 mg (0.04 mmol, 1.5 eq.) of phenol-activated SN38 was added thereto. The resulting mixture was stirred for an hour, and then concentrated under reduced pressure to remove a reaction solution. The concentrate was purified by column chromatography (10% MeOH in DCM) to obtain a target compound. TLC (DCM:MeOH = 10:1); R_f = 0.2.

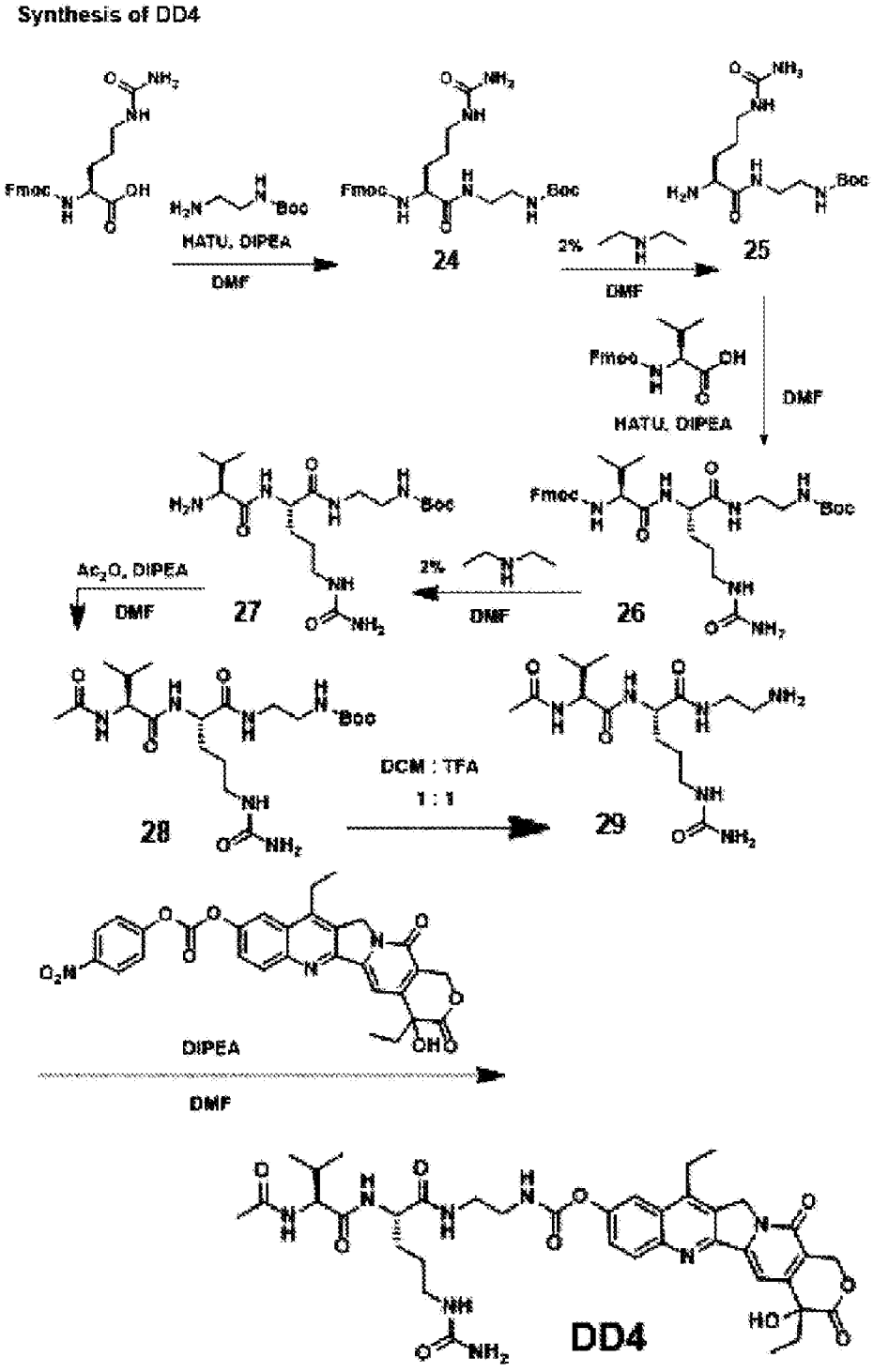
[1140] Confirmation of structure of VC linker (DD3)

[1141] LRMS (ESI): m/z 926.4 [$M+H^+$]

[1142] The results are shown in FIG. 29.

[1143] 1.3.3. Synthesis and confirmation of structure of VC linker (DD4)

[1144] [Scheme 12]



[1145] Synthesis of VC linker (DD4)

[1146] Synthesis of Compound 24

[1147] 5 g (14.7 mmol, 1.0 eq.) of Fmoc-Val-OH and 1.7 g (14.7 mmol, 1.0 eq.) of N-hydroxysuccinimide (NHS) were dissolved in 140 mL of dimethoxyethane (DME), and stirred. 2.5 mL (16.2 mmol, 1.1 eq.) of N,N'-diisopropylcarbodiimide (DIC) was added dropwise at 0°C, and stirred for 16 hours. The reaction solution was filtered under reduced pressure to remove floating matter, and the filtrate was concentrated under reduced pressure. The residue was dissolved in acetone, and stored at a low temperature for 4 hours in a refrigerator. Thereafter, the resulting solution was filtered under reduced pressure to remove the re-formed floating matter, and used in the next reaction without any purification (crude yield: 5.5 g, 86%). TLC (EA:Hex = 1:1); R_f = 0.5.

[1148] Synthesis of Compound 25

[1149] 2.0 g (11.5 mmol, 1.0 eq.) of L-citrulline was dissolved in 100 mL of a 1:1 mixed solution of tetrahydrofuran (THF) and water, and stirred. 988 mg of sodium hydrogen carbonate was added thereto, and stirred. Thereafter, 5.0 g of Compound 21 (11.5 mmol, 1.0 eq.) was dissolved in 80 mL of acetone, added dropwise to the reaction solution, and then stirred. The resulting mixture was stirred for 21 hours, and then concentrated under reduced pressure to remove the organic solvent. An aqueous layer was washed with ethyl acetate (EA), and then titrated to pH 3 by slow dropwise addition of 2 N HCl. EA was added thereto so that the precipitate in an organic layer was extracted. Then, the organic layer was dried over a saturated saline solution and sodium sulfate, and used in the next reaction without any purification (crude yield: 5.6 g, 98%). TLC (DCM:MeOH = 10:1, one drop of formic acid); R_f = 0.1.

[1150] Synthesis of Compound 26

[1151] 50 mL of 10% piperidine in N,N-dimethylformamide (DMF) was added dropwise to 2.84 g (5.72 mmol, 1.0 eq.) of Compound 25, and stirred. After 4 hours, the resulting mixture was concentrated under reduced pressure to remove a reaction solution, and the residue was dissolved in water, and filtered under reduced pressure to remove the formed floating matter. An aqueous layer was concentrated to obtain a target compound, which was used in the next reaction without any purification. TLC (DCM:MeOH = 10:1); R_f = 0.01.

[1152] Synthesis of Compound 27

[1153] 54 mg (0.22 mmol, 1.0 eq.) of Compound 26 was dissolved in 2 mL of DMF, and 0.05 mL (0.264 mmol, 1.2 eq.) of N,N-diisopropylethylamine (DIPEA) was added dropwise thereto. Compound 23 was completely dissolved by adding 2 mL of water, and 0.05 mL (0.528 mmol, 2.4 eq.) of an acetic anhydride was added dropwise thereto at room temperature. After 3 hours, the resulting mixture was concentrated under reduced pressure to remove a reaction solution, and the concentrate was purified by reversed-phase column chromatography to obtain a target

compound. TLC (DCM:MeOH = 10:1); R_f = 0.05.

[1154] Synthesis of Compound 28

[1155] 300 mg (0.95 mmol, 1.0 eq.) of Compound 27 was dissolved in 6 mL of DMF, and 550 mg (1.43 mmol, 1.5 eq.) of 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (HATU) was then added thereto. Thereafter, 0.25 mL (1.43 mmol, 1.5 eq.) of DIPEA was added dropwise, and 230 mg (1.43 mmol, 1.5 eq.) of 1-[(tert-butoxycarbonyl)amino]-2-aminoethane was dissolved in 3.5 mL of DMF, and added dropwise at room temperature. After 7 hours, the reaction solution was diluted with ethyl acetate (EA), and an organic layer was washed with a saturated sodium hydrogen carbonate solution. Then, the organic layer was dried over a saturated saline solution and sodium sulfate, and filtered to obtain a target compound. The obtained target compound was used in the next reaction without any purification. TLC (DCM:MeOH = 10:1); R_f = 0.5.

[1156] Synthesis of Compound 29

[1157] Compound 28 was dissolved in a 2:1 mixed solution of DCM and TFA, and a reaction was then carried out at 0°C. Thereafter, the reaction mixture was concentrated under reduced pressure to remove a reaction solution such as DCM, and this concentration was then repeated three times. The residue was dried under high vacuum, and purified by reversed-phase column chromatography to obtain 370 mg of a target compound. TLC (DCM:MeOH = 10:1); R_f = 0.01.

[1158] Synthesis of Compound DD4

[1159] 0.03 mL (0.17 mmol, 1.2 eq.) of DIPEA was added dropwise to a solution obtained by dissolving 50 mg (0.14 mmol, 1.0 eq.) of Compound 26 in 3 mL of DMF, and 107 mg (0.21 mmol, 1.5 eq.) of phenol-activated SN38 was added thereto. The resulting mixture was stirred for an hour, and then concentrated under reduced pressure to remove a reaction solution. The concentrate was purified by column chromatography (10% MeOH in DCM) to obtain a target compound. TLC (DCM:MeOH = 10:1); R_f = 0.1.

[1160] Confirmation of structure of VC linker (DD4)

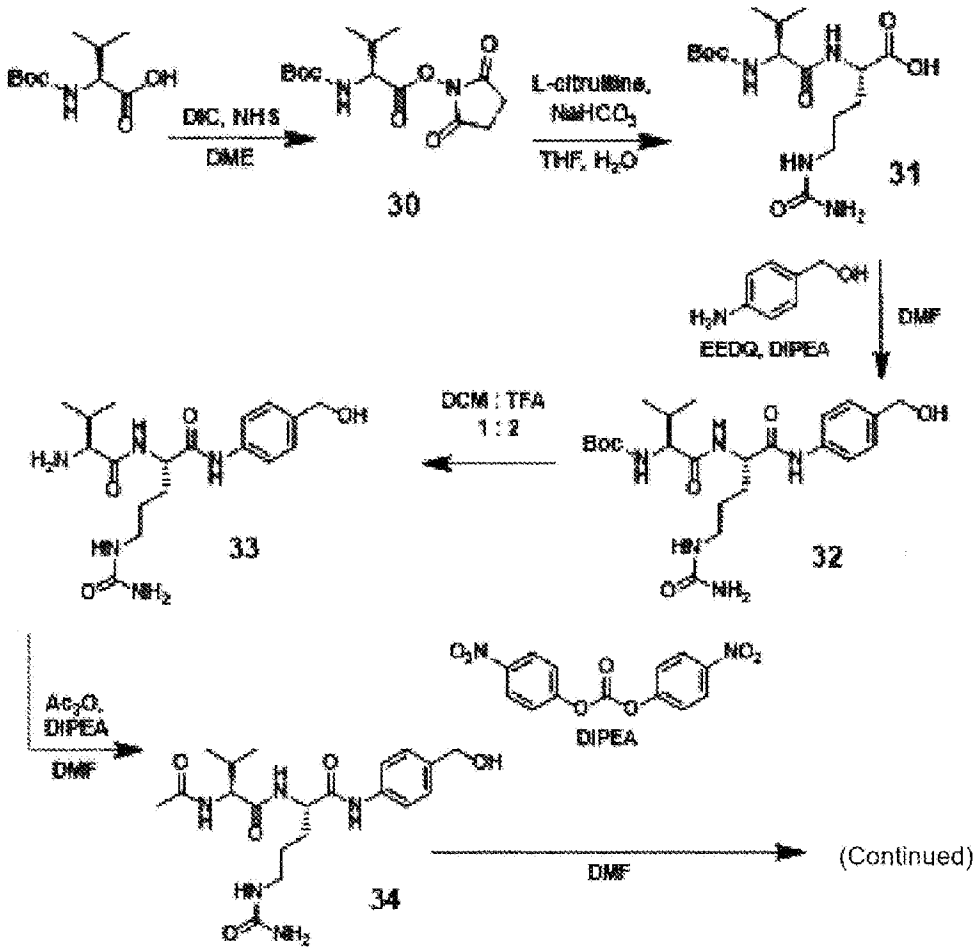
[1161] LRMS (ESI): m/z 777.3 [$M+H^+$]

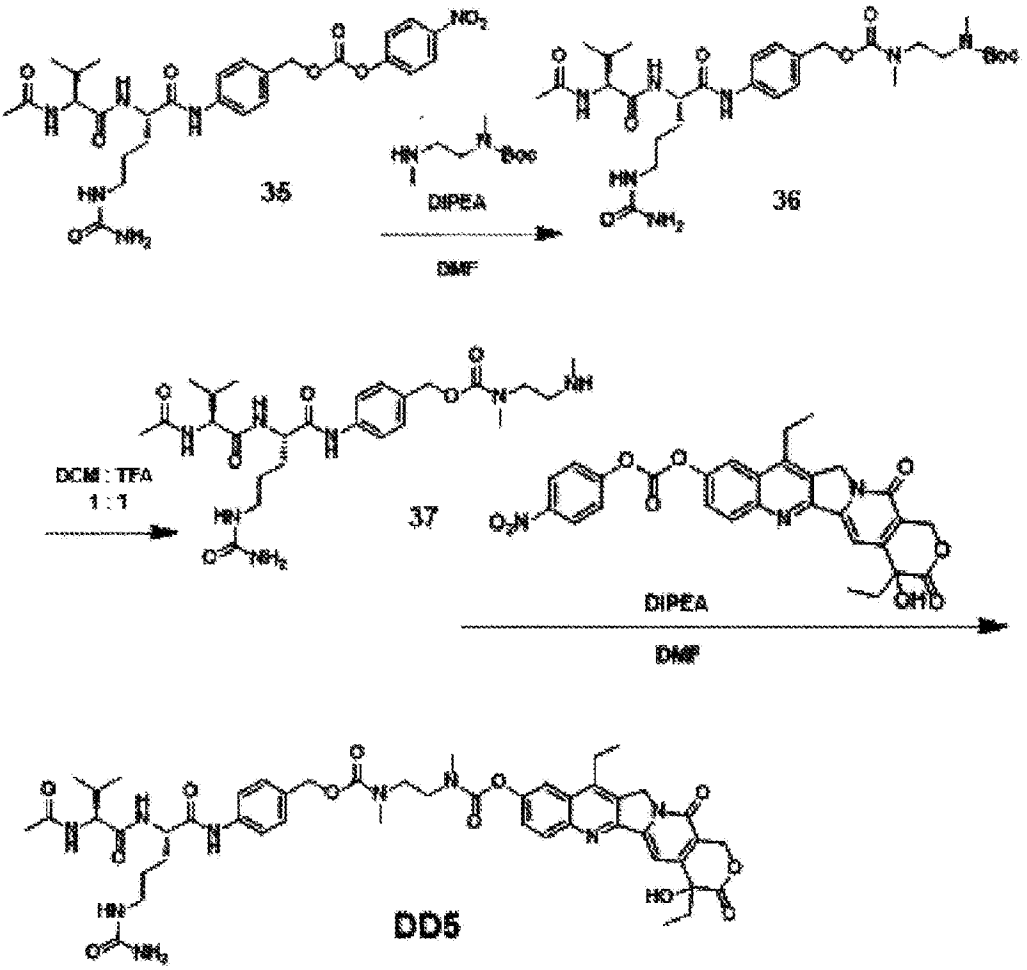
[1162] The results are shown in FIG. 30.

[1163] 1.3.4. Synthesis and confirmation of structure of VC linker (DD5)

[1164] [Scheme 13]

Synthesis of DD5





[1165] Synthesis of VC linker (DD5)

[1166] Synthesis of Compound 30

[1167] 5 g (14.7 mmol, 1.0 eq.) of Fmoc-Val-OH and 1.7 g (14.7 mmol, 1.0 eq.) of N-hydroxysuccinimide (NHS) were dissolved in 140 mL of dimethoxyethane (DME), and stirred. 2.5 mL (16.2 mmol, 1.1 eq.) of N,N'-diisopropylcarbodiimide (DIC) was added dropwise at 0°C, and stirred for 16 hours. The reaction solution was filtered under reduced pressure to remove floating matter, and the filtrate was concentrated under reduced pressure. The residue was dissolved in acetone, and stored at a low temperature for 4 hours in a refrigerator. Thereafter, the resulting solution was filtered under reduced pressure to remove the re-formed floating matter, and used in the next reaction without any purification (crude yield: 5.5 g, 86%). TLC (EA:Hex = 1:1); R_f = 0.5.

[1168] Synthesis of Compound 31

[1169] 2.0 g (11.5 mmol, 1.0 eq.) of L-citrulline was dissolved in 100 mL of a 1:1 mixed

solution of tetrahydrofuran (THF) and water, and stirred. 988 mg of sodium hydrogen carbonate was added thereto, and stirred. Thereafter, 5.0 g (11.5 mmol, 1.0 eq.) of Compound 30 was dissolved in 80 mL of acetone, added dropwise to the reaction solution, and then stirred. The resulting mixture was stirred for 21 hours, and then concentrated under reduced pressure to remove the organic solvent. An aqueous layer was washed with ethyl acetate (EA), and then titrated to pH 3 by slow dropwise addition of 2 N HCl. EA was added thereto so that the precipitate in an organic layer was extracted. Then, the organic layer was dried over a saturated saline solution and sodium sulfate, and used in the next reaction without any purification (crude yield: 5.6 g, 98%). TLC (DCM:MeOH = 10:1, one drop of formic acid); R_f = 0.1.

[1170] Synthesis of Compound 32

[1171] 50 mL of 10% piperidine in N,N-dimethylformamide (DMF) was added dropwise to 2.84 g (5.72 mmol, 1.0 eq.) of Compound 31, and stirred. After 4 hours, the resulting mixture was concentrated under reduced pressure to remove a reaction solution, and the residue was dissolved in water, and filtered under reduced pressure to remove the formed floating matter. An aqueous layer was concentrated to obtain a target compound, which was used in the next reaction without any purification. TLC (DCM:MeOH = 10:1); R_f = 0.01.

[1172] Synthesis of Compound 33

[1173] 54 mg (0.22 mmol, 1.0 eq.) of Compound 29 was dissolved in 2 mL of DMF, and 0.05 mL (0.264 mmol, 1.2 eq.) of N,N-diisopropylethylamine (DIPEA) was added dropwise thereto. Compound 32 was completely dissolved by adding 2 mL of water, and 0.05 mL (0.528 mmol, 2.4 eq.) of an acetic anhydride was added dropwise thereto at room temperature. After 3 hours, the resulting mixture was concentrated under reduced pressure to remove a reaction solution, and the concentrate was purified by reversed-phase column chromatography to obtain a target compound. TLC (DCM:MeOH = 10:1); R_f = 0.05.

[1174] Synthesis of Compound 34

[1175] 1 g (3.16 mmol, 1.0 eq.) of Compound 33 was dissolved in 30 mL of a 2:1 mixed solution of DCM and methanol, and 868 mg (3.48 mmol, 1.1 eq.) of N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) was added thereto, and stirred. 451 mg (3.66 mmol, 1.16 eq.) of 4-aminobenzyl alcohol was added thereto, and stirred for 5 hours. The resulting mixture was concentrated under reduced pressure to remove a reaction solution, and the concentrate was purified by column chromatography (10% MeOH in DCM) to obtain 419 mg of a target compound (yield: 32%). TLC (DCM:MeOH = 10:1); R_f = 0.1.

[1176] Synthesis of Compound 35

[1177] 240 mg (0.55 mmol, 1.0 eq.) of Compound 34 was dissolved in 10 mL of DMF, and

0.3 mL (1.65 mmol, 3.0 eq.) of DIPEA was added dropwise thereto, and stirred. Thereafter, a reaction was carried out by adding 509 mg (1.65 mmol, 3.0 eq.) of bis-(4-aminophenyl) carbonate. After 3 hours, the resulting mixture was concentrated under reduced pressure to remove a reaction solution, and diethyl ether was added thereto to precipitate a target compound, which was filtered under reduced pressure. The obtained target compound was used in the next reaction without any purification.

[1178] Synthesis of Compound 36

[1179] 33 mg (0.06 mmol, 1.0 eq.) of Compound 35 was dissolved in 5 mL of DMF, and 16 mg (0.08 mmol, 1.3 eq.) of N-[(tert-butoxy)carbonyl]-N,N'-dimethylethylenediamine and 0.02 mL (0.08 mmol, 1.3 eq.) of DIPEA were added dropwise thereto, and stirred. The resulting mixture was stirred for 7 hours, and then concentrated under reduced pressure to remove a reaction solution, and diethyl ether was added thereto to precipitate a target compound, which was filtered under reduced pressure. The obtained target compound was used in the next reaction without any purification. TLC (DCM:MeOH = 10:1); R_f = 0.1.

[1180] Synthesis of Compound 37

[1181] 17 mg (0.03 mmol, 1.0 eq.) of Compound 36 was dissolved in 1 mL of DCM, and 1 mL of trifluoroacetic acid (TFA) was then added dropwise thereto at 0°C. A reaction was carried out by stirring the resulting mixture at 0°C for 30 minutes, followed by stirring at room temperature for 30 minutes. Thereafter, the mixture was concentrated under reduced pressure to remove a reaction solution such as DCM, and this concentration was then repeated three times. The concentrated matter was dried under high vacuum, and purified by reversed-phase column chromatography to obtain 13 mg of a target compound. TLC (DCM:MeOH = 10:1); R_f = 0.01.

[1182] Synthesis of Compound DD5

[1183] 0.01 mL (0.04 mmol, 1.5 eq.) of DIPEA was added dropwise to a solution obtained by dissolving 13 mg (0.03 mmol, 1.0 eq.) of Compound 37 in 3 mL of DMF, and 22 mg (0.04 mmol, 1.5 eq.) of phenol-activated SN38 was added thereto. The resulting mixture was stirred for an hour, and then concentrated under reduced pressure to remove a reaction solution. The concentrate was purified by column chromatography (10% MeOH in DCM) to obtain a target compound. TLC (DCM:MeOH = 10:1); R_f = 0.2.

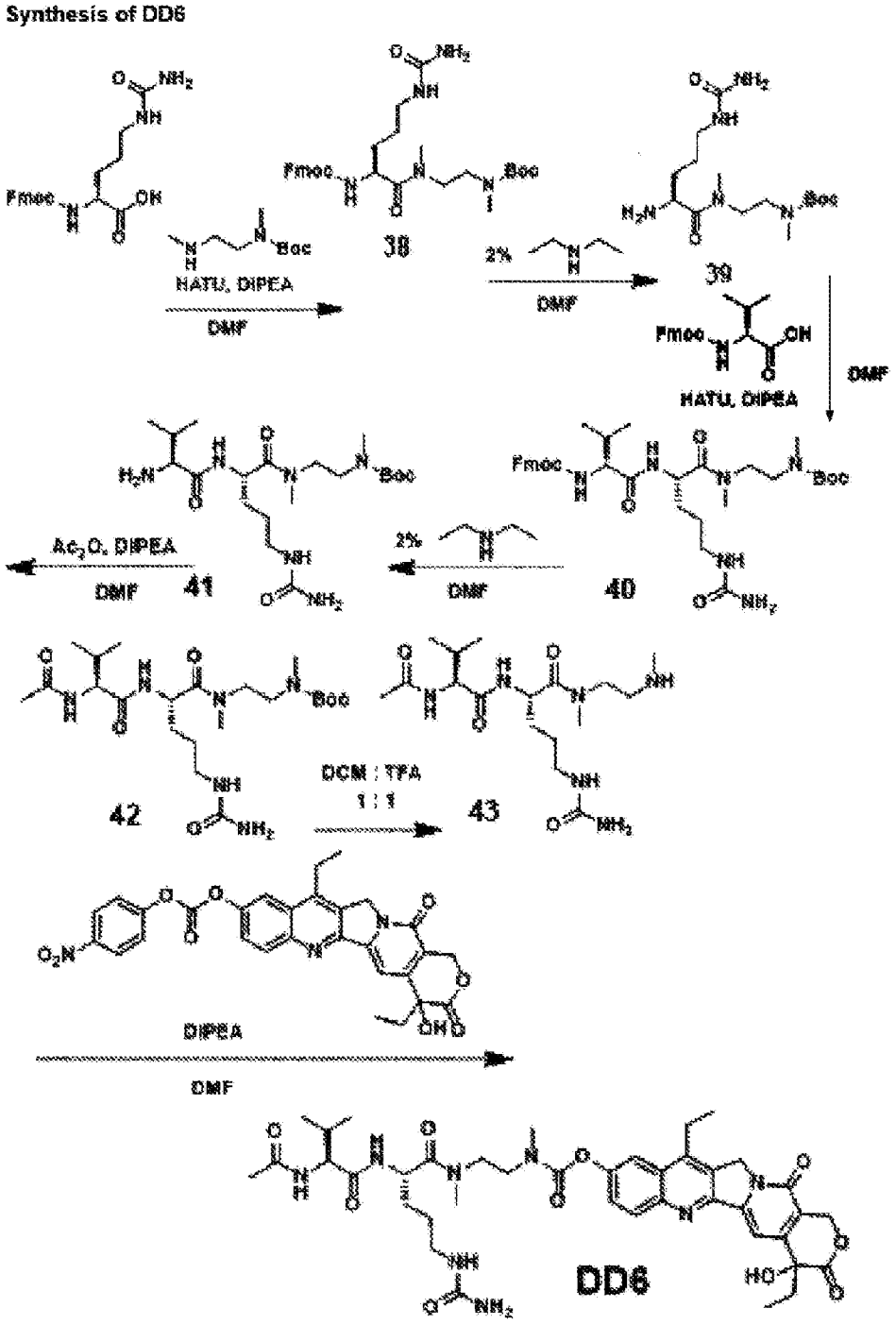
[1184] 3-4-2: Confirmation of structure of VC linker (DD5)

[1185] LRMS (ESI): m/z 954.4 [$M+H^+$]

[1186] The results are shown in FIG. 31.

[1187] 1.3.5. Synthesis and confirmation of structure of VC linker (DD6)

[1188] [Scheme 14]



[1189] Synthesis of VC linker (DD6)

[1190] Synthesis of Compound 38

[1191] 5 g (14.7 mmol, 1.0 eq.) of Fmoc-Val-OH and 1.7 g (14.7 mmol, 1.0 eq.) of N-hydroxysuccinimide (NHS) were dissolved in 140 mL of dimethoxyethane (DME), and stirred.

2.5 mL (16.2 mmol, 1.1 eq.) of N,N'-diisopropylcarbodiimide (DIC) was added dropwise at 0°C, and stirred for 16 hours. The reaction solution was filtered under reduced pressure to remove floating matter, and the filtrate was concentrated under reduced pressure. The residue was dissolved in acetone, and stored at a low temperature for 4 hours in a refrigerator. Thereafter, the resulting solution was filtered under reduced pressure to remove the re-formed floating matter, and used in the next reaction without any purification (crude yield: 5.5 g, 86%). TLC (EA:Hex = 1:1); R_f = 0.5.

[1192] Synthesis of Compound 39

[1193] 2.0 g (11.5 mmol, 1.0 eq.) of L-citrulline was dissolved in 100 mL of a 1:1 mixed solution of tetrahydrofuran (THF) and water, and stirred. 988 mg of sodium hydrogen carbonate was added thereto, and stirred. Thereafter, 5.0 g (11.5 mmol, 1.0 eq.) of Compound 38 was dissolved in 80 mL of acetone, added dropwise to the reaction solution, and then stirred. The resulting mixture was stirred for 21 hours, and then concentrated under reduced pressure to remove the organic solvent. An aqueous layer was washed with ethyl acetate (EA), and then titrated to pH 3 by slow dropwise addition of 2 N HCl. EA was added thereto so that the precipitate in an organic layer was extracted. Then, the organic layer was dried over a saturated saline solution and sodium sulfate, and used in the next reaction without any purification (crude yield: 5.6 g, 98%). TLC (DCM:MeOH = 10:1, one drop of formic acid); R_f = 0.1.

[1194] Synthesis of Compound 40

[1195] 50 mL of 10% piperidine in N,N-dimethylformamide (DMF) was added dropwise to 2.84 g (5.72 mmol, 1.0 eq.) of Compound 39, and stirred. After 4 hours, the resulting mixture was concentrated under reduced pressure to remove a reaction solution, and the residue was dissolved in water, and filtered under reduced pressure to remove the formed floating matter. An aqueous layer was concentrated to obtain a target compound, which was used in the next reaction without any purification. TLC (DCM:MeOH = 10:1); R_f = 0.01.

[1196] Synthesis of Compound 41

[1197] 54 mg (0.22 mmol, 1.0 eq.) of Compound 40 was dissolved in 2 mL of DMF, and 0.05 mL (0.264 mmol, 1.2 eq.) of N,N-diisopropylethylamine (DIPEA) was added dropwise thereto. Compound 37 was completely dissolved by adding 2 mL of water, and 0.05 mL (0.528 mmol, 2.4 eq.) of an acetic anhydride was added dropwise thereto at room temperature. After 3 hours, the resulting mixture was concentrated under reduced pressure to remove a reaction solution, and the concentrate was purified by reversed-phase column chromatography to obtain a target compound. TLC (DCM:MeOH = 10:1); R_f = 0.05.

[1198] Synthesis of Compound 42

[1199] 300 mg (0.95 mmol, 1.0 eq.) of Compound 41 was dissolved in 6 mL of DMF, and 550 mg (1.43 mmol, 1.5 eq.) of 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (HATU) was then added thereto. Thereafter, 0.25 mL (1.43 mmol, 1.5 eq.) of DIPEA was added dropwise, and 230 mg (1.43 mmol, 1.5 eq.) of N-[(tert-butoxy)carbonyl]-N,N'-dimethylethylenediamine was dissolved in 3.5 mL of DMF, and added dropwise at room temperature. After 7 hours, the reaction solution was diluted with ethyl acetate (EA), and an organic layer was washed with a saturated sodium hydrogen carbonate solution. Then, the organic layer was dried over a saturated saline solution and sodium sulfate, and filtered to obtain a target compound. The obtained target compound was used in the next reaction without any purification. TLC (DCM:MeOH = 10:1); R_f = 0.5.

[1200] Synthesis of Compound 43

[1201] Compound 42 was dissolved in a 2:1 mixed solution of DCM and TFA, and a reaction was then carried out at 0°C. Thereafter, the reaction mixture was concentrated under reduced pressure to remove a reaction solution such as DCM, and this concentration was then repeated three times. The residue was dried under high vacuum, and purified by reversed-phase column chromatography to obtain 370 mg of a target compound. TLC (DCM:MeOH = 10:1); R_f = 0.01.

[1202] Synthesis of Compound DD6

[1203] 0.03 mL (0.17 mmol, 1.2 eq.) of DIPEA was added dropwise to a solution obtained by dissolving 50 mg (0.14 mmol, 1.0 eq.) of Compound 43 in 3 mL of DMF, and 107 mg (0.21 mmol, 1.5 eq.) of phenol-activated SN38 was added thereto. The resulting mixture was stirred for an hour, and then concentrated under reduced pressure to remove a reaction solution. The concentrate was purified by column chromatography (10% MeOH in DCM) to obtain a target compound. TLC (DCM:MeOH = 10:1); R_f = 0.1.

[1204] Confirmation of structure of VC linker (DD6)

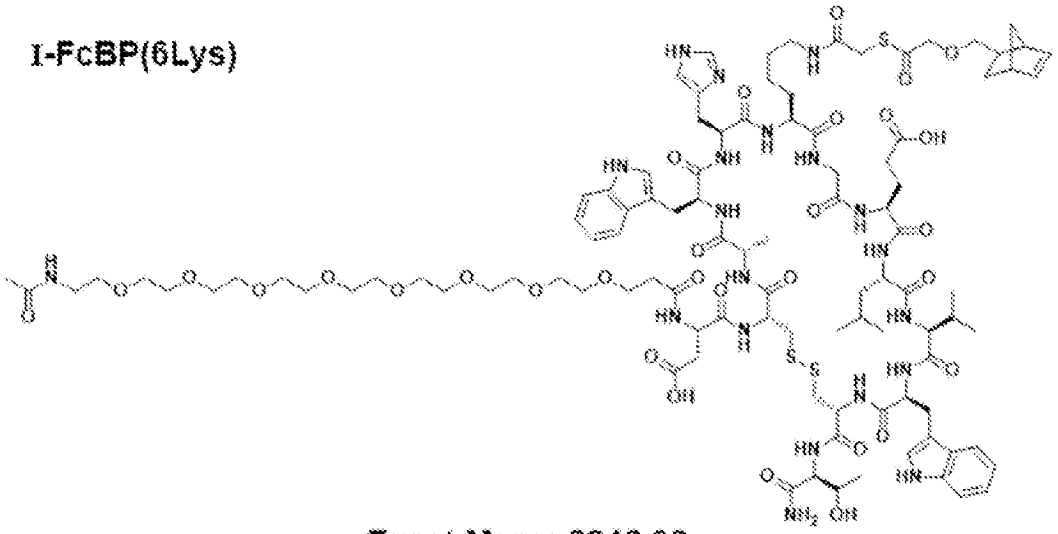
[1205] LRMS (ESI): m/z 805.5 [$M+H^+$]

[1206] The results are shown in FIG. 32.

[1207] 2. Preparation of agent for transferring site-specific first click-chemistry functional group to antibody (H_1-L_2-SSFI)

[1208] 2.1. Synthesis and confirmation of structure of Compound I-SSFI (where Xa_1 is lysine)

I-FcBP(6Lys)



Exact Mass: 2246.99

[1209] Synthesis method of Compound I-SSFI (Lys)

[1210] Synthesis of Compound I-SSFI was carried out in DMF, and 3 equivalents of DIPEA and 8.4 μmol of Compound I were dissolved in 7.3 μmol of SSFI, and stirred to introduce Compound I into SSFI.

[1211] After the termination of the reaction was confirmed, the reaction solution was concentrated, and purification of Compound I-SSFI by preparative-HPLC was attempted. After the purification, the Compound I-SSFI was freeze-dried to obtain a target compound, the amount of which was confirmed to be 12 mg (purity: >95% up (HPLC), and yield: 71%).

[1212] Confirmation of structure of Compound I-SSFI

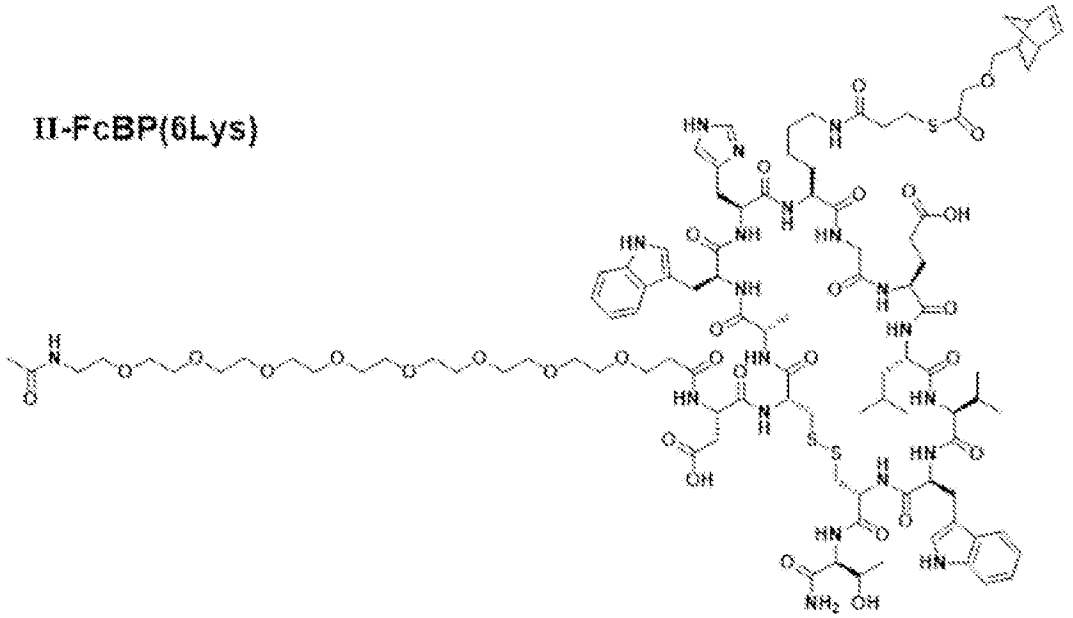
[1213] Measuring equipment: Waters Quattro Premier Xe

[1214] Calculated molecular weight: 2246.99 g/mol

[1215] Measured molecular weight $(M/2+H)^{2+}$: 1124.90 g/mol

[1216] The results are shown in FIG. 33.

[1217] 2.2. Synthesis and confirmation of structure of Compound II-SSFI (wherein Xa_1 is lysine)



[1218] Synthesis method of Compound II-SSFI (Lys)

[1219] Synthesis of Compound II-SSFI was carried out in DMF, and 3 equivalents of DIPEA and 8.4 μmol of Compound II were dissolved in 7.3 μmol of SSFI, and stirred to introduce Compound II into SSFI.

[1220] After the termination of the reaction was confirmed, the reaction solution was concentrated, and purification of Compound II-SSFI by preparative-HPLC was attempted. After the purification, the Compound II-SSFI was freeze-dried to obtain a target compound, the amount of which was confirmed to be 13 mg (purity: >95% up (HPLC), and yield: 78%).

[1221] Confirmation of structure of Compound II-SSFI (Lys)

[1222] Measuring equipment: Ultraflextreme (Bruker)

[1223] Measuring matrix: CHCA (α-Cyano-4-hydroxycinnamic acid) & DHB (2,5-Dihydroxybenzoic acid)

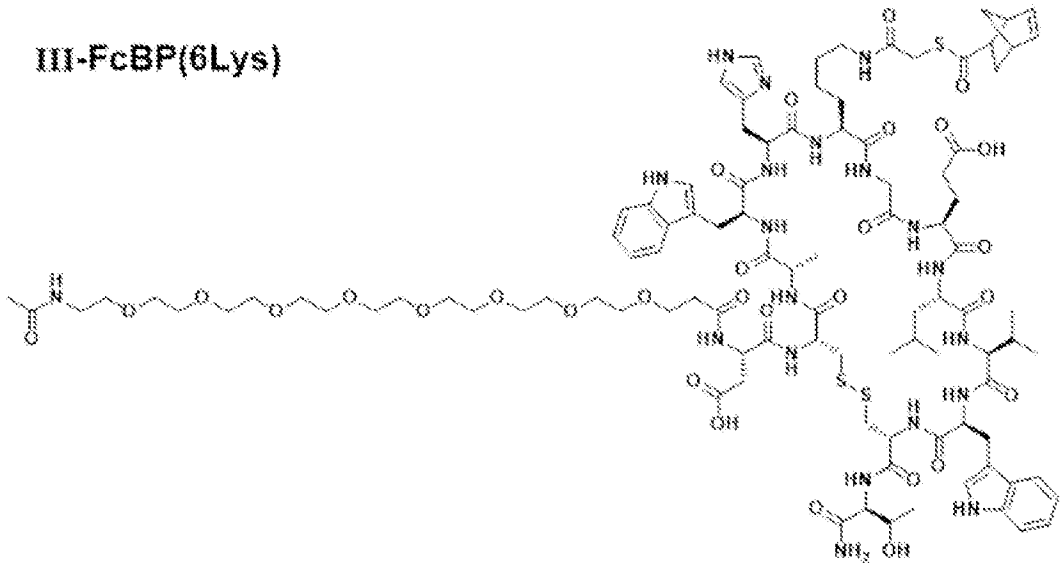
[1224] Calculated molecular weight: 2261.01 g/mol

[1225] Measured molecular weight $(M/2+H)^{2+}$: 2263.26 g/mol

[1226] The results are shown in FIG. 34.

[1227] 2.3. Synthesis and confirmation of structure of Compound III-SSFI (wherein Xa_1 is lysine)

III-FcBP(6Lys)



Exact Mass: 2202.97

[1228] Synthesis method of Compound III-SSFI (Lys)

[1229] Synthesis of Compound III-SSFI was carried out in DMF, and 3 equivalents of DIPEA and 8.4 μmol of Compound III were dissolved in 7.3 μmol of SSFI, and stirred to introduce Compound III into SSFI.

[1230] After the termination of the reaction was confirmed, the reaction solution was concentrated, and purification of Compound III-SSFI by preparative-HPLC was attempted. After the purification, the Compound III-SSFI was freeze-dried to obtain a target compound, the amount of which was confirmed to be 12 mg (purity: >95% up (HPLC), and yield: 75%).

[1231] Confirmation of structure of Compound III-SSFI (Lys)

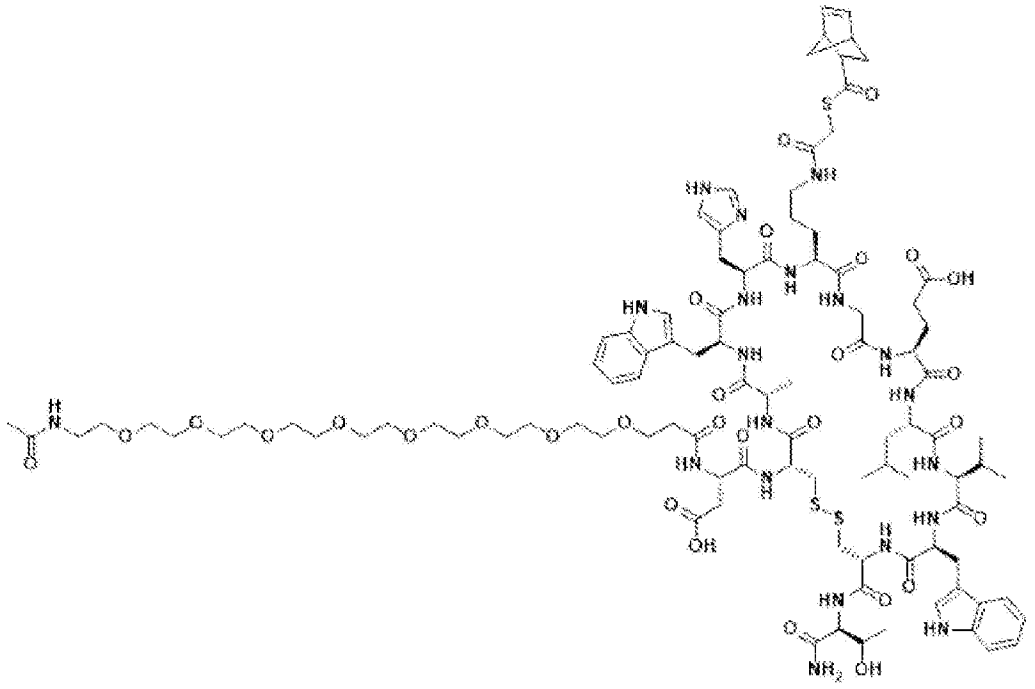
[1232] Measuring equipment: Waters Quattro Premier Xe

[1233] Calculated molecular weight: 2202.97 g/mol

[1234] Measured molecular weight $(M/2+H)^{2+}$: 1103.64 g/mol

[1235] The results are shown in FIG. 35.

[1236] 2.4. Synthesis and confirmation of structure of Compound III-SSFI (wherein Xa_1 is ornithine)



Exact Mass: 2188.95

[1237] Synthesis method of Compound III-SSFI (Orn)

[1238] Synthesis of Compound III-SSFI was carried out in DMF, and 3 equivalents of DIPEA and 8.4 μmol of Compound III were dissolved in 7.3 μmol of SSFI, and stirred to introduce Compound III into SSFI.

[1239] After the termination of the reaction was confirmed, the reaction solution was concentrated, and purification of Compound III-SSFI by preparative-HPLC was attempted. After the purification, the Compound III-SSFI was freeze-dried to obtain a target compound, the amount of which was confirmed to be 14 mg (purity: >95% up (HPLC), and yield: 87%).

[1240] Confirmation of structure of Compound III-SSFI (Orn)

[1241] Measuring equipment: Waters Quattro Premier Xe

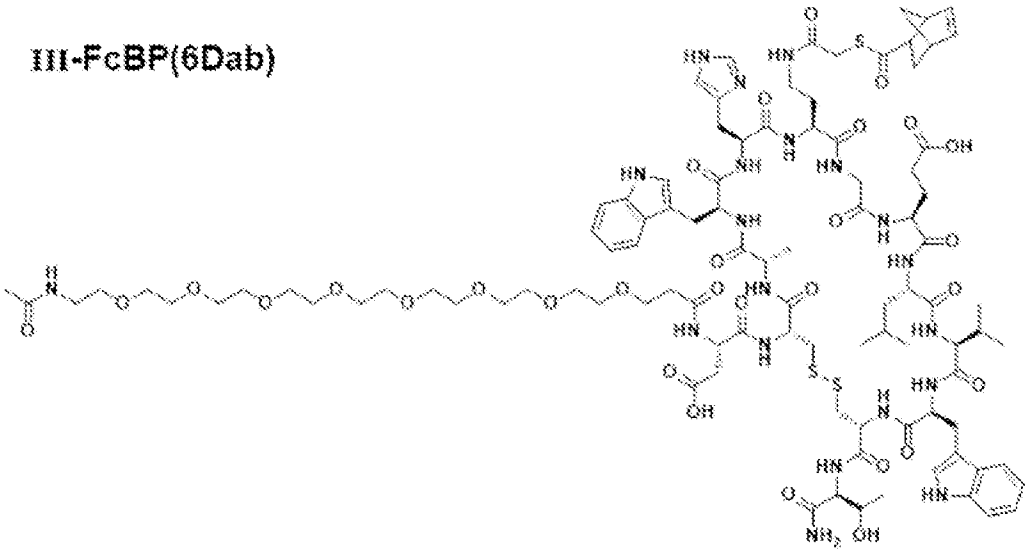
[1242] Calculated molecular weight: 2188.97 g/mol

[1243] Measured molecular weight $(M/2+H)^{2+}$: 1096.64 g/mol

[1244] The results are shown in FIG. 36.

[1245] 2.5. Synthesis and confirmation of structure of Compound III-SSFI (wherein Xa_1 is 2,4-diaminobutanoic acid (Dab))

III-FcBP(6Dab)



Exact Mass: 2174.94

[1246] Synthesis method of Compound III-SSFI (Dab)

[1247] Synthesis of Compound III-SSFI was carried out in DMF, and 3 equivalents of DIPEA and 8.4 μmol of Compound III were dissolved in 7.3 μmol of SSFI, and stirred to introduce Compound III into SSFI.

[1248] After the termination of the reaction was confirmed, the reaction solution was concentrated, and purification of Compound III-SSFI by preparative-HPLC was attempted. After the purification, the Compound III-SSFI was freeze-dried to obtain a target compound, the amount of which was confirmed to be 12 mg (purity: >95% up (HPLC), and yield: 75%).

[1249] Confirmation of structure of Compound III-SSFI (Dab)

[1250] Measuring equipment: Waters Quattro Premier Xe

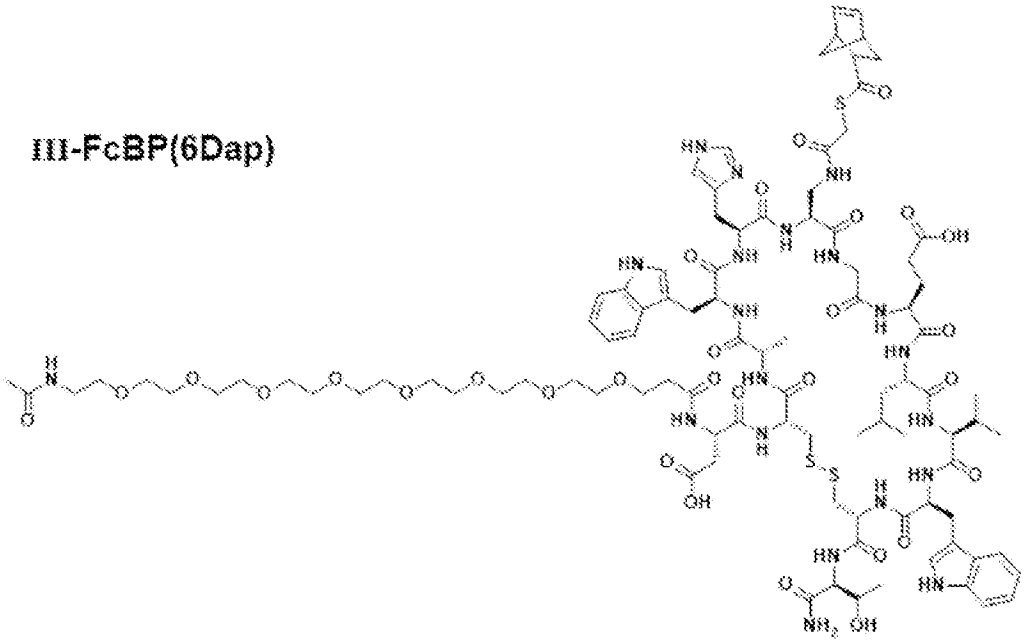
[1251] Calculated molecular weight: 2174.94 g/mol

[1252] Measured molecular weight $(M/2+H)^{2+}$: 1089.94 g/mol

[1253] The results are shown in FIG. 37.

[1254] 2.6. Synthesis and confirmation of structure of Compound III-SSFI (wherein Xa_1 is 2,3-diaminopropionic acid (Dap))

III-FcBP(6Dap)



Exact Mass: 2160.92

[1255] Synthesis method of Compound III-SSFI (Dap)

[1256] Synthesis of Compound III-SSFI was carried out in DMF, and 3 equivalents of DIPEA and 8.4 μmol of Compound III were dissolved in 7.3 μmol of SSFI, and stirred to introduce Compound III into SSFI.

[1257] After the termination of the reaction was confirmed, the reaction solution was concentrated, and purification of Compound III-SSFI by preparative-HPLC was attempted. After the purification, the Compound III-SSFI was freeze-dried to obtain a target compound, the amount of which was confirmed to be 12 mg (purity: >95% up (HPLC), and yield: 75%).

[1258] Confirmation of structure of Compound III-SSFI (Dap)

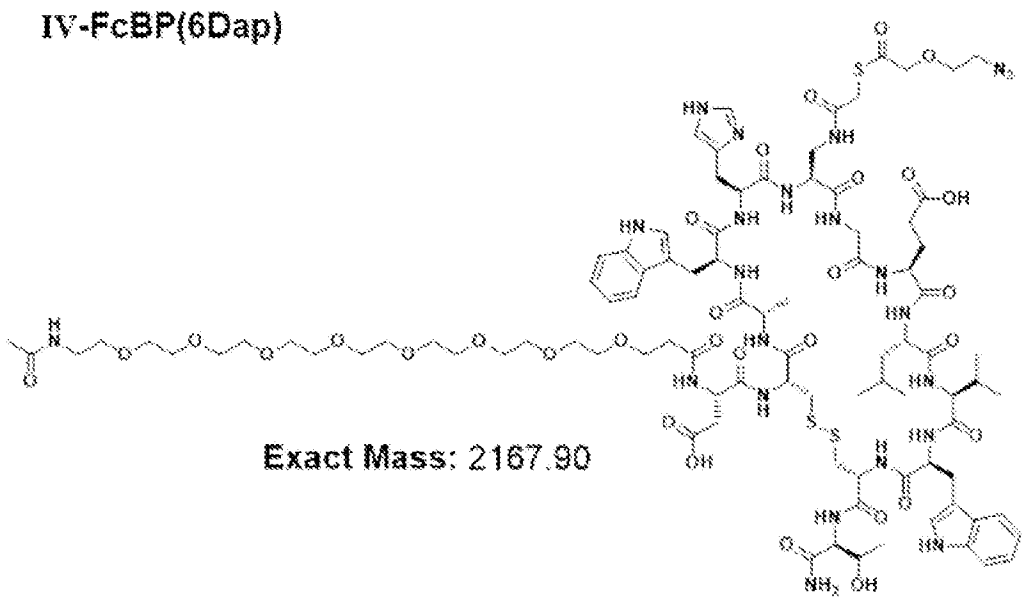
[1259] Measuring equipment: Waters Quattro Premier Xe

[1260] Calculated molecular weight: 2160.92 g/mol

[1261] Measured molecular weight $(M/2+H)^{2+}$: 1082.34 g/mol

[1262] The results are shown in FIG. 38.

[1263] 2.7. Synthesis and confirmation of structure of Compound IV-SSFI (wherein Xa_1 is Dap)



[1264] Synthesis method of Compound IV-SSFI (Dap)

[1265] Synthesis of Compound IV-SSFI was carried out in DMF, and 3 equivalents of DIPEA and 8.4 μmol of Compound IV were dissolved in 7.3 μmol of SSFI, and stirred to introduce Compound IV into SSFI.

[1266] After the termination of the reaction was confirmed, the reaction solution was concentrated, and purification of Compound IV-SSFI by preparative-HPLC was attempted. After the purification, the Compound IV-SSFI was freeze-dried to obtain a target compound, the amount of which was confirmed to be 12 mg (purity: >95% up (HPLC), and yield: 76%).

[1267] Confirmation of structure of Compound IV-SSFI (Dap)

[1268] Measuring equipment: Waters Quattro Premier Xe

[1269] Calculated molecular weight: 2167.90 g/mol

[1270] Measured molecular weight $(M/2+H)^{2+}$: 1085.60 g/mol

[1271] The results are shown in FIG. 39.

[1272] 3. Synthesis and confirmation of structure of antibody containing first click-chemistry functional group in site-specific manner

[1273] 3.1. Synthesis of Antibody-Norbornene

[1274] Synthesis method of Trastuzumab-Norbornene (1)

[1275] Introduction reaction using Compound I-SSFI (Lys)

[1276] Synthesis of Ab (246/248 Lys)-norbornene was carried out in a buffer using Compound I-SSFI (Lys). A 1× PBS buffer (0.01% Tween 20, pH 7.4) was used as the reaction buffer. To introduce norbornene at a certain site of an antibody (trastzumab, 4 mg/mL), 8

equivalents of Compound I-SSFI (Lys) was added per one antibody, and reacted at room temperature for 72 hours, and the reaction monitoring and termination were confirmed by HIC-HPLC. As the binding of norbornene to the antibody proceeds, the peaks on the chromatogram were observed to shift from 4 minutes to 4.5 minutes and 5 minutes, thereby confirming a degree of progression of the reaction. The Ab-norbornene conjugate was dialyzed three times using a 1× PBS buffer (pH 7.4), and purified using a size exclusion chromatography technique.

[1277] Observation of the binding reaction between trastuzumab and Compound I-SSFI (Lys) according to reaction time is shown in FIG. 40.

[1278] Synthesis method of Trastuzumab-Norbornene (2)

[1279] Introduction reaction using Compound II-SSFI (Lys)

[1280] Synthesis of Ab (246/248 Lys)-norbornene was carried out in a buffer using Compound II-SSFI (Lys). A 1× PBS buffer (0.01% Tween 20, pH 7.4) was used as the reaction buffer. To introduce norbornene at a certain site of an antibody (trastzumab, 4 mg/mL), 8 equivalents of Compound II-SSFI (Lys) was added per one antibody, and reacted at room temperature for 72 hours, and the reaction monitoring and termination were confirmed by HIC-HPLC. As the binding of norbornene to the antibody proceeds, the peaks on the chromatogram were observed to shift from 4 minutes to 4.5 minutes, thereby confirming a degree of progression of the reaction. The Ab-norbornene conjugate was dialyzed three times using a 1× PBS buffer (pH 7.4), and purified using a size exclusion chromatography technique.

[1281] Observation of the binding reaction between trastuzumab and Compound II-SSFI (Lys) according to reaction time is shown in FIG. 41.

[1282] Synthesis method of Trastuzumab-Norbornene (3)

[1283] Introduction reaction using Compound III-SSFI (Dap, Dab, Orn, or Lys)

[1284] Synthesis of Ab (246/248 Lys)-norbornene was carried out in a buffer using Compound III-SSFI (Lys). A 1× PBS buffer (0.01% Tween 20, pH 7.4) was used as the reaction buffer. To introduce norbornene at a certain site of an antibody (Herceptin or trastzumab, 4 mg/mL), 10 equivalents of Compound III-SSFI (Dap, Dab, Orn, or Lys) was added per one antibody, and reacted at room temperature for 28 hours, and the reaction monitoring and termination were confirmed by HIC-HPLC. As the binding of norbornene to the antibody proceeds, the peaks on the chromatogram were observed to shift from 6.2 minutes to 6.4 to 6.7 minutes, thereby confirming a degree of progression of the reaction. The Ab-norbornene conjugate was dialyzed using a 1× PBS buffer (pH 7.4), and purified using a size exclusion chromatography technique.

[1285] The reaction between Compound III-SSFI (Dap, Dab, Orn, or Lys) and the antibody is shown in FIG. 42, and the structure of the final product (i.e., Ab(246/248)-Norbornene) is shown in FIG. 43.

[1286] Observation of the binding reaction between trastuzumab and Compound III-SSFI (Dap) according to reaction time is shown in FIG. 44.

[1287] Observation of the binding reaction between trastuzumab and Compound III-SSFI (Dab) according to reaction time is shown in FIG. 45.

[1288] Observation of the binding reaction between trastuzumab and Compound III-SSFI (Orn) according to reaction time is shown in FIG. 46.

[1289] Observation of the binding reaction between trastuzumab and Compound III-SSFI (Lys) according to reaction time is shown in FIG. 47.

[1290] 3.2. Synthesis of Antibody-Azide

[1291] Synthesis method of Trastuzumab-Azide (4)

[1292] Introduction reaction using Compound IV-SSFI (Dap)

[1293] Synthesis of Ab (246/248 Lys)-azide was carried out in a buffer using Compound IV-SSFI (Dap). A 1× PBS buffer (0.01% Tween 20, pH 7.4) was used as the reaction buffer. To introduce an azide at a certain site of an antibody (trastzumab, 4 mg/mL), 10 equivalents of Compound IV-SSFI (Dap) was added per one antibody, and reacted at room temperature for 24 hours, and the reaction monitoring and termination were confirmed by HIC-HPLC. As the binding of norbornene to the antibody proceeds, the peaks on the chromatogram were observed to shift from 6 minutes to 6.3 minutes, thereby confirming a degree of progression of the reaction. The Ab-norbornene conjugate was dialyzed three times using a 1× PBS buffer (pH 7.4), and purified using a size exclusion chromatography technique.

[1294] Observation of the binding reaction between trastuzumab and Compound IV-SSFI (Dap) according to reaction time is shown in FIG. 48.

[1295] 3.3. Confirmation of structure and site-specific binding of trastuzumab-norbornene conjugate

[1296] An antibody-norbornene complex in which a norbornene linker was bound to an antibody via Compound III-SSFI (Dap) was confirmed by mass spectrometry. It was determined to which site of the antibody the norbornene was bound via F(ab')2 and Fc/2 after the antibody-norbornene complex was treated with an IdeS enzyme. It was confirmed that the Ides Fc/2-norbornene complex was found to have an "Obs-Theo" value of 2991.68, a value which is an exact match in consideration of the combined value of the mass value of N-glycan, the value of a

loss of one lysine residue, and the value of one norbornene molecule, which corresponded to the molecular weight of 120 Da, (a value of -0.10 was considered to be a system error in the high-mass molecular weight analysis). The observed mass spectrum of the Ides_F(ab')2 was detected, but the mass value of one charge was not shifted due to the complexity of the respective mass spectra, thereby making impossible to obtain the corresponding mass value.

[1297] The spectra of the complex having an increased molecular weight due to the antibody-norbornene binding are shown in FIGS. 49 and 50, and changes in molecular weights due to the norbornene conjugation are as listed in Table 12.

<Table 12> Changes in molecular weights of antibody-norbornene conjugates

Sample		Theoretical mass (peptide full seq.)	Observed mass	Δ mass (Obs-Theo) (continued-)
Trastuzumab	Ides Fc/2	23916.08	25231.10	1315.02
Trastuzumab-norbornene conjugate	Ides Fc/2	23916.08	25353.53	1437.45

(-continued) N-glcan (G0F/G0F)	C-term (-2K)	Norbornene conjugate	Δ mass	
1445.00	128	ND	-1.98	(G0F/-K)
1445.00	128	122	-1.55	(G0F/-K)

[1298] Also, the norbornene binding site of the antibody-norbornene complex was confirmed through comparison of the MS/MS spectrum with trastuzumab. As a result, it was

confirmed that the norbornene molecule was bound to a K248 site of the sequence LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF **(SEQ ID NO: 26)**. The MS/MS chromatograms are shown FIGS. 51 to 54, and the sequence matching results based on the MS/MS spectra are shown in FIG. 55.

[1299] The conditions for the analysis are as follows:

[1300] UPLC conditions

[1301] Measuring equipment: Acquity UPLC I-Class system

[1302] 1. Column: Thermo MAbPac™ RP (2.1 mm × 5 mm)

[1303] 2. Column Temp.: 60 °C

[1304] 3. Mobile phase

[1305] A. 0.1% Formic acid in water

[1306] B. 0.1% Formic acid in acetonitrile

[1307] Mass spectroscopy conditions

[1308] Measuring equipment: LTQ Elite (Thermo)

[1309] Source type: HESI

[1310] Capillary Temp.: 320 °C

[1311] Source heater temp.: 300 °C

[1312] Sheath gas flow: 40.00

[1313] Aux gas flow: 20.00

[1314] Sweep gas flow: 5.00

[1315] Source voltage (KV): 4.00

[1316] FTMS resolution: 120,000

[1317] FTMS mass range: 400 to 4,000

[1318] 3.4. Confirmation of structure of Trastuzumab-Azide

[1319] The antibody-azide complex in which an azide linker was bound to an antibody via Compound IV-SSFI (Dap) was confirmed using mass spectrometry. A molecular weight increase of 249 Da was confirmed when two molecules of an azide compound were bound to trastuzumab. The measured mass spectrum of trastuzumab is shown in FIG. 56, and the mass spectrum of the antibody-azide complex to which two azide molecules were bound is shown in FIG. 57.

[1320] Measuring equipment: Ultraflex III (TOF/TOF)

[1321] Analysis mode: Linear mode

[1322] Polarity: Positive

[1323] Detection: m/z 2,000 to 300,000

[1324] Laser repetition rate: 100 Hz

[1325] Number of shots: 1,000 shots

[1326] Deflection: On, 5,000 Da

[1327] Voltage: Ion Source kV, Ion Source II 23.00 kV, Lens 9.00 kV

[1328] Calculated molecular weight: 148,271 g/mol

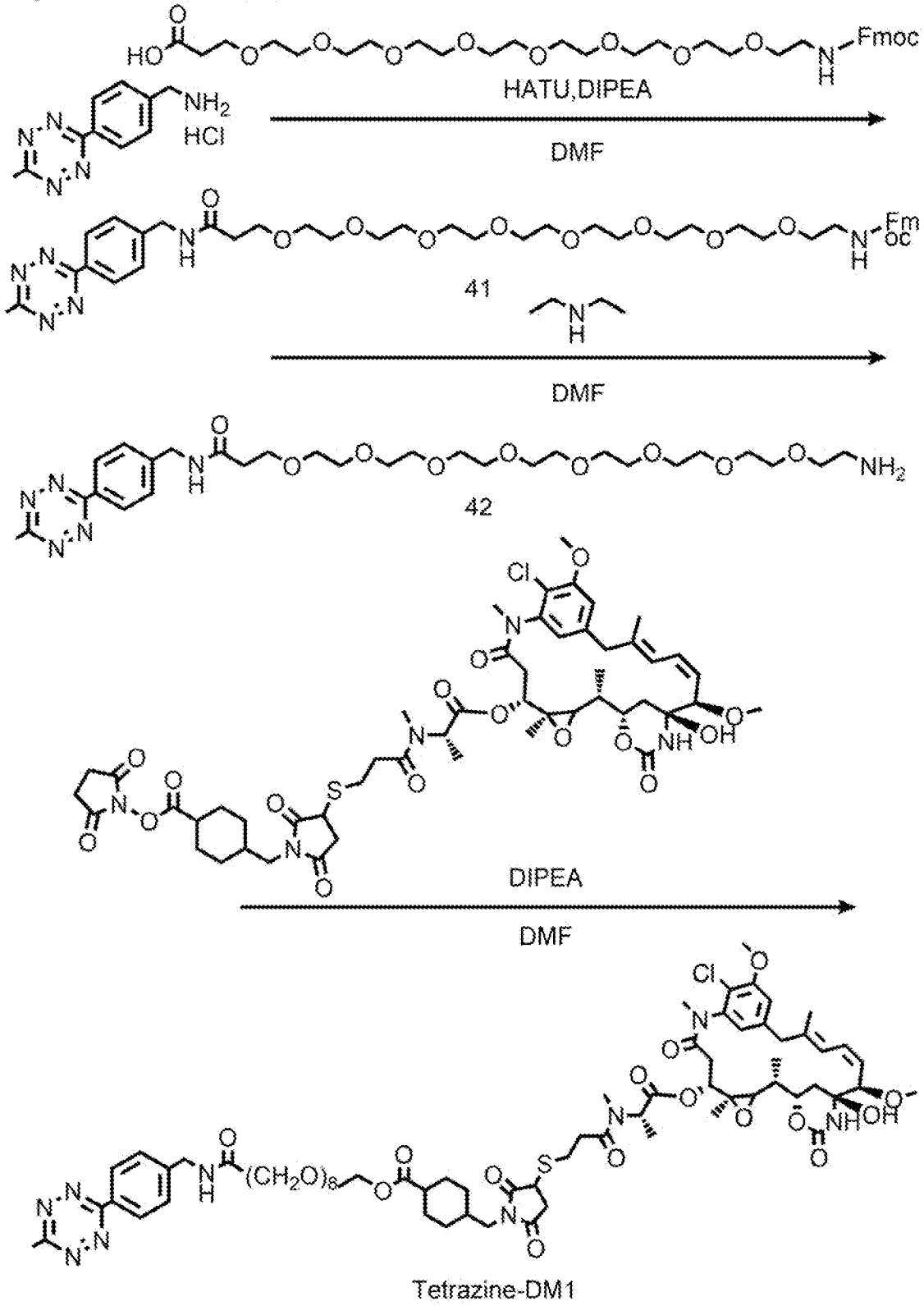
[1329] Measured molecular weight: 148,262 g/mol

[1330] 4. Synthesis and confirmation of structure of antibody-payload conjugate

[1331] 4.1. Synthesis method and confirmation of structure of payload

[1332] [Scheme 15]

Synthesis of Tetrazine-DM1



Tetrazine-DM1

[1333] Synthesis method of Tetrazine-DM1

[1334] Synthesis of Compound 41

[1335] 0.53 g (0.8 mmol, 1.0 eq.) of Fmoc-PEG_8-OH was dissolved in 7 mL of DMF, and then stirred. Thereafter, 0.38 g (1.0 mmol, 1.25 eq.) of HATU and 0.3 mL (1.7 mmol, 2.1 eq.) of DIPEA were added dropwise thereto. 0.205 g (0.86 mmol, 1.1 eq.) of methyltetrazine-amine·HCl was added dropwise, and 0.3 mL (1.7 mmol, 2.1 eq.) of DIPEA was added dropwise thereto. The resulting mixture was stirred for 4 hours, and then concentrated under reduced pressure to remove the solvent. The mixture was purified by column chromatography (5% MeOH in DCM) to obtain a target compound. TLC (DCM:MeOH = 20:1); R_f = 0.3.

[1336] Synthesis of Compound 42

[1337] 0.527 g (0.62 mmol, 1.0 eq.) of Compound 41 was dissolved in 6 mL of DMF, and 0.3 mL of diethylamine was added dropwise thereto. The resulting mixture was stirred for 2.5 hours, and then concentrated under reduced pressure to remove the solvent. The mixture was purified by column chromatography (DCM:MeOH:NH_4OH= 80:25:2.5) to obtain a target compound, the amount of which was confirmed to be 0.273 g (yield: 70%). TLC (DCM:MeOH:NH_4OH = 80:25:2.5); R_f = 0.1.

[1338] Synthesis of Tetrazine-DM1

[1339] 0.355 g (0.57 mmol, 1.5 eq.) of Compound 42 was dissolved in 25 mL of DMF, and then stirred. 0.405 g (0.38 mmol, 1.0 eq.) of DM1-SMCC-NHS was added thereto, and DIPEA was added dropwise until the pH of the mixture reached pH 9.0. Thereafter, the mixture was stirred for 2 hours. The reaction solution was concentrated under reduced pressure, and then purified by column chromatography (10% MeOH in DCM) to obtain a target compound, the amount of which was confirmed to be 0.434 g (yield: 73%). TLC (DCM:MeOH = 10:1); R_f = 0.5.

[1340] Confirmation of structure of Tetrazine-DM1

[1341] LRMS (ESI): m/z 782.6 [$M+2H^+$]

[1342] The results are shown in FIG. 58.

[1343] 4.2. Synthesis and confirmation of structure of antibody-payload conjugate

[1344] 4.2.1. Antibody-Norbornene-Tetrazine-DM1

[1345] Synthesis method of Trastuzumab-Norbornene-Tetrazine-DM1 (1)

[1346] Introduction reaction of Tetrazine-DM1 into Trastuzumab-Norbornene using Compound III-SSFI (Dap)

[1347] An antibody-payload conjugate was constructed using trastuzumab into which two norbornene molecules were introduced via Compound III-SSFI (Dap). A reaction was carried

out using 25 mL of the antibody-payload conjugate at a concentration of 4.5 mg/mL, and conjugation of a tetrazine-PEG8-DM1 drug was attempted for a biorthogonal reaction (i.e., biorthogonal chemistry) with norbornene conjugated to the antibody. 40 Equivalents of the drug was used per one antibody, and the conjugation reaction was performed at room temperature for 24 hours in a 20 mM histidine acetate solution (pH 5.5). The conjugation reaction was monitored by HIC-HPLC, and it was observed that the peaks appearing at 7.7 minutes after only a norbornene molecule was bound to trastuzumab were shifted to 8.7 minutes (DAR 1) and 10.5 minutes (DAR 2) as the tetrazine-PEG8-DM1 drug reacted with the antibody-norbornene linker. As a result, formation of the antibody-payload conjugate was observed.

[1348] The structure of the product 'antibody-payload conjugate' is shown in FIG. 59, and the reaction monitoring by HIC-HPLC is shown in FIG. 60 (Table 13).

<Table 13>

Reaction monitoring by HIC-HPLC for formation of antibody-payload conjugate

Retention time	Composition
6.6 min	Trastuzumab
7.7 min	Trastuzumab-norbornene conjugate
8.7 min	Trastuzumab-DM1 conjugate (1 site, DAR1)
10.5 min	Trastuzumab-DM1 conjugate (2 site, DAR2)

[1349] Confirmation of Trastuzumab-Norbornene-Tetrazine-DM1 structure (2)

[1350] An antibody-drug conjugate in which tetrazine-DM1 was bound to Trastuzumab-Norbornene was confirmed by mass spectrometry. It was determined to which site of the antibody the tetrazine-DM1 was bound via F(ab')2 and Fc/2 after the antibody-drug conjugate was treated with an IdeS enzyme. It was confirmed that the Ides Fc/2-DM1 complex was found to have an "Obs-Theo" value of 1673.5, indicating that the drug was bound to the conjugate in consideration of the combined value of one tetrazine-DM1 molecule, which corresponded to the molecular weight of 1,688.80 Da (a dehydration reaction was observed in the molecular weight analysis of the DM1-series drug). The observed mass spectrum of the Ides_F(ab')2 was detected, but the mass value of one charge was not shifted due to the complexity of the respective mass spectra, thereby making impossible to obtain the corresponding mass value.

[1351] The spectra of the complex having an increased molecular weight due to the trastuzumab-norbornene-tetrazine-DM1 binding are shown in FIGS. 61 and 62.

[1352] UPLC conditions

[1353] Measuring equipment: Acquity UPLC I-Class system

[1354] 1. Column: Thermo MAbPac™ RP (2.1 mm × 5 mm)

[1355] 2. Column Temp: 60 °C

[1356] 3. Mobile phase

[1357] A. 0.1% Formic acid in water

[1358] B. 0.1% Formic acid in acetonitrile

[1359] Mass spectroscopy conditions

[1360] Measuring equipment: LTQ Elite (Thermo)

[1361] Source type: HESI

[1362] Capillary Temp.: 320 °C

[1363] Source heater temp.: 300 °C

[1364] Sheath gas flow: 40.00

[1365] Aux gas flow: 20.00

[1366] Sweep gas flow: 5.00

[1367] Source voltage (KV): 4.00

[1368] FTMS resolution: 120,000

[1369] FTMS mass range: 400 to 4,000

[1370] 5. FcRn binding analysis of antibody-drug conjugate

[1371] The antibody-drug conjugate constructed in this patent was named 'AbClick®Pro.'

[1372] To check whether or not the antibody-drug conjugate (see FIG. 59; AbClick®Pro (NC, DM1)) had an antibody recycling function, the binding affinity of the conjugate for a neonatal Fc receptor (FcRn), which is closely associated with the *in vivo* antibody recycling, was analyzed. As one of the biomolecular interaction systems, Octet (model: Octet QK384) was used to determine the reaction affinity of FcRn for the constructed ADC. As a difference in reflection of light caused by a surface change, which occurs according to a degree of reaction between molecules attached to a surface of a sensor and molecules in a sample, was recognized as being free of a label, kinetic analysis was performed. Trastuzumab, Kadcyla, and AbClick®Pro (NC, DM1) were used as the samples to measure binding affinities for FcRn under a condition of pH 6.0. As a result, it was confirmed that the measured KD values were 1.12×10^{-7} M, 1.12×10^{-7} M, and 8.84×10^{-8} M, respectively, indicating that AbClick®Pro had a similar or superior binding affinity, compared to Kadcyla (Table 14).

<Table 14> FcRn binding analysis of antibody-drug conjugate

pH 6.0	FcRn binding		
	KD_Kinetics (M)	Ka (1/Ms)	Kd (1/S)
Trastuzumab	1.12E-7	3.31E+05	3.69E-02
Kadcyla	1.12E-7	2.10E+05	2.37E-02
*AbClick® Pro	8.84E-8	2.26E+05	1.99E-02

[1373] 6. Antigen binding analysis of antibody-drug conjugate

[1374] It was determined through enzyme-linked immunosorbent assay (ELISA) analysis whether or not a Her2 protein was bound to the antibody-drug conjugate. The antibody-norbornene complex constructed using trastuzumab and Compound III-SSFI (Dap) was used as the antibody-drug conjugate for measurement of antigen binding affinity. Structures of three drugs to be bound to the antibody-norbornene complex are shown in FIG. 63.

[1375] The dissociation constants (Kd) of Herceptin (trastuzumab), Kadcyla, AbClick®Pro (NC, DM1), AbClick®Pro (VC, DM1), and AbClick®Pro (VC, MMAE) from the antigen were confirmed to be approximately 98.6 pM, approximately 94.8 pM, approximately 121.4 pM, approximately 137.0 pM, and approximately 117.3 pM, respectively. Based on the results, it was confirmed that the binding site of the drug did not affect the binding affinity of the constructed ADC for the antigen.

[1376] A graph of measuring the antigen binding affinities of the antibody-drug conjugates is shown in FIG. 64 (Table 15).

<Table 15> Antigen binding analysis of antibody-drug conjugate

Antibody	K_D Value (binding affinity, pM)
Herceptin	98.6
Kadcyla	94.7
AbClick®Pro (NC,DM1)	121.4
AbClick®Pro (VC,DM1)	137.0
AbClick®Pro (VC,MMAE)	117.3

[1377] 7. Confirmation of serum stability of antibody-drug conjugate

[1378] It was determined through enzyme-linked immunosorbent assay (ELISA) analysis whether or not the antibody-drug conjugate was stable in serum. The stabilities of the antibody-drug conjugates in rat, rabbit and human sera were tested at room temperature. It was confirmed that a pattern of an intact ADC (i.e., drug-bound ADC) residual level of the self-constructed ADC "AbClick®Pro (NC, DM1)" over time was consistent with that of the Kadcyla® group.

[1379] The results of serum stability analysis of the antibody-drug conjugate are shown in FIGS. 65 to 67.

[1380] 8. Evaluation of medicinal effect of antibody-drug conjugate (AbClick®Pro) at cellular level (*in vitro* cytotoxicity test)

[1381] Medicinal effects of the three antibody-drug conjugates (ADC, AbClick®Pro series) using trastuzumab were evaluated according to a level of a target marker in cancer cells, and a cytotoxicity test was performed using NCI-N87 and MDA-MB-468 as a positive cell line expressing the target antigen (Her2) 'cell line' and a negative cell line expressing BT474, respectively. The three antibody-drug conjugates (AbClick®Pro (NC, DM1), AbClick®Pro (VC, DM1), and AbClick®Pro (VC, MMAE)) constructed in the Her2-overexpressing cells NCI-N87 were observed after the cells were treated with different concentrations of the antibody-drug conjugates. As a result, it was confirmed that the antibody-drug conjugates had IC50 values of 207.7 ng/mL, 93.9 ng/mL, and 57.7 ng/mL, indicating that the antibody-drug conjugates had an excellent anti-cancer effect. The three antibody-drug conjugates (AbClick®Pro (NC, DM1), AbClick®Pro (VC, DM1), and AbClick®Pro (VC, MMAE)) constructed in another Her2-overexpressing cell line BT474 were

observed after the cells were treated with different concentrations of the antibody-drug conjugates. As a result, it was confirmed that the antibody-drug conjugates had IC50 values of 47.7 ng/mL, 44.6 ng/mL, and 1.09 ng/mL, indicating that the antibody-drug conjugates had an excellent anti-cancer effect. The utility of the AbClick®Pro (VC, MMAE) as the novel ADC was confirmed because the AbClick®Pro (VC, MMAE) was observed to have an equal or superior apoptotic effect, compared to Kadcyla as the commercially available Herceptin ADC.

[1382] The test results are shown in FIGS. 68 to 70.

[1383] 9. Evaluation of medicinal effect of antibody-drug conjugate (AbClick®Pro) at animal level (*in vitro* cytotoxicity test)

[1384] A target antigen-overexpressing NCI-N87 gastric cancer cell line was subcutaneously transplanted into mice to establish a xenograft model, and the mice were divided into 4 groups to perform an anti-cancer efficacy test on the materials administered. Because the BALB/c nude mice used in this test were deficient in T cells, cancer cells were easily transplanted into the BALB/c nude mice. Therefore, the BALB/c nude mice were used as a model suitable for an anti-cancer efficacy test using a rodent.

[1385] (a) Preparation of cell line

[1386] One vial of a human tumor cell line (NCI-N87 cell line) was put into a cell culture flask containing an RPMI1640 medium (Gibco, 22400-089) supplemented with heat-inactivated 10% fetal bovine serum (FBS; Gibco, 10082-742), and cultured at 37°C in a 5% CO_2 incubator. The cell culture medium was washed with PBS, and diluted 10-fold with 2.5% trypsin-EDTA (Gibco, 15090). Thereafter, the diluted cell culture medium was added to separate the cells, and the cells were centrifuged (at 1,000 rpm for 5 minutes) to discard a supernatant. A fresh medium was added to the cell pellets to obtain a cell suspension. The viability of the cells was confirmed using a microscope, and the cells were diluted to a concentration of 1.25×10^7 cells/mL with a solution obtained by mixing a medium and Matrigel at a mixing ratio of 1:1 to prepare a cell line.

[1387] (b) Transplantation of cell line

[1388] A cell line was prepared using a method described in the section "4. 3) (4) Preparation of cell line." The cells were re-suspended and homogenized to prepare a cell line, and the prepared cell line was immediately administered to animals. For transplantation of the cell line, the back region of the animal was sterilized with 70% alcohol, the dorsal skin was pulled with the thumb and forefinger to form a space between the skin and muscles. Thereafter, a syringe with a 26 gauge needle was stuck in a subcutaneous pocket between the thumb and forefinger, and the cell line was then subcutaneously administered at a dose of 2.5×10^6 cells/0.2 mL/head from the front of the animal. During an acclimation period, healthy animals were

selected, and subjected to cell line inoculation. When the size of a tumor in the cell line-transplanted site reached approximately 100 to 150 mm^3, the mice were divided according to the size of a ranked tumor so that the tumor sizes in each group were distributed as uniformly as possible.

[1389] (c) Configuration of experimental groups and determination of dose and administration method

[1390] Cell line = NCI-N87

[1391] Mouse type = BALB/c nude (CAnN.Cg-Foxn1nu/CrljOri)

[1392] Number per group = 5

[1393] Administration method = Intravenous injection (using a 26-gauge needle syringe)

[1394] Dose = 5 mg/kg

[1395] Number of administration = Once/ 3 day, administered three times

[1396] Observation period = 5 weeks

[1397] Group 1: PBS; Group 2: Herceptin (trastuzumab); Group 3: Kadcyla; Group 4 : AbClick®Pro(NC, DM1); Group 5 : AbClick®Pro(VC, DM1); and Group 6: AbClick®Pro(VC, MMAE)

[1398] (d) Observation and inspection items

[1399] General symptoms

[1400] During the administration and observation periods, the type of general symptoms (including death), the symptom onset date, and the severity of symptoms were observed once a day, and recorded for each individual. The individuals whose general symptoms worsened were quarantined.

[1401] Body weight

[1402] The body weight was measured on the grouping date or the start date for administration of the test substance, and then measured twice a week.

[1403] Measurement of tumor size

[1404] The tumor size was measured twice a week for 5 weeks from the start date for administration of the test substance. The major- and minor-axis lengths of a tumor were measured using calipers, and the tumor size was calculated according to the following equation.

[1405] Tumor size = $ab^2/2$ (a: a major-axis length; and b: a minor-axis length)

[1406] (e) Results

[1407] The mice in the groups into which phosphate buffered saline (PBS) and Herceptin (trastuzumab) were injected did not show a tendency to suppress the growth of tumor for an observation period of 5 weeks. It was confirmed that the AbClick®Pro-series antibody-drug

conjugates constructed according to the present invention has a superior ability to suppress the growth of tumor compared to Herceptin, indicating that the antibody-drug conjugates according to the present invention successfully function as the ADC.

[1408] The related results are shown in FIGS. 71 and 72.

[1409] 10. Pharmacokinetics (PK) test of antibody-drug conjugate

[1410] To analyze the pharmacokinetic characteristics of the antibody-drug conjugate (ADC) manufactured using the AbClick®Pro, an enzyme-linked immunosorbent assay (ELISA) was performed to confirm the pharmacokinetics of the antibody-drug conjugate. The manufactured ADC had an AbClick®Pro (NC, DM1) structure as shown in FIG. 59, and comparative validation with Kadcyla was carried out. Rats (n = 3) were used for an animal model. In this case, the rats were intravenously injected with 5 mg/kg of ADC, and then monitored over time. It was confirmed that the intact ADC form (an intact ADC to which a drug was bound) of the administered AbClick®Pro (NC, DM1) was reduced over time. It was judged that the intact ADC had similar or improved drug characteristics, compared to Kadcyla.

[1411] The results are shown in FIG. 73.

[1412] This application contains references to amino acid sequences and/or nucleic acid sequences which have been submitted concurrently herewith as the sequence listing entitled "000072uscoa_SequenceListing.XML", file size 62 kilobytes (KB), created on **13 November** ~~**16 January**~~ 2023. The aforementioned sequence listing is hereby incorporated by reference in its entirety pursuant to 37 C.F.R. §1.52(e)(5).

Docket No.: 18896-000072-US-COA
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Sang Jeon CHUNG et al.

Application No.: 18/154,998 Confirmation No.: 5143

Filed: January 16, 2023 Art Unit: 1642

For: SITE-SPECIFIC ANTIBODY CONJUGATION AND ANTIBODY-DRUG CONJUGATE AS SPECIFIC EMBODIMENT THEREOF

Examiner: Y. Xiao

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT UNDER 37 C.F.R. §1.97(C)(1)

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

Dear Commissioner:

Applicant hereby submits a Supplemental Information Disclosure Statement (IDS) under 37 C.F.R. §1.97(c)(1): An IDS shall be considered by the Office if filed after the period specified in paragraph (b) of this section, provided that the IDS is filed before the mailing date of any of a final action under §1.113, a notice of allowance under §1.311, or an action that otherwise closes prosecution in the application, and it is accompanied by: (1) The Statement specified in paragraph (e) of this section. No fee is believed payable with respect to this IDS. However, if any fee should be found payable, please charge such fee to Deposit Account No. 08-0750.

37 C.F.R. §1.97(e)(1) Statement: Each item of information contained in the IDS was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the IDS.

PEZ/ei

This IDS further supplements the IDSs previously submitted on 16 January 2023 and 4 October 2023.

In accordance with 37 C.F.R. §1.97(g) and (h), this submission shall not be construed as a representation that a search has been made or as admission that information presented or cited herein is, or is considered to be, material to patentability as defined in 37 C.F.R. §1.56(b).

In accordance with 37 C.F.R. §1.98, Applicant submits herewith a listing on Form HDP-1449 of documents requested to be considered by the Examiner. Consideration by the Examiner of the present submission is requested.

Applicant believes no fee is due with this response. However, if a fee is due, please charge our Deposit Account No. 08-0750, under Order No. 18896-000072-US-COA from which the undersigned is authorized to draw.

Dated: November 13, 2023

Respectfully submitted,

By: /Paul E. Zarek/
Paul E. Zarek
Registration No.: 75,190
HARNESS, DICKEY & PIERCE, P.L.C.
7700 Bonhomme
Suite 400
Clayton, Missouri 63105
314.446.7668
Attorney for Applicant

Enclosures:
HDP Form 1449
Cited documents

UNITED STATES
PATENT AND TRADEMARK OFFICE

P.O. Box 1450
Alexandria, VA 22313 - 1450
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ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
18/154,998	**11/13/2023 04:50:06 PM Z ET**	**18896-000072-US-COA**

Title of Invention

SITE-SPECIFIC ANTIBODY CONJUGATION AND ANTIBODY-DRUG CONJUGATE AS SPECIFIC EMBODIMENT THEREOF

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	5143	FILED BY	Erica Imergoot
PATENT CENTER #	63233371	FILING DATE	01/16/2023
CUSTOMER #	28997	FIRST NAMED INVENTOR	Sang Jeon CHUNG
CORRESPONDENCE ADDRESS	-	AUTHORIZED BY	Paul Zarek

Documents

TOTAL DOCUMENTS: 12

DOCUMENT	PAGES	DESCRIPTION	SIZE (KB)
000072uscoa_2ndSupplementalForm1449.pdf	1	Information Disclosure Statement (IDS) Form (SB08)	130 KB
Warning: This is not a USPTO supplied IDS fillable form. Data in the form cannot be automatically loaded to other USPTO systems.			
000072uscoa_2ndSupplementalIDSTransmittal.pdf	2	Transmittal Letter	85 KB
Office Action of Australian Pantet Application No. 2020234394 issued on 26 September 2023.pdf	2	Other reference-Patent/Application/Search Documents	200 KB

WO 2020-184944 A1.pdf		263	Foreign Reference	10068 KB
Tamura.pdf		12	Non Patent Literature	1536 KB
000072uscoa_ResponseToNFOADated16August2023.pdf		12	-	214 KB
000072uscoa_ResponseToNFOADated16August2023-A....pdf	(1-1)	1	Amendment/Request for Reconsideration-After Non-Final Rejection	86 KB
000072uscoa_ResponseToNFOADated16August2023-SPEC.pdf	(2-2)	1	Specification	83 KB
000072uscoa_ResponseToNFOADated16August2023-CLM.pdf	(3-9)	7	Claims	191 KB
000072uscoa_ResponseToNFOADated16August2023-REM.pdf	(10-12)	3	Applicant Arguments/Remarks Made in an Amendment	90 KB
000072uscoa_SequenceListing.xml		-	Sequence Listing Supp (XML File)	62 KB

Warning: Please note that ST.23 and ST.25 sequence listings are required for applications with a filing date before July 1, 2022.

000072uscoa_SubstituteSpecification_Clean.pdf		171	Specification	5681 KB
000072uscoa_SubstituteSpecification_MarkedUp.pdf		171	Specification	5710 KB

Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)

000072uscoa_2ndSupplementalForm1449.pdf	685EC0EAF0FBB480A9AC4A84B9CAEFEEE4FCE60E38DD1E5979DFB8B8C8565EF9F6B9D3AB25A988676D8B73A3958AD907E0A2F61D5177AE09BC6E449C157B2303
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Office Action of Australian Pantet Application No. 2020234394 issued on 26 September 2023.pdf	1C01D69D9DC0EB6A780C0BFCB5E28D03BFA8EC5B30CB8C91BED9039F230474DD0AD7A8AA2371B60F743F2B487AD38F9C6D343C65732F8BB4E452CD0384FC0D5F
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This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application No.:	18/154,998
Filing Date:	16 January 2023
Applicant:	Sang Jeon CHUNG
Group Art Unit:	1642
Confirmation No.:	5143
Examiner:	Yan XIAO
Title:	SITE-SPECIFIC ANTIBODY CONJUGATION AND ANTIBODY-DRUG CONJUGATE AS SPECIFIC EMBODIMENT THEREOF
Attorney Docket:	18896-000072-US-COA

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

13 November 2023

RESPONSE TO OFFICE ACTION DATED 16 AUGUST 2023

This paper is in response to the Office Action dated 16 August 2023 in the above referenced application, in which a three-month shortened statutory period was set for reply. This response is timely and no fee is believed due. If a fee is due, authorization is hereby provided to charge any fee due to Deposit Account No. **08-0750**.

* * *

AMENDMENTS to the specification begin on page 2 of this paper.

AMENDMENTS to the claims begin on page 3 of this paper.

REMARKS on the present amendment begin on page 10 of this paper.

AMENDMENTS TO THE SPECIFICATION

Please replace the existing specification with the attached substitute specification. As required by 37 C.F.R. §1.121(b)(3), a substitute specification is attached hereto in both "marked-up" and "clean" form in compliance with 37 C.F.R. §1.52, §1.115, §1.125 and §1.215. Applicant states in accordance with 37 C.F.R. §1.125(b) that the substitute specification includes no new matter.

AMENDMENT TO THE CLAIMS

The following listing of claims will replace all prior versions and listings of claims in the application.

1.-53. (Cancelled)

54. (Currently Amended) A modified antibody or its fragment comprising bio-orthogonal click chemistry functional group,

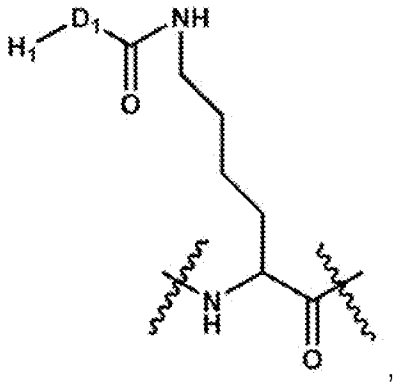
wherein the modified antibody comprises one or more modified heavy chain,

wherein each of the modified heavy chain comprises a modified lysine residue at 248 position of heavy chain amino acid sequence, wherein the 248 position is determined by an EU numbering system, and

wherein the modified lysine residue comprises a bio-orthogonal click chemistry functional group at terminal end**, and**

wherein the modified lysine residue does not comprise a terminal bio-reactive functional group selected from terminal hydroxy group, terminal carboxyl group, terminal thiol group, terminal amino group, and terminal aldehyde group.

55. (Previously Presented) The modified antibody of claim 54, wherein the modified lysine residue has following structure:



,

wherein H_1 is a group having the bio-orthogonal click chemistry functional group, and

wherein D_1 is bond, or is selected from C_{1-4} alkylene, C_{2-4} alkenylene, C_{2-4} alkynylene and C_{3-8} cycloalkylene.

56. (Previously Presented) The modified antibody of claim 55, wherein D_1 is bond, or is selected from unsubstituted C_{1-4} alkylene, unsubstituted C_{2-4} alkenylene, unsubstituted C_{2-4} alkynylene and unsubstituted C_{3-8} cycloalkylene.

57. (Cancelled)

58. (Cancelled)

59. (Currently Amended) The modified antibody of claim 54, wherein the modified heavy chain comprises an amino acid sequence having a structure of formula 8-2:

[formula 8-2]

G—P—S—V—F—L—F—P—P—K—P—(K)'—D—T—L—M—I **(SEQ ID NO: 22)**,

wherein K' is the modified lysine residue.

60. (Previously Presented) The modified antibody of claim 55, wherein D_1 comprises one or more heteroatoms.

61. (Previously Presented) The modified antibody of claim 55,wherein D_1 is $-[CH_2]_a-O-[CH_2]_b-$,

wherein each of a and b is independently selected from 0 to 4, and each of a and b is integer, and

wherein sum of a and b is 0 or more and 4 or less.

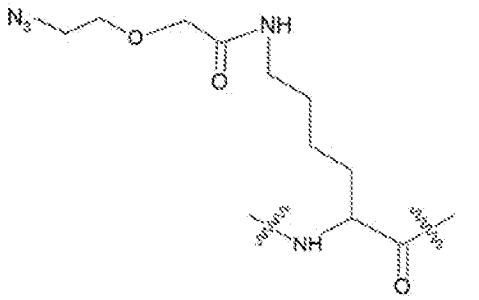
62. (Previously Presented) The modified antibody of claim 55, wherein D_1 is -$CH_2CH_2OCH_2$- or -CH_2OCH_2-.

63. (Previously Presented) The modified antibody of claim 54, wherein the modified antibody comprises two modified heavy chains.

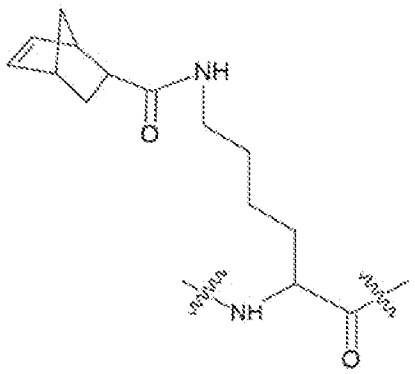
64. (Previously Presented) The modified antibody of claim 54, wherein the bio-orthogonal click chemistry functional group is a group capable of participating in a click chemistry reaction selected from Huisgen 1,3-dipolar cycloaddition and Diels-Alder reaction.

65. (Currently Amended) The modified antibody of claim 54, wherein the bio-orthogonal click chemistry functional group is selected from azide group**, tetrazine group, dibenzocyclooctyne group,** and norbornene group.

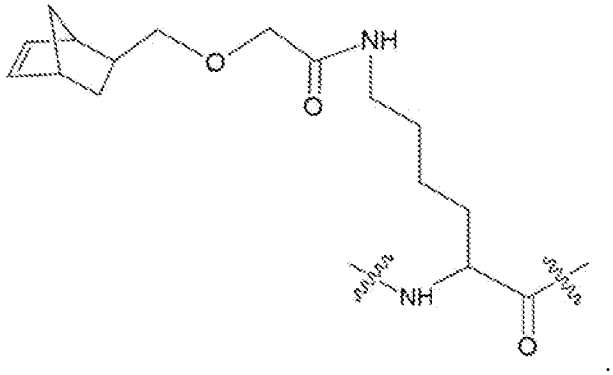
66. (Previously Presented) The modified antibody of claim 54, wherein the modified lysine residue has following structure:



,



, or



67. (Withdrawn) A method for preparing antibody-payload conjugate, comprising:

contacting a modified antibody or its fragment comprising a first click chemistry functional group with a payload comprising a cargo unit and a second click chemistry functional group,

wherein the antibody-payload conjugate is formed by the click chemistry reaction between the first click chemistry functional group of the modified antibody and the second click chemistry functional group of the payload,

wherein the modified antibody comprises:

one or more modified heavy chain,

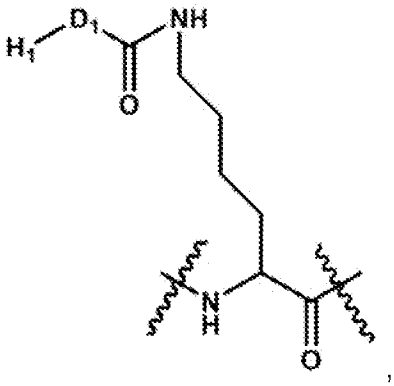
wherein each of the modified heavy chain comprises a modified lysine residue at 248 position of heavy chain amino acid sequence, wherein the 248 position is determined based on EU numbering system,

wherein the modified lysine residue comprises the first click chemistry functional group which is a bio-orthogonal click chemistry functional group at terminal end,

wherein the antibody-payload conjugate comprises an antibody unit derived from the modified antibody and a payload unit derived from the payload,

wherein the payload unit of the antibody-payload conjugate is linked to the antibody unit through K248 of the antibody unit.

68. (Withdrawn) The method of claim 67, wherein the modified lysine residue has the following structure:



wherein H_1 is a group having the bio-orthogonal click chemistry functional group,

wherein D_1 is bond, or is selected from C_{1-4} alkylene, C_{2-4} alkenylene, C_{2-4} alkynylene and C_{3-8} cycloalkylene.

69. (Withdrawn) The method of claim 68, wherein D_1 is bond, or is selected from unsubstituted C_{1-4} alkylene, unsubstituted C_{2-4} alkenylene, unsubstituted C_{2-4} alkynylene and unsubstituted C_{3-8} cycloalkylene.

70. (Withdrawn) The method of claim 68, wherein the modified lysine residue does not comprise terminal bio-reactive group selected from terminal hydroxy group, terminal carboxyl group, terminal thiol group, terminal amino group, and terminal aldehyde group.

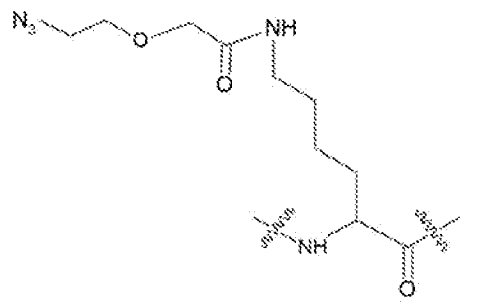
71. (Withdrawn) The method of claim 68, wherein D_1 comprises one or more heteroatoms.

72. (Withdrawn) The method of claim 69, wherein D_1 is $-CH_2CH_2OCH_2-$ or $-CH_2OCH_2-$.

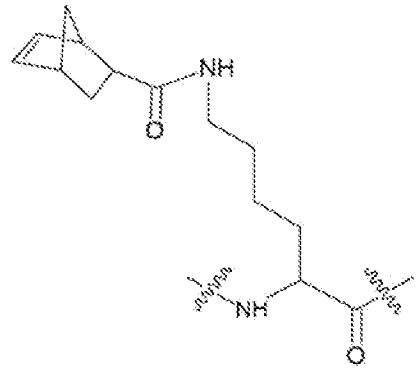
73. (Withdrawn) The method of claim 68, wherein the bio-orthogonal click chemistry functional group is a group capable of participating in a click chemistry reaction selected from Huisgen 1,3-dipolar cycloaddition and Diels-Alder reaction.

74. (Withdrawn) The method of claim 68, wherein the bio-orthogonal click chemistry functional group is selected from azide group and norbornene group.

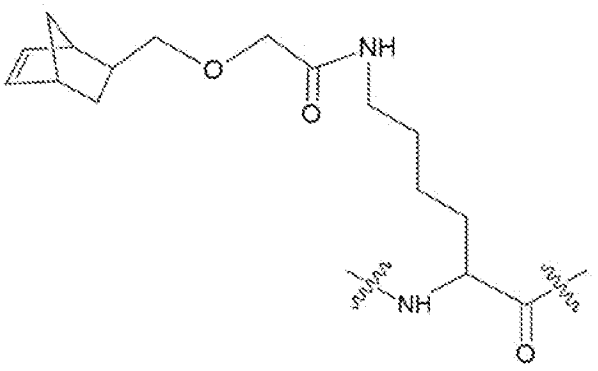
75. (Withdrawn) The method of claim 68, wherein the modified lysine residue has following structure:



,



, or



.

76. (Withdrawn) The method of claim 68, wherein the cargo unit comprises a carrier moiety, a fluorescent moiety, a drug moiety, or a radioactive moiety.

77. (New) The modified antibody of claim 56, wherein H_1 is the bio-orthogonal click chemistry functional group.

78. (New) The modified antibody of claim 77, wherein the bio-orthogonal click chemistry group is a group capable of participating in a click chemistry reaction selected from Huisgen 1,3-dipolar cycloaddition and Diels-Alder reaction.

79. (New) The modified antibody of claim 77, wherein the bio-orthogonal click chemistry functional group is selected from the group consisting of an azide group, a tetrazine group, a dibenzocyclooctyne group, and a norbornene group.

STATUS OF THE CLAIMS

Following the amendment as requested herein, Claims 54, 55, and 58-79 are pending in the present application. Of the pending claims, Claims 67-78 are withdrawn. Of the pending claims, Claims 54-56 and 59-65 are rejected.

Claims 54, 59, and 65 are amended.

Support for the amendment to Claims 54 and 65 can be found throughout the originally filed specification, for example at previously filed Claims 57 and 58.

Claim 59 is amended to include the corresponding SEQ ID NO.

Claims 77-79 are added.

Support for newly added claims 77-79 can be found throughout the originally filed specification, for example at previously filed Claims 57 and 58.

Claims 57 and 58 are cancelled without prejudice.

No new matter is added. Applicant respectfully requests entry of these amendments and favorable consideration of the pending claims.

RESPONSE TO OFFICE ACTION DATED 16 AUGUST 2023

1. Sequence listing

Submitted with this response is an amended sequence listing to include SEQ ID NO: 26 for the sequence described on page 161, paragraph [1298]. The revised sequence listing includes no new matter, as required by 37 CFR §§ 1.821(e), 1.821(f), 1.821(g), 1.824(a)-(d), 1.825(b), or 1.825(d). Applicant requests the revised sequence listing be entered into the application.

2. Objection to drawings

The drawings are objected to for disclosing an amino acid sequence without a corresponding SEQ ID NO in the figure or in the description of the drawing. With this response, the specification is amended in the Brief Description of the Drawings to clarify that the sequence of Fig. 1 is SEQ ID NO: 1.

Withdrawal of this objection is respectfully requested.

3. Objection to specification

The specification stands objected to for containing sequences without corresponding SEQ ID NOs. With this response, marked up and clean substitute specifications are submitted to include a SEQ ID NO with each relevant sequence.

Withdrawal of this objection is respectfully requested.

4. Claim objections

Claim 59 stands objected to for reciting a sequence without a corresponding SEQ ID NO. With this response, Claim 59 is amended to recite that the sequence is SEQ ID NO: 22.

Withdrawal of this objection is respectfully requested.

5. Claims 54-56 and 59-65 under 35 U.S.C. § 102(a)(1)

Claims 54-56 and 59-65 stand rejected under 35 U.S.C. § 102(a)(1) as allegedly anticipated by Yamada (WO 2018/199337).

With this response, and solely to advance prosecution, Claim 54 is amended to include all the elements of Claim 58, which was not rejected as anticipated by Yamada. Therefore, Claim 54, as amended, is not anticipated by Yamada. Claims 55, 56, and 59-65 depend upon Claim 54 and are not anticipated for at least the same reasons.

Withdrawal of this rejection is respectfully requested.

6. Obviousness double patenting

Claims 54, 63, and 65 stand rejected on the grounds of obviousness-type double patenting over Claims 21-33 of co-pending Application no. 17/424,528.

With this response, Claim 54 is amended to include the elements of Claim 58, which was not rejected on the grounds of obviousness-type double patenting. Therefore, Claim 54, as amended, is not subject to an obviousness-type double patenting rejection over the co-pending ‘528 application.

Withdrawal of this rejection is respectfully requested.

7. Conclusion

It is believed that all of the stated grounds of rejections are properly traversed, accommodated or rendered moot herein. Applicant therefore respectfully requests that the Examiner reconsider and withdraw all presently outstanding objection and rejections. It is believed that a full and complete response has been made to the outstanding Office Action and that the application is in condition for allowance. Thus, prompt and favorable consideration of this amendment is respectfully requested.

Respectfully submitted,

Dated: 13 November 2023

By: / Paul Zarek /
Paul Zarek, Reg. No. 75,190

HARNESS, DICKEY & PIERCE, P.L.C.
7700 Bonhomme, Suite 400
St. Louis, MO 63105
314-446-7668 (telephone)
314-726-7501 (facsimile)

UNITED STATES
PATENT AND TRADEMARK OFFICE

P.O. Box 1450
Alexandria, VA 22313 - 1450
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ELECTRONIC PAYMENT RECEIPT

APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
18/154,998	**11/13/2023 04:50:06 PM Z ET**	**18896-000072-US-COA**

Title of Invention

SITE-SPECIFIC ANTIBODY CONJUGATION AND ANTIBODY-DRUG CONJUGATE AS SPECIFIC EMBODIMENT THEREOF

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	5143	FILED BY	Erica Imergoot
PATENT CENTER #	63233371	AUTHORIZED BY	Paul Zarek
CUSTOMER #	28997	FILING DATE	01/16/2023
CORRESPONDENCE ADDRESS	-	FIRST NAMED INVENTOR	Sang Jeon CHUNG

Payment Information

PAYMENT METHOD	PAYMENT TRANSACTION ID	PAYMENT AUTHORIZED BY
DA / 080750	**E2023ACG50295633**	**Paul Zarek**

FEE CODE	DESCRIPTION	ITEM PRICE($)	QUANTITY	ITEM TOTAL($)
2202	EACH CLAIM IN EXCESS OF 20	40.00	1	40.00
			TOTAL AMOUNT:	**$40.00**

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New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for 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.

Used in Lieu of PTO/SB/08A/B
(Based on PTO 10-07 version)

Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 1

Complete if Known	
Application Number	18/154,998-Conf. #5143
Filing Date	January 16, 2023
First Named Inventor	Sang Jeon CHUNG
Art Unit	1642
Examiner Name	Y. Xiao
Attorney Docket Number	18896-000072-US-COA

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (*if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	AA	US-2021/0139541-A1	05-13-2021	MATSUDA et al.	

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]-Number[4]-Kind Code[5] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
	BA	WO-2020/184944-A1	09-17-2020	Abtis Co Ltd		

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. * CITE NO.: Those application(s) which are marked with an single asterisk (*) next to the Cite No. are not supplied (under 37 CFR 1.98(a)(2)(iii)) because that application was filed after June 30, 2003 or is available in the IFW. [1] Applicant's unique citation designation number (optional). [2] See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [6] Applicant is to place a check mark here if English language Translation is attached.

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	CA	Office Action from corresponding Australian Patent Application No. 2020234394 issued on 26 September 2023.	
	CB	TAMURA, T., et al.; "Rapid labelling and covalent inhibition of intracellular native proteins using ligand-directed N-acyl-N-alkyl sulfonamide", Nature Communications", 2018, 9, 1870, pp. 1-12.	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1]Applicant's unique citation designation number (optional). [2]Applicant is to place a check mark here if English language Translation is attached.

Examiner Signature		Date Considered	

KL/ei

Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 1

Complete if Known	
Application Number	18/154,998-Conf. #5143
Filing Date	January 16, 2023
First Named Inventor	Sang Jeon CHUNG
Art Unit	1642
Examiner Name	Y. Xiao
Attorney Docket Number	18896-000072-US-COA

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (*if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]-Number[4]-Kind Code[5] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
	BA**	KR-10-2020-0002858-A	01-08-2020	Ajinomoto KK		√

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NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	CA**	Notice of Allowance from corresponding Korean Application No. 10-2020-0029224, dated 25 July 2023.	√

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

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Examiner Signature		Date Considered	

63282689 KL/aa

Electronic Acknowledgement Receipt

EFS ID:	48678610
Application Number:	18154998
International Application Number:	
Confirmation Number:	5143
Title of Invention:	SITE-SPECIFIC ANTIBODY CONJUGATION AND ANTIBODY-DRUG CONJUGATE AS SPECIFIC EMBODIMENT THEREOF
First Named Inventor/Applicant Name:	Sang Jeon CHUNG
Customer Number:	28997
Filer:	Kisuk Lee/Erica Imergoot
Filer Authorized By:	Kisuk Lee
Attorney Docket Number:	18896-000072-US-COA
Receipt Date:	04-OCT-2023
Filing Date:	16-JAN-2023
Time Stamp:	13:01:07
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Foreign Reference	KR_10-2020-0002858_Apt1.pdf	7260231 b4e181bd604821088264d593efff9e988ed726b4	no	169

Warnings:

Information:					
2	Foreign Reference	KR_10-2020-0002858_Apt2.pdf	22539352 510f29ac364e72692750b70d6fc9a68f9637cabb	no	169
Warnings:					
Information:					
3	Other reference-Patent/Application/ Search Documents	KR_10-2020-0029224_Notice_of_Allowance.PDF	382924 079466b574e296b562450e229bc190951856b99c	no	3
Warnings:					
Information:					
4	Transmittal Letter	000072uscoa_SupplementalIDSTransmittal.PDF	17579 1f5e1ab96f68a1772eb53d1870060969593 3fdd4	no	2
Warnings:					
Information:					
5	Information Disclosure Statement (IDS) Form (SB08)	000072uscoa_SupplementalForm1449.PDF	28298 fecba3d1bd1705840d29f4a53310f2fe8470d2c5	no	1
Warnings:					
Information:					
This is not an USPTO supplied IDS fillable form					
		Total Files Size (in bytes):	30228384		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Docket No.: 18896-000072-US-COA
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Sang Jeon CHUNG et al.

Application No.: 18/154,998 Confirmation No.: 5143

Filed: January 16, 2023 Art Unit: 1642

For: SITE-SPECIFIC ANTIBODY CONJUGATION AND ANTIBODY-DRUG CONJUGATE AS SPECIFIC EMBODIMENT THEREOF

Examiner: Yan XIAO

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT UNDER 37 C.F.R. §1.97(C)(1)

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

Dear Commissioner:

Applicant hereby submits a Supplemental Information Disclosure Statement (IDS) under 37 C.F.R. §1.97(c)(1): An IDS shall be considered by the Office if filed after the period specified in paragraph (b) of this section, provided that the IDS is filed before the mailing date of any of a final action under §1.113, a notice of allowance under §1.311, or an action that otherwise closes prosecution in the application, and it is accompanied by: (1) The Statement specified in paragraph (e) of this section. No fee is believed payable with respect to this IDS. However, if any fee should be found payable, please charge such fee to Deposit Account No. 08-0750.

37 C.F.R. §1.97(e)(1) Statement: Each item of information contained in the IDS was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the IDS.

This IDS further supplements the IDS(s) previously submitted on 16 January 2023.

In accordance with 37 C.F.R. §1.97(g) and (h), this submission shall not be construed as a representation that a search has been made or as admission that information presented or cited herein is, or is considered to be, material to patentability as defined in 37 C.F.R. §1.56(b).

In accordance with 37 C.F.R. §1.98, Applicant submits herewith a listing on Form HDP-1449 of documents requested to be considered by the Examiner. Consideration by the Examiner of the present submission is requested.

Applicant believes no fee is due with this response. However, if a fee is due, please charge our Deposit Account No. 08-0750, under Order No. 18896-000072-US-COA from which the undersigned is authorized to draw.

Dated: October 4, 2023

Respectfully submitted,

Electronic signature: /Kisuk Lee/
Kisuk Lee
Registration No.: 66,861
HARNESS, DICKEY & PIERCE, P.L.C.
7700 Bonhomme
Suite 400
Clayton, Missouri 63105
314.446.7670
Attorney for Applicant

Enclosures:
HDP Form 1449
Cited documents

UNITED STATES PATENT AND TRADEMARK OFFICE

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
18/154,998	01/16/2023	Sang Jeon CHUNG	18896-000072-US-COA	5143

28997 7590 08/16/2023

Harness Dickey (St. Louis)
7700 Bonhomme, Suite 400
St. Louis, MO 63105

EXAMINER
XIAO, YAN

ART UNIT	PAPER NUMBER
1642	

NOTIFICATION DATE	DELIVERY MODE
08/16/2023	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):

bkamer@hdp.com
stldocket@hdp.com

Office Action Summary	Application No. 18/154,998	Applicant(s) CHUNG et al.	
	Examiner YAN XIAO	Art Unit 1642	AIA (FITF) Status Yes

-- 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 MONTHS 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 07/31/2023.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

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

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5) ☑ Claim(s) 54-76 is/are pending in the application.

5a) Of the above claim(s) 67-76 is/are withdrawn from consideration.

6) ☐ Claim(s) _____ is/are allowed.

7) ☑ Claim(s) 54-56 and 59-65 is/are rejected.

8) ☑ Claim(s) 57-58 and 66 is/are objected to.

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

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to **PPHfeedback@uspto.gov.**

Application Papers

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

11)☑ The drawing(s) filed on 05/02/2023 is/are: a)☐ accepted or b)☑ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

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)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☑ Notice of References Cited (PTO-892)

2) ☑ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

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

4) ☑ Other: PTO-2301.

Notice of Pre-AIA or AIA Status

1. The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

DETAILED ACTION

2. The election without traverse filed 07/31/2023 in response to the Office Action of 06/09/2023 is acknowledged and has been entered.

Applicant has elected Group I, claims 54-66, drawn to a modified antibody or its fragment comprising bio-orthogonal click chemistry functional group, wherein the modified antibody comprises one or more modified heavy chain, wherein each of the modified heavy chain comprises a modified lysine residue at 248 position of heavy chain amino acid sequence, wherein the 248 position is determined by an EU numbering system, and wherein the modified lysine residue comprises a bio-orthogonal click chemistry functional group at terminal end.

3. Claims 54-76 are pending in the application. Claims 67-76 have been withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected invention, there being no allowable generic or linking claim. Election was made without traverse in the reply filed on 07/31/2023.

4. Claims 54-66 are currently under prosecution.

Priority

5. Applicant's claim under 35 U.S.C. §§ 119(e), 120 and 365(c) for benefit of the earlier filing date of application, is acknowledged.

Drawings

6. The drawing set forth as Figure 1 is objected to because the figure depicts an amino acid sequence, which is not identified by sequence identification number, either in the figure or in the brief description of figure at page 17. Sequences appearing in the specification and/or drawings must be identified by a sequence identifier in accordance

with 37 C.F.R. 1.821(d); sequence identifiers for sequences appearing in the drawings may appear in the drawings or in the brief description of the drawings.

A replacement drawing sheet, including the correction, is required, if the drawings are objected to. See 37 CFR 1.121(d). However, this ground of objection would be withdrawn, so that a replacement drawing would be not being required, if Applicant were to amend the brief description of the figure at page 17 of the specification to include sequence identification number.

Specification

7. The disclosure is objected to for the following reason: The specification contains sequence disclosures that are encompassed by the definitions for nucleotide and/or amino acid sequences set forth in 37 C.F.R. § 1.821(a)(1) and (a)(2). Sequences appearing in the specification and/or drawings must be identified by sequence identifier in accordance with 37 C.F.R. 1.821(d). According to 37 CFR § 1.821(a), an unbranched sequence of four or more specifically identified amino acids or an unbranched sequence of ten or more nucleotides must be identified by sequence identification numbers. See MPEP § 2422.01.

In this instance, the sequence depicted in Figure 1 is not identified by sequence identification number, either in the figure or in the brief description of figure at page 17. In addition, there are sequences disclosed in Table 1, which are also not identified by sequence identification numbers.

Applicant must provide appropriate amendments to the specification or drawings inserting the required sequence identifiers. Sequence identifiers for sequences appearing in the drawings may appear in the drawings or in the brief description of the drawings.

As noted in the attached Notice to Comply, appropriate action correcting this deficiency is required. If necessary to correct the deficiency, Applicant must submit paper and computer-readable copies of a substitute sequence listing, together with an

amendment directing its entry into the specification and a statement that the content of both copies are the same and, where applicable, include no new matter.

Claim Objections

8. Claim 59 is objected because the claim contains sequence disclosure that is encompassed by the definition for nucleotide and/or amino acid sequences set forth in 37 C.F.R. § 1.821(a) (1) and (a) (2). The claim fails to comply with the requirements of 37 C.F.R. §§ 1.821-1.825. In particular claim 59 discloses sequence without SEQ ID NO or improper SEQ ID NO. Applicant is reminded to review the entire disclosure to ensure that the present application is in sequence compliance.

Any questions regarding compliance with the sequence rules requirements specifically should be directed to the departments listed at the bottom of the Notice to Comply.

APPLICANT IS GIVEN THE TIME ALLOTED IN THIS OFFICE ACTION WITHIN WHICH TO COMPLY WITH THE SEQUENCE RULES, 37 C.R.F. §§ 1.821-1.825. Failure to comply with these requirements will result in ABANDONMENT of the application under 37 C.F.R. § 1.821(g). Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 C.F.R. § 1.136. In no case may an applicant extend the period for response beyond the six-month statutory period. Direct the response to the undersigned.

9. In the event the determination of the status of the application as subject to AIA 35 U.S.C. 102 and 103 (or as subject to pre-AIA 35 U.S.C. 102 and 103) is incorrect, any correction of the statutory basis for the rejection will not be considered a new ground of rejection if the prior art relied upon, and the rationale supporting the rejection, would be the same under either status.

Claim Rejections - 35 USC § 102

10. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –

> (a)(1) the claimed invention was patented, described in a printed publication, or in public use, on sale or otherwise available to the public before the effective filing date of the claimed invention.

11. Claims 54-56 and 59-65 are rejected under 35 U.S.C. 102(a)(1) as being anticipated by Yamada, et al. (WO 2018199337, published on Nov. 1, 2018, IDS) evidenced by machine translation of WO 2018199337 (08/04/2023).

Claims 54-56 and 59-65 are drawn to a modified antibody or its fragment comprising bio-orthogonal click chemistry functional group, wherein the modified antibody comprises one or more modified heavy chain, wherein each of the modified heavy chain comprises a modified lysine residue at 248 position of heavy chain amino acid sequence, wherein the 248 position is determined by an EU numbering system, and wherein the modified lysine residue comprises a bio-orthogonal click chemistry functional group at terminal end.

Yamada, et al. teach a compound containing an affinity agent for soluble protein (e.g. antibody) and a bioorthogonal functional group; see entire document, e.g. [0027], [0030] of machine translation of WO 2018199337. Yamada, et al. teach modification of lysine residue (positions 248) of the antibody; see [0037-0040] of machine translation of WO 2018199337.

For claims 55-56 and 60-62, Yamada, et al. teach the structure of the instant claim 55; see [0572].

For claim 59, Yamada, et al. teach THTCPPCPAPELLGGPSVFLFPPKPKDTLMISR (SEQ ID NO: 40); see page 39 of machine translation of WO 2018199337. SEQ ID NO: 40 of Yamada, et al. comprising the instant claimed Formula 8-2.

For claim 63, Yamada, et al. teach modified trastuzumab (which comprises two heavy chains); see page 39 of machine translation of WO 2018199337.

For claims 64-65, Yamada, et al. teach the bioorthogonal functional group is an azide residue (which is a group capable of participating cycloaddition reaction); see page 7 of machine translation of WO 2018199337.

Double Patenting

12. 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 double patenting rejection is appropriate where the claims at issue 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 reference 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. A terminal disclaimer must be signed in compliance with 37 CFR 1.321(b).

The USPTO internet Web site contains terminal disclaimer forms which may be used. Please visit http://www.uspto.gov/forms/. The filing date of the application will determine what form should be used. A web-based eTerminal Disclaimer may be filled out completely online using web-screens. An eTerminal Disclaimer that meets all requirements is auto-processed and approved immediately upon submission. For more information about eTerminal Disclaimers, refer to http://www.uspto.gov/patents/process/file/efs/guidance/eTD-info-I.jsp.

13. Claims 54, 63 and 65 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 21-33 of copending Application No. 17/424528. Although the conflicting claims are not identical, they are not patentably distinct from each other for the following reasons:

Claims 54, 63 and 65 are drawn to a modified antibody or its fragment comprising bio-orthogonal click chemistry functional group, wherein the modified antibody comprises one or more modified heavy chain, wherein each of the modified heavy chain comprises a modified lysine residue at 248 position of heavy chain amino acid sequence, wherein the 248 position is determined by an EU numbering system, and wherein the modified lysine residue comprises a bio-orthogonal click chemistry functional group at terminal end, wherein the bio-orthogonal click chemistry functional group is azide group.

Claims 21-33 of copending Application No. 17/424528 are drawn to an antibody-payload conjugate comprising click chemistry functional groups, wherein the click chemistry functional group is azide, wherein the nitrogen atom linked to the Ab is derived from the lysine 246 or the lysine 248 of each Fc region of the antibody.

This is a provisional obviousness-type double patenting rejection because the conflicting claims have not in fact been patented.

Conclusion

14. Claims 54-56 and 59-65 are rejected. Claims 57-58 and 66 are objected to as being dependent upon a rejected base claim.

15. Any inquiry concerning this communication or earlier communications from the examiner should be directed to YAN XIAO whose telephone number is (571)270-3578. The examiner can normally be reached M-F 8-5 EST.

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at http://www.uspto.gov/interviewpractice.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Misook Yu can be reached on 571-272-0839. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of published or unpublished applications may be obtained from Patent Center. Unpublished application information in Patent Center is available to registered users. To file and manage patent submissions in Patent Center,

visit: https://patentcenter.uspto.gov. Visit https://www.uspto.gov/patents/apply/patent-center for more information about Patent Center and https://www.uspto.gov/patents/docx for information about filing in DOCX format. For additional questions, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/YAN XIAO/
Primary Examiner, Art Unit 1642

Notice to Comply	Application No. 18/154,998	Applicant(s) CHUNG et al.	
	Examiner YAN XIAO	Art Unit 1642	AIA (FITF) Status Yes

NOTICE TO COMPLY WITH REQUIREMENTS FOR PATENT APPLICATIONS CONTAINING NUCLEOTIDE SEQUENCE AND/OR AMINO ACID SEQUENCE DISCLOSURES

Applicant must file the items indicated below within the time period set in the Office action to which the Notice is attached to avoid abandonment under 35 U.S.C. § 133 (extensions of time may be obtained under the provisions of 37 CFR 1.136(a)).

The nucleotide and/or amino acid sequence disclosure contained in this application does not comply with the requirements for such a disclosure as set forth in 37 C.F.R.1.8211.825 for the following reason(s):

☑ 1. This application clearly fails to comply with the requirements of 37 C.F.R. 1.821-1.825. Applicant's attention is directed to the final rulemaking notice published at 55 FR 18230 (May 1, 1990), and 1114 OG 29 (May 15, 1990).
If the effective filing date is on or after July 1, 1998, see the final rulemaking notice published at 63 FR 29620 (June 1, 1998) and 1211 OG 82 (June 23, 1998).

☐ 2.This application does not contain, as a separate part of the disclosure on paper copy, a “Sequence Listing” as required by 37 C.F.R.1.821(c).

☐ 3. A copy of the “Sequence Listing” in computer readable form has not been submitted as required by 37 C.F.R.1.821(e).

☐ 4. A copy of the “Sequence Listing” in computer readable form has been submitted. However, the content of the computer readable form does not comply with the requirements of 37 C.F.R.1.822 and/or 1.823, as indicated on the attached copy of the marked -up “Raw Sequence Listing.”

☐ 5. The computer readable form that has been filed with this application has been found to be damaged and/or unreadable as indicated on the attached CRF Diskette Problem Report. A Substitute computer readable form must be submitted as required by 37 C.F.R.1.825(d).

☐ 6. The paper copy of the “Sequence Listing” is not the same as the computer readable form of the “Sequence Listing” as required by 37 C.F.R.1.821(e).

☐ 7. Other: _____

Applicant Must Provide:

☑ An initial or substitute computer readable form (CRF) copy of the “Sequence Listing”.

☑ An initial or substitute paper copy of the “Sequence Listing”, **as an amendment specifically directing its entry into the application.**

☑ A statement that the content of the paper and computer readable copies are the same and, where applicable , include no new matter, as required by 37 C.F.R.1.821(e) or 1.821(f) or 1.821(g) or 1.825(b) or 1.825(d).

For questions regarding compliance to these requirements, please contact:

For Rules Interpretation, call (571) 270-5300
For CRF Submission Help, call (571) 272-2510
PatentIn Software Program Support
Technical Assistance.1-866-217-9197
PatentIn Software is Available At www.USPTO.gov

PLEASE RETURN A COPY OF THIS NOTICE WITH YOUR REPLY

/YAN XIAO/ Primary Examiner, Art Unit 1642	

Notice of References Cited	Application/Control No. 18/154,998	Applicant(s)/Patent Under Reexamination CHUNG et al.	
	Examiner YAN XIAO	Art Unit 1642	Page 1 of 1

U.S. PATENT DOCUMENTS

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

FOREIGN PATENT DOCUMENTS

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

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Machine translation WO2018199337 (08/04/2023, pages 1-378)
	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.

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	18/154,998	CHUNG et al.
	Examiner	Art Unit
	YAN XIAO	1642

CPC - Searched*		
Symbol	**Date**	**Examiner**
A61K 47/6803	08/09/2023	YX
A61K 47/6855	08/09/2023	YX
A61K 47/6889	08/09/2023	YX
A61P 35/00	08/09/2023	YX
C07D 207/46	08/09/2023	YX
C07K 16/32	08/09/2023	YX

CPC Combination Sets - Searched*		
Symbol	**Date**	**Examiner**

US Classification - Searched*			
Class	**Subclass**	**Date**	**Examiner**
424	133.1	08/09/2023	YX

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

Search Notes		
Search Notes	**Date**	**Examiner**
Search through PE2ESearch and STN	08/09/2023	YX
Performed and recorded inventors search	08/09/2023	YX
STIC (please see PE2E-DAV for sequences search results)	08/04/2023	YX

/YAN XIAO/ Primary Examiner, Art Unit 1642	

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	18/154,998	CHUNG et al.
	Examiner	Art Unit
	YAN XIAO	1642

Interference Search			
US Class/CPC Symbol	US Subclass/CPC Group	Date	Examiner

/YAN XIAO/ Primary Examiner, Art Unit 1642	

* * * * * * * * * * Welcome to STN International * * * * * * * * * *

NEWS 1 MAR 25 New Get Prior Art Analysis Feature in CA/CAplus Provides an Efficient Means to Expand Search Results
NEWS 2 MAR 25 Searching Chemical Compounds with Fragmentation Codes in Derwent World Patents Index (R) Subscriber Files
NEWS 3 MAR 30 New Projects Feature Allows STNext Users to Define and Manage Search Projects
NEWS 4 APR 4 The enhanced Derwent World Patents Index(R) is now available on STNext
NEWS 5 APR 4 The Derwent Chemistry Resource (DCR) is now available as a standalone database on STNext
NEWS 6 JUN 22 Claims for Four Additional Patent Authorities Added to CA/CAplus
NEWS 7 JUN 24 SMARTracker SDI Option Now Supported in Derwent Files
NEWS 8 JUN 24 Expanded Claims Coverage and Enhanced HIT Displays of Claims Data in Derwent World Patents Index(R)
NEWS 9 SEP 9 Enhanced Coverage of Malaysia, Saudi Arabia, and Georgia Increases Comprehensiveness of INPADOC
NEWS 10 SEP 14 New Preference Setting for Transcript Options Helps Better Manage Transcripts, Increase Their Efficiency
NEWS 11 SEP 22 CAS STNext Structure Editor Update Preserves Functionality, Increases Flexibility
NEWS 12 OCT 24 Links to National Patent Registers Now Available in Patent Databases on CAS STNext
NEWS 13 NOV 7 PCTFULL Reload Provides Additional Coverage, Independent Claims and Assignee Searching Enhancements
NEWS 14 NOV 7 EPFULL Reload on STNext Features New Independent Claims Searching Capabilities
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NEWS 23 MAY 12 Claim Tags Enhancement Extended to Include WO Patents
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NEWS 25 JUL 05 Reloaded Japanese Patent Full-Text Database JPFULL Provides Expanded Content, New Search Options
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=> S lysine (A) 248

L1          14 FILE BIOSIS
L2           7 FILE EMBASE
L3           0 FILE IPA
L4           6 FILE MEDLINE
L5           7 FILE PCTFULL
L6           4 FILE PQSCITECH
L7           0 FILE PS
L8           6 FILE SCISEARCH

TOTAL FOR ALL FILES
L9          44 LYSINE (A) 248

=> S bio-orthogonal OR bioorthogonal

L10       1962 FILE BIOSIS
L11       3157 FILE EMBASE
L12         32 FILE IPA
L13       2948 FILE MEDLINE
L14       1343 FILE PCTFULL
L15        560 FILE PQSCITECH
L16          0 FILE PS
L17       3607 FILE SCISEARCH

TOTAL FOR ALL FILES
L18      13609 BIO-ORTHOGONAL OR BIOORTHOGONAL

=> S L9 AND L18

L19          0 FILE BIOSIS
L20          0 FILE EMBASE
L21          0 FILE IPA
L22          0 FILE MEDLINE
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L23           1 FILE PCTFULL
L24           0 FILE PQSCITECH
L25           0 FILE PS
L26           0 FILE SCISEARCH

TOTAL FOR ALL FILES
L27           1 L9 AND L18

=> S L27 AND PY<=2019

L28           0 FILE BIOSIS
L29           0 FILE EMBASE
L30           0 FILE IPA
L31           0 FILE MEDLINE
L32           0 FILE PCTFULL
L33           0 FILE PQSCITECH
'2019' NOT A VALID FIELD CODE
L34           0 FILE PS
L35           0 FILE SCISEARCH

TOTAL FOR ALL FILES
L36           0 L27 AND PY<=2019

=>
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(Based on PTO 10-07 version)

Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 1

Complete if Known	
Application Number	Not Yet Assigned
Filing Date	Concurrently Herewith
First Named Inventor	Sang Jeon CHUNG
Art Unit	Not Yet Assigned
Examiner Name	Not Yet Assigned
Attorney Docket Number	18896-000072-US-COA

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (*if known*)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	AA	US-2012-0172575-A1	07-05-2012	BOONS et al.	
	AB	US-2018-0141976-A1	05-24-2018	ITO	Corresponding to KR 10-2018-0002734 A
	AC	US-2020-0190165-A1	06-18-2020	Yamada et al.	Corresponding to WO 2018-199337 A1

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]-Number[4]-Kind Code[5] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
	BA**	CN-107496978-A	12-22-2017	Liu, Yunhui		√
	BB**	EP-3 299 383-A1	03-28-2018	Univ Kagoshima		
	BC**	EP-3617235-A1	03-04-2020	Ajinomoto Co., Inc		
	BD**	KR-10-2018-0002734-A	01-08-2018	Univ Kagoshima		√
	BE**	WO-2018-199337-A1	11-01-2018	Ajinomoto KK		

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. * CITE NO.: Those application(s) which are marked with an single asterisk (*) next to the Cite No. are not supplied (under 37 CFR 1.98(a)(2)(iii)) because that application was filed after June 30, 2003 or is available in the IFW. ** CITE NO.: Those document(s) which are marked with an double asterisk (**) next to the Cite No. are not supplied because they were previously cited by or submitted to the Office in a prior application relied upon in this application for an earlier filing date under 35 U.S.C. 120. [1] Applicant's unique citation designation number (optional). [2] See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [6] Applicant is to place a check mark here if English language Translation is attached.

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	CA**	VAN GEEL, R. et al.; Chemoenzymatic Conjugation of Toxic Payloads to the Globally Conserved NGlycan of Native mAbs Provides Homogeneous and Highly Efficacious AntibodyDrug Conjugates", Bioconjugate Chemistry, 2015, 26, pgs. 2233-2242.	
	CB**	International Search Report from corresponding PCT Application No. PCT/KR2020/003282, dated 16 June 2020.	
	CC**	Written Opinion from corresponding PCT Application No. PCT/KR2020/003282, dated 16 June 2020.	
	CD**	Extended European Search Report from corresponding European Patent Application No. 20770235.8, dated 10 October 2022.	
	CE**	KUROSAKI HIROMASA et al: "Irreversable Inhibition of Metallo-[beta]-lactamase (IMP-1) by 3-(30Mercaptopropionylsulfanyl)propionic Acid Pentafluorophenyl Ester", Angewandte Chemie International Edition, vol. 44, no. 25, 10 June 2005, pp. 3861-3864.	
	CF**	Office Action from corresponding Japanese Patent Application No. 2021-553121, dated 21 October 2022.	
	CG**	Yamaguchi, Y., et al.; "Structure-Function Analysis and Development of Inhibitors of Metallo-β-lactamases Conferring Drug Resistance in Bacteria", Yakugaku Zasshi, vol. 135, no. 11, 2015, pp. 1299-1305.	

Examiner Signature	/YAN XIAO/	Date Considered	08/08/2023

KL/ei

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /Y.X/

PE2E SEARCH - Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	British Equivalents	Time Stamp
L1	1	"18154998"	(US-PGPUB; USPAT)	ADJ	ON	ON	2023/08/03 10:04 PM
L2	13	"10751826"	(US-PGPUB; USPAT)	ADJ	ON	ON	2023/08/04 08:45 PM
L3	2	"15520573"	(US-PGPUB; USPAT)	ADJ	ON	ON	2023/08/04 08:54 PM
L4	2	"10584692"	(US-PGPUB; USPAT)	ADJ	ON	ON	2023/08/04 09:13 PM
L5	2	"16029979"	(US-PGPUB; USPAT)	ADJ	ON	ON	2023/08/04 09:48 PM
L6	3	"14898126"	(US-PGPUB; USPAT)	ADJ	ON	ON	2023/08/04 09:50 PM
L7	1	"16080903"	(US-PGPUB; USPAT)	ADJ	ON	ON	2023/08/04 09:53 PM
L8	1	"16663791"	(US-PGPUB; USPAT)	ADJ	ON	ON	2023/08/04 09:56 PM
L9	1	"17119495"	(US-PGPUB; USPAT)	ADJ	ON	ON	2023/08/04 10:01 PM
L10	2692	lysine WITH "248"	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)	ADJ	ON	ON	2023/08/09 10:47 AM
L11	39	L10 AND (bio-orthogonal OR biorthogonal)	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)	ADJ	ON	ON	2023/08/09 10:48 AM

L12	7	L11 AND @PY<="2019"	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)	ADJ	ON	ON	2023/08/09 10:49 AM
L13	1749903	(A61K47/6803 OR A61K47/6855 OR A61K47/6889 OR A61P35/00 OR C07D207/46 OR C07K16/32).cpc.	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)	ADJ	ON	ON	2023/08/09 10:53 AM
L14	18814	424/133.1	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)	ADJ	ON	ON	2023/08/09 10:54 AM
L15	5	L12 AND (L13 OR L14)	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO,	ADJ	ON	ON	2023/08/09 10:54 AM

			MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)				
L16	14	CHUNG, Sang Jeon .IN.	(US-PGPUB; USPAT)	ADJ	ON	ON	2023/08/09 09:25 PM
L17	59	Kim, Ju Hwan .IN.	(US-PGPUB; USPAT)	ADJ	ON	ON	2023/08/09 09:26 PM
L18	25	Lee, Young Geun .IN.	(US-PGPUB; USPAT)	ADJ	ON	ON	2023/08/09 09:29 PM
L19	547	Lee, Tae Jin .IN.	(US-PGPUB; USPAT)	ADJ	ON	ON	2023/08/09 09:29 PM
L20	22	Seo, Jin Woo .IN.	(US-PGPUB; USPAT)	ADJ	ON	ON	2023/08/09 09:30 PM
L21	4	L16 AND lysine	(US-PGPUB; USPAT)	ADJ	ON	ON	2023/08/09 09:32 PM
L22	3	L16 AND lysine .CLM.	(US-PGPUB; USPAT)	ADJ	ON	ON	2023/08/09 09:32 PM
L23	1	L17 AND lysine .CLM.	(US-PGPUB; USPAT)	ADJ	ON	ON	2023/08/09 09:33 PM
L24	1	L18 AND lysine .CLM.	(US-PGPUB; USPAT)	ADJ	ON	ON	2023/08/09 09:33 PM
L25	1	L19 AND lysine .CLM.	(US-PGPUB; USPAT)	ADJ	ON	ON	2023/08/09 09:33 PM
L26	1	L20 AND lysine .CLM.	(US-PGPUB; USPAT)	ADJ	ON	ON	2023/08/09 09:33 PM

PE2E SEARCH - Search History (Interference)

There are no Interference searches to show.

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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
18/154,998	01/16/2023	Sang Jeon CHUNG	18896-000072-US-COA

CONFIRMATION NO. 5143

28997
Harness Dickey (St. Louis)
7700 Bonhomme, Suite 400
St. Louis, MO 63105

PUBLICATION NOTICE

OC00000006042730Z

Date Mailed: 08/11/2023

Title:SITE-SPECIFIC ANTIBODY CONJUGATION AND ANTIBODY-DRUG CONJUGATE AS SPECIFIC EMBODIMENT THEREOF

Publication No.US-2023-0248842-A1
Publication Date:08/10/2023

NOTICE OF PUBLICATION OF APPLICATION

The above-identified application will be electronically published as a patent application publication pursuant to 37 CFR 1.211, et seq. The patent application publication number and publication date are set forth above.

The publication may be accessed through the USPTO's publically available Searchable Databases via the Internet at www.uspto.gov. The direct link to access the publication is currently http://www.uspto.gov/patft/.

The publication process established by the Office does not provide for mailing a copy of the publication to applicant. A copy of the publication may be obtained from the Office upon payment of the appropriate fee set forth in 37 CFR 1.19(a)(1). Orders for copies of patent application publications are handled by the USPTO's Public Records Division. The Public Records Division can be reached by telephone at (571) 272-3150 or (800) 972-6382, by facsimile at (571) 273-3250, by mail addressed to the United States Patent and Trademark Office, Public Records Division, Alexandria, VA 22313-1450 or via the Internet.

In addition, information on the status of the application, including the mailing date of Office actions and the dates of receipt of correspondence filed in the Office, may also be accessed via the Internet through the Patent Electronic Business Center at www.uspto.gov using the public side of the Patent Application Information and Retrieval (PAIR) system. The direct link to access this status information is currently https://portal.uspto.gov/pair/PublicPair. Prior to publication, such status information is confidential and may only be obtained by applicant using the private side of PAIR.

Further assistance in electronically accessing the publication, or about PAIR, is available by calling the Patent Electronic Business Center at 1-866-217-9197.

Office of Data Managment, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

Electronic Acknowledgement Receipt

EFS ID:	48370728
Application Number:	18154998
International Application Number:	
Confirmation Number:	5143
Title of Invention:	SITE-SPECIFIC ANTIBODY CONJUGATION AND ANTIBODY-DRUG CONJUGATE AS SPECIFIC EMBODIMENT THEREOF
First Named Inventor/Applicant Name:	Sang Jeon CHUNG
Customer Number:	28997
Filer:	Paul Zarek
Filer Authorized By:	
Attorney Docket Number:	18896-000072-US-COA
Receipt Date:	31-JUL-2023
Filing Date:	16-JAN-2023
Time Stamp:	12:21:32
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Response to Election / Restriction Filed	Response.pdf	92755 05fd57d739b1194ff87f046ae9138dc340d52248	no	2

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New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application No.:	18/154,998
Filing Date:	16 January 2023
Applicant:	Sang Jeon CHUNG
Group Art Unit:	1642
Confirmation No.:	5143
Examiner:	Yan XIAO
Title:	SITE-SPECIFIC ANTIBODY CONJUGATION AND ANTIBODY-DRUG CONJUGATE AS SPECIFIC EMBODIMENT THEREOF
Attorney Docket:	18896-000072-US-COA

Commissioner for Patent
P.O. Box 1450
Alexandria, Virginia 22313-1450

31 July 2023

RESPONSE TO OFFICE ACTION DATED 9 JUNE 2023

This paper is in response to the Office Action (Restriction/Election Requirement) dated 9 June 2023 in the above referenced application, in which a two-month shortened statutory period was set for reply. This response is timely and no fee is believed due. If a fee is due, authorization is hereby provided to charge any fee due to Deposit Account No. **08-0750**.

* * *

REMARKS on the present amendment begin on page 2 of this paper.

STATUS OF THE CLAIMS

Claims 54-76 are pending in the present application.

RESPONSE TO OFFICE ACTION DATED 9 JUNE 2023

The Office is requiring Applicant to restrict these claims, under 35 U.S.C. §121 to one of the following groups of inventions:

I. Claims 54-66 drawn to a modified antibody or fragment; and

II. Claims 67-76, drawn to a method for preparing an antibody-payload conjugate.

Applicant provisionally elects, without traverse, Group I (i.e., Claims 54-66) for further prosecution. Applicant reserves the right to pursue one or more continuing applications directed toward any unelected subject matter.

Applicant believes the application is now in condition for examination on the merits. If personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number below at (314) 446-7668.

Respectfully submitted,

Dated: 31 July 2021

By: /Paul Zarek /
Paul Zarek, Reg. No. 75,190

HARNESS, DICKEY & PIERCE, P.L.C.
7700 Bonhomme, Suite 400
St. Louis, MO 63105
314-446-7668 (telephone)
314-726-7501 (facsimile)

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
18/154,998	01/16/2023	Sang Jeon CHUNG	18896-000072-US-COA	5143

28997 7590 06/09/2023

Harness Dickey (St. Louis)
7700 Bonhomme, Suite 400
St. Louis, MO 63105

EXAMINER
XIAO, YAN

ART UNIT	PAPER NUMBER
1642	

NOTIFICATION DATE	DELIVERY MODE
06/09/2023	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):

bkamer@hdp.com
stldocket@hdp.com

Office Action Summary	Application No. 18/154,998	Applicant(s) CHUNG et al.	
	Examiner YAN XIAO	Art Unit 1642	AIA (FITF) Status Yes

-- 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 2 MONTHS 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 01/16/2023.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

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

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5) ☑ Claim(s) 54-76 is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6) ☐ Claim(s) _____ is/are allowed.

7) ☐ Claim(s) _____ is/are rejected.

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

9) ☑ Claim(s) 54-76 are subject to restriction and/or election requirement

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to **PPHfeedback@uspto.gov.**

Application Papers

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

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

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)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)

2) ☐ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

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

4) ☐ Other: _____.

Notice of Pre-AIA or AIA Status

1. The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

DETAILED ACTION

2. Claims 54-76 are pending in the application and are currently subject to the following restriction and election requirement.

Election/Restrictions

3. Restriction is required under 35 U.S.C. 121 and 372.

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1.

In accordance with 37 CFR 1.499, applicant is required, in reply to this action, to elect a single invention to which the claims must be restricted.

Group I, claim(s) 54-66, drawn to a modified antibody or its fragment comprising bio-orthogonal click chemistry functional group, wherein the modified antibody comprises one or more modified heavy chain, wherein each of the modified heavy chain comprises a modified lysine residue at 248 position of heavy chain amino acid sequence, wherein the 248 position is determined by an EU numbering system, and wherein the modified lysine residue comprises a bio-orthogonal click chemistry functional group at terminal end.

Group II, claim(s) 67-76, drawn to a method for preparing antibody-payload conjugate, comprising: contacting a modified antibody or its fragment comprising a first click chemistry functional group with a payload comprising a cargo unit and a second click chemistry functional group, wherein the antibody-payload conjugate is formed by the click chemistry reaction between the first click chemistry functional group of the modified antibody and the second click chemistry functional group of the payload, wherein the modified antibody comprises: one or more modified heavy chain, wherein each of the modified heavy chain comprises a modified lysine residue at 248 position of

heavy chain amino acid sequence, wherein the 248 position is determined based on EU numbering system, wherein the modified lysine residue comprises the first click chemistry functional group which is a bio-orthogonal click chemistry functional group at terminal end, wherein the antibody-payload conjugate comprises an antibody unit derived from the modified antibody and a payload unit derived from the payload, wherein the payload unit of the antibody-payload conjugate is linked to the antibody unit through K248 of the antibody unit.

4. The inventions listed as Groups I-II do not relate to a single general inventive concept under PCT Rule 13.1 because unity of invention between different categories of inventions will only be found to exist if specific combinations of inventions are present. Those combinations include:

A) A product and a special process of manufacture of said product.

B) A product and a process of use of said product.

C) A product, a special process of manufacture of said product, and a process of use of said product.

D) A process and an apparatus specially designed to carry out said process.

E) A product, a special process of manufacture of said product, and an apparatus specially designed to carry out said process.

The groups of inventions listed above do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The technical feature linking Groups I-II is a modified antibody comprises a modified lysine residue at 248 position of heavy chain amino acid sequence. However, WO 2018199337 (published on Nov, 1, 2018, IDS) teaches site-selectively modulate lysine 246 or 248 in an Fc domain using an analogue of Fc-III and use a biorthogonal functional group (see entire document, e.g. [0506]. Therefore, the technical feature linking the inventions of Groups I-II does not constitute a special technical feature as defined by PCT Rule 13.2 as it does not define a contribution over the prior art.

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.

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).

5. 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 of the other invention.

6. 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).

7. The examiner has required restriction between product or apparatus claims and process claims. Where applicant elects claims directed to the product/apparatus, and all product/apparatus claims are subsequently found allowable, withdrawn process claims

that include all the limitations of the allowable product/apparatus claims should be considered for rejoinder. All claims directed to a nonelected process invention must include all the limitations of an allowable product/apparatus claim for that process invention to be rejoined.

In the event of rejoinder, the requirement for restriction between the product/apparatus claims and the rejoined process claims will be withdrawn, and the rejoined process claims will be fully examined for patentability in accordance with 37 CFR 1.104. Thus, to be allowable, the rejoined claims must meet all criteria for patentability including the requirements of 35 U.S.C. 101, 102, 103 and 112. Until all claims to the elected product/apparatus are found allowable, an otherwise proper restriction requirement between product/apparatus claims and process claims may be maintained. Withdrawn process claims that are not commensurate in scope with an allowable product/apparatus claim will not be rejoined. See MPEP § 821.04. Additionally, in order for rejoinder to occur, applicant is advised that the process claims should be amended during prosecution to require the limitations of the product/apparatus claims. **Failure to do so may result in no rejoinder.** Further, note that the prohibition against double patenting rejections of 35 U.S.C. 121 does not apply where the restriction requirement is withdrawn by the examiner before the patent issues. See MPEP § 804.01.

Conclusion

8. Any inquiry concerning this communication or earlier communications from the examiner should be directed to YAN XIAO whose telephone number is (571)270-3578. The examiner can normally be reached M-F 8-5 EST.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Misook Yu can be reached on 571-272-0839. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of published or unpublished applications may be obtained from Patent Center. Unpublished application information in Patent Center is available to registered users. To file and manage patent submissions in Patent Center, visit: https://patentcenter.uspto.gov. Visit https://www.uspto.gov/patents/apply/patent-center for more information about Patent Center and

https://www.uspto.gov/patents/docx for information about filing in DOCX format. For additional questions, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/YAN XIAO/
Primary Examiner, Art Unit 1642

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.
18/154,998	01/16/2023	Sang Jeon CHUNG	18896-000072-US-COA	5143

28997 7590 05/16/2023

Harness Dickey (St. Louis)
7700 Bonhomme, Suite 400
St. Louis, MO 63105

EXAMINER
CENTRAL, DOCKET

ART UNIT	PAPER NUMBER
1643	

NOTIFICATION DATE	DELIVERY MODE
05/16/2023	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):

bkamer@hdp.com
stldocket@hdp.com

Decision Granting Request for Prioritized Examination (Track I)	**Application No.** 18/154,998	**Applicant(s)** CHUNG et al.	
	Examiner Dale A Hall	**Art Unit** OPET	**AIA (FITF) Status** Yes

1. THE REQUEST FILED 16 January 2023 IS **GRANTED** .

The above-identified application has met the requirements for prioritized examination

A. ☑ for an original nonprovisional application (Track I).
B. ☐ for an application undergoing continued examination (RCE).

2. **The above-identified application will undergo prioritized examination**. The application will be accorded special status throughout its entire course of prosecution until one of the following occurs:

A. filing a **petition for extension of time** to extend the time period for filing a reply;

B. filing an **amendment to amend the application to contain more than four independent claims, more than thirty total claims** , or a multiple dependent claim;

C. filing a **request for continued examination** ;

D. filing a notice of appeal;

E. filing a request for suspension of action;

F. mailing of a notice of allowance;

G. mailing of a final Office action;

H. completion of examination as defined in 37 CFR 41.102; or

I. abandonment of the application.

Telephone inquiries with regard to this decision should be directed to Dale Hall at (571)272-3586. In his/her absence, calls may be directed to Petition Help Desk at (571) 272-3282.

/Dale A. Hall/ Paralegal Specialist, OPET	

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
18/154,998	01/16/2023		1288	18896-000072-US-COA	23	2

CONFIRMATION NO. 5143

UPDATED FILING RECEIPT

28997
Harness Dickey (St. Louis)
7700 Bonhomme, Suite 400
St. Louis, MO 63105

OC000000056726185

Date Mailed: 05/05/2023

Receipt is acknowledged of this non-provisional utility patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF FIRST INVENTOR, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection.

Please verify the accuracy of the data presented on this receipt. If an error is noted on this Filing Receipt, please submit a written request for a corrected Filing Receipt, including a properly marked-up ADS showing the changes with strike-through for deletions and underlining for additions. If you received a "Notice to File Missing Parts" or other Notice requiring a response for this application, please submit any request for correction to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections provided that the request is grantable.

Inventor(s)

Sang Jeon CHUNG, Gyeonggi-do, KOREA, REPUBLIC OF;
Ju Hwan KIM, Gyeonggi-do, KOREA, REPUBLIC OF;
Young Geun LEE, Gyeonggi-do, KOREA, REPUBLIC OF;
Tae Jin LEE, Gyeonggi-do, KOREA, REPUBLIC OF;
Jin Woo SEO, Gyeonggi-do, KOREA, REPUBLIC OF;

Applicant(s)

AbTis Co., Ltd., Gyeonggi-do, KOREA, REPUBLIC OF;

Power of Attorney: The patent practitioners associated with Customer Number 28997

Domestic Priority data as claimed by applicant

This application is a CON of 16/980,503 09/14/2020
which is a 371 of PCT/KR20/03282 03/09/2020
which claims benefit of 62/815,557 03/08/2019

Foreign Applications for which priority is claimed (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.) - None.
Foreign application information must be provided in an Application Data Sheet in order to constitute a claim to foreign priority. See 37 CFR 1.55 and 1.76.

Permission to Access Application via Priority Document Exchange: Yes

Permission to Access Search Results: Yes

Applicant may provide or rescind an authorization for access using Form PTO/SB/39 or Form PTO/SB/69 as appropriate.

If Required, Foreign Filing License Granted: 04/11/2023
The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 18/154,998**
Projected Publication Date: 08/10/2023
Non-Publication Request: No
Early Publication Request: No
**** SMALL ENTITY ****
Title

SITE-SPECIFIC ANTIBODY CONJUGATION AND ANTIBODY-DRUG CONJUGATE AS SPECIFIC EMBODIMENT THEREOF

Preliminary Class

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative,

this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4258).

LICENSE FOR FOREIGN FILING UNDER

Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where the conditions for issuance of a license have been met, regardless of whether or not a license may be required as set forth in 37 CFR 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign AssetsControl, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

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The United States represents the largest, most dynamic marketplace in the world and is an unparalleled location for business investment, innovation, and commercialization of new technologies. The U.S. offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to promote and facilitate business investment. SelectUSA provides information assistance to the international investor community; serves as an ombudsman for existing and potential investors; advocates on behalf of U.S. cities, states, and regions competing for global investment; and counsels U.S. economic development organizations on investment attraction best practices. To learn more about why the United States is the best country in the world to develop

technology, manufacture products, deliver services, and grow your business, visit http://www.SelectUSA.gov or call +1-202-482-6800.

PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 18/154,998

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	64		N/A	
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	280		N/A	
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	320		N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	23 minus 20 =	* 3	x 40 =	120	OR		
INDEPENDENT CLAIMS (37 CFR 1.16(h))	2 minus 3 =	*	x =	0			
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			504			
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				0			
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	1168		TOTAL	

APPLICATION AS AMENDED - PART II

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT A	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE($)	ADDITIONAL FEE($)	OR	RATE($)	ADDITIONAL FEE($)
AMENDMENT B	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.

** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".

*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".

The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application No.:	18/154,998
Filing Date:	16 January 2023
First Named Inventor:	Sang Jeon CHUNG
Group Art Unit:	TBD
Examiner:	TBD
Confirmation No.:	5143
Title:	SITE-SPECIFIC ANTIBODY CONJUGATION AND ANTIBODY-DRUG CONJUGATE AS SPECIFIC EMBODIMENT THEREOF
Attorney Docket:	18896-000072-US-COA

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

RESPONSE TO NOTICE TO FILE CORRECTED APPLICATION PAPERS

This paper is in response to the Notice to File Corrected Application Papers, dated 12 April 2023 in the above referenced application, in which a two-month shortened statutory period was set for reply. This response is timely and no fee for extension of time is believed payable.

* * *

Amendments to the Drawings begin on page **2** of this paper.

Remarks begin on page **3** of this paper.

AMENDMENTS TO THE DRAWINGS

The attached "Replacement Sheets" of drawings include changes to Figures 49, 50, 55, 60, 61 and 62. The attached "Replacement Sheets" which include Figures 1-73, replace the original sheets including Figures 1-73.

Attachment: Replacement Sheets

REMARKS

Applicant has amended the drawings (Figs. 49, 50, 55, 60-62) to remove the extraneous marks and strike thru marks as required by the Notice to File Corrected Application Papers received.

Although Applicant believes no fee is due in connection with this filing, the Patent Office is hereby authorized to charge any required fee or credit any overpayment to Deposit Account No. **08-0750**.

If the Examiner believes that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at (314) 446-7670.

Respectfully submitted,

Dated: 2 May 2023

By: / Kisuk Lee /
Kisuk Lee, Reg. No. 66,861

HARNESS, DICKEY & PIERCE, P.L.C.
7700 Bonhomme, Suite 400
St. Louis, MO 63105
314-446-7670 (telephone)
314-726-7501 (facsimile)

Electronic Acknowledgement Receipt

EFS ID:	47937977
Application Number:	18154998
International Application Number:	
Confirmation Number:	5143
Title of Invention:	SITE-SPECIFIC ANTIBODY CONJUGATION AND ANTIBODY-DRUG CONJUGATE AS SPECIFIC EMBODIMENT THEREOF
First Named Inventor/Applicant Name:	Sang Jeon CHUNG
Customer Number:	28997
Filer:	Kisuk Lee/Erica Imergoot
Filer Authorized By:	Kisuk Lee
Attorney Docket Number:	18896-000072-US-COA
Receipt Date:	02-MAY-2023
Filing Date:	16-JAN-2023
Time Stamp:	13:57:21
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Drawings-only black and white line drawings	000072uscoa_ReplacementDrawings.PDF	13465422 8cfd7cbf4920d6407e8d5444c885acb8e3ab4d10	no	73

Warnings:

Information:					
2		000072uscoa_ResponsetoNoticetoFileCorrectedApplicationPapers.PDF	19041 6b0043f11d987638cdf94858beb1b843902 12230	yes	3

Multipart Description/PDF files in .zip description		
Document Description	**Start**	**End**
Applicant Response to Pre-Exam Formalities Notice	1	1
Drawings-only black and white line drawings	2	2
Applicant Arguments/Remarks Made in an Amendment	3	3

Warnings:	
Information:	
Total Files Size (in bytes):	13484463

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.

amino acid sequence :

G—P—S—V—F—L—F—P—P—K—P—K—D—T—L—M—I

EU numbering for heavy chain :

237 238 239 240 241 242 243 244 245 246 247 248 249 250 251 252 253

FIG. 1

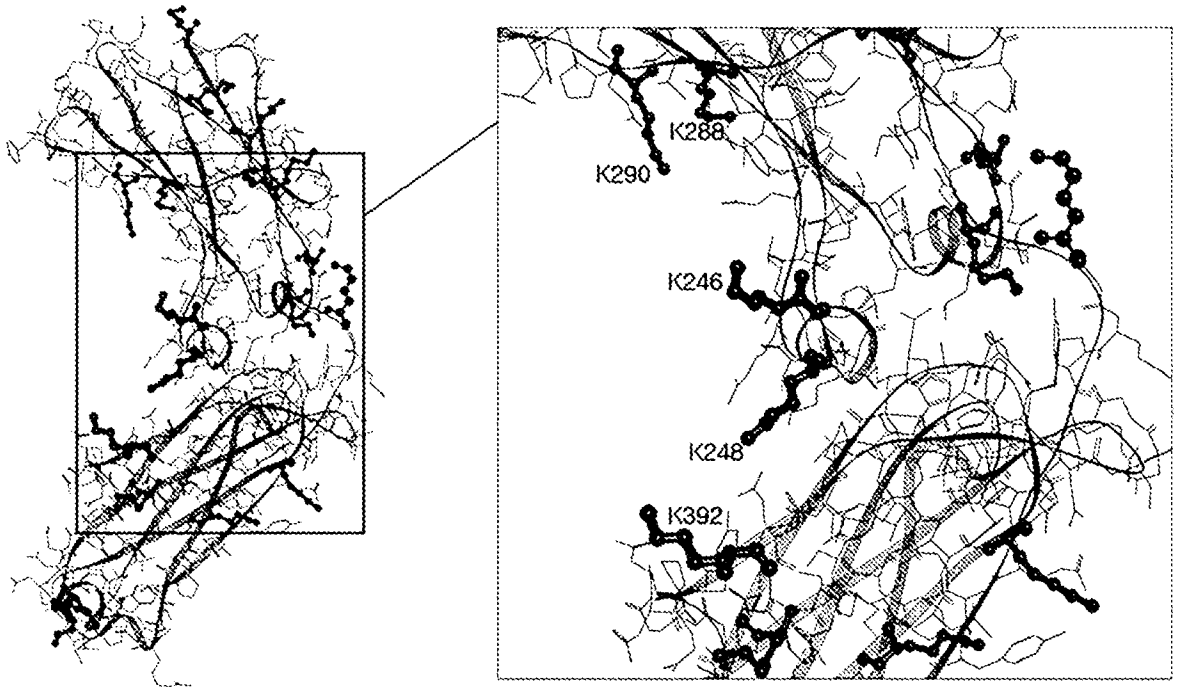


FIG. 2

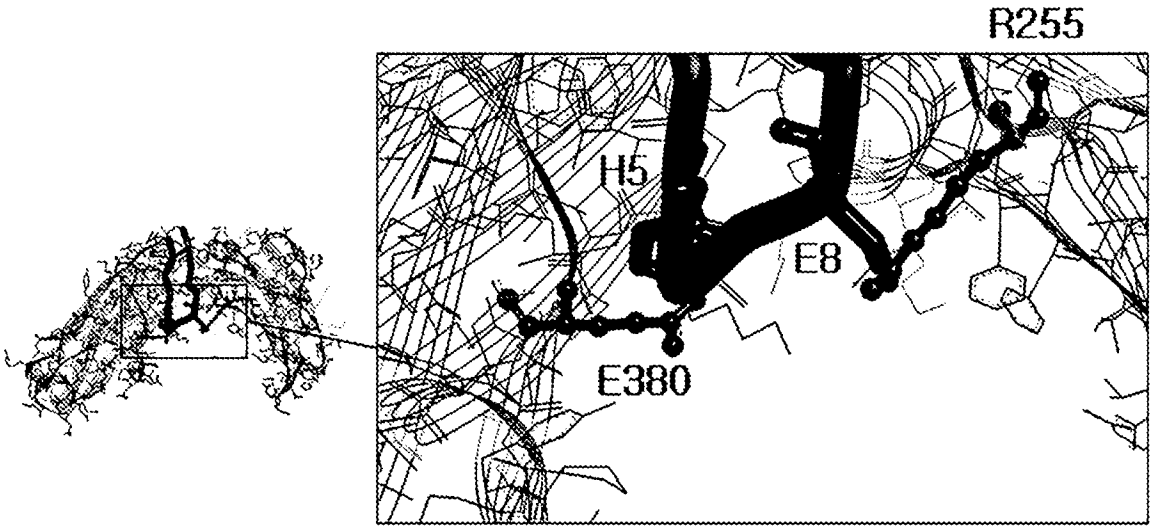


FIG. 3

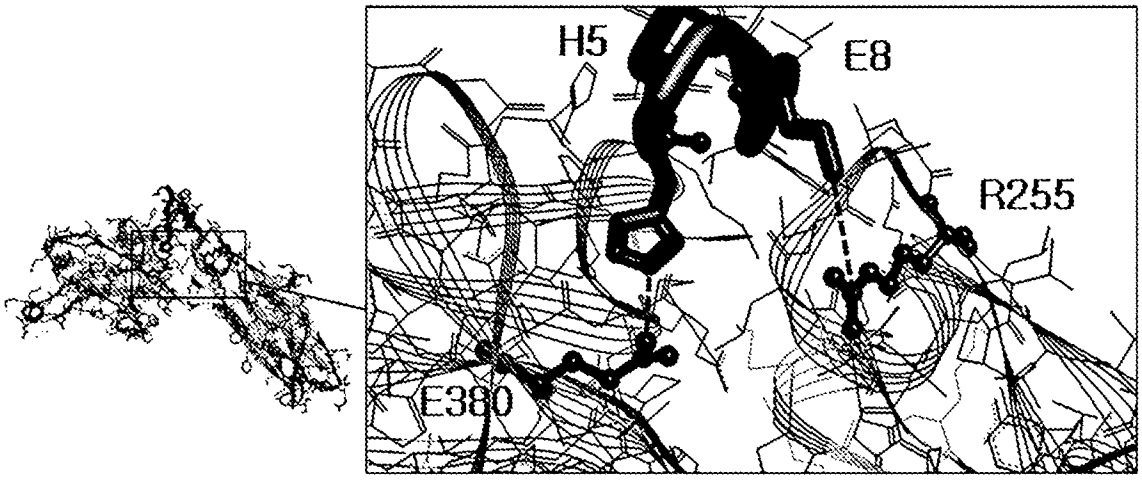


FIG. 4

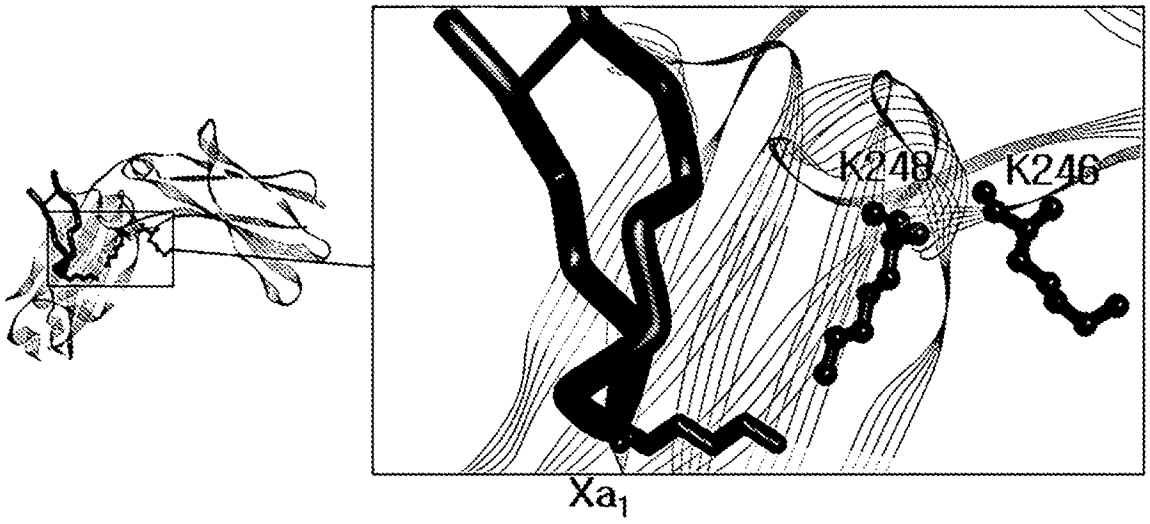


FIG. 5

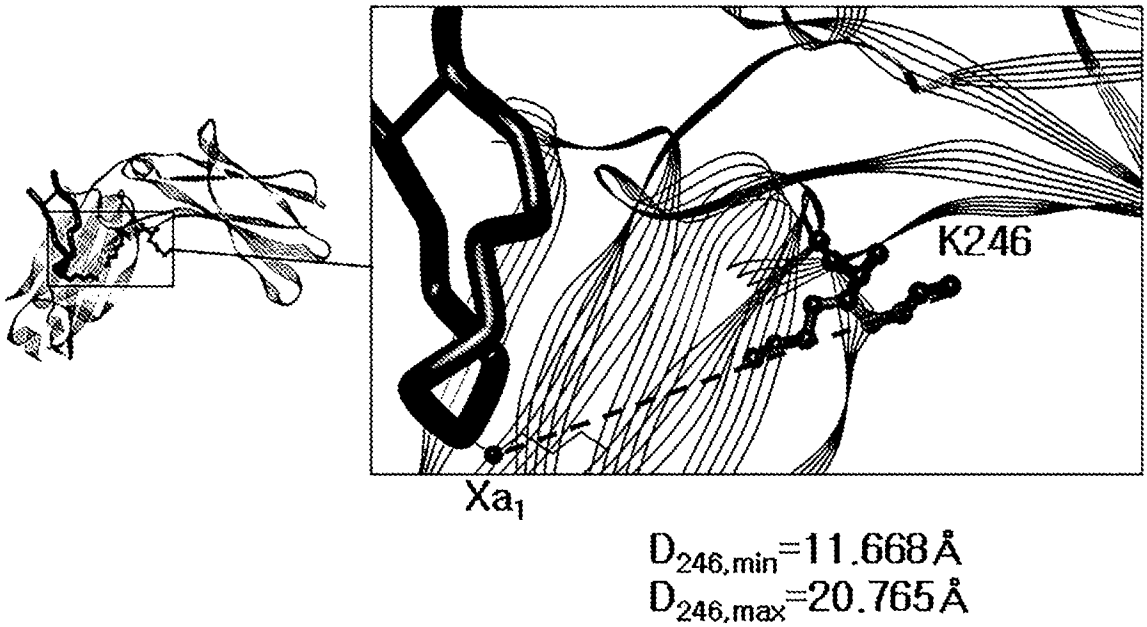


FIG. 6

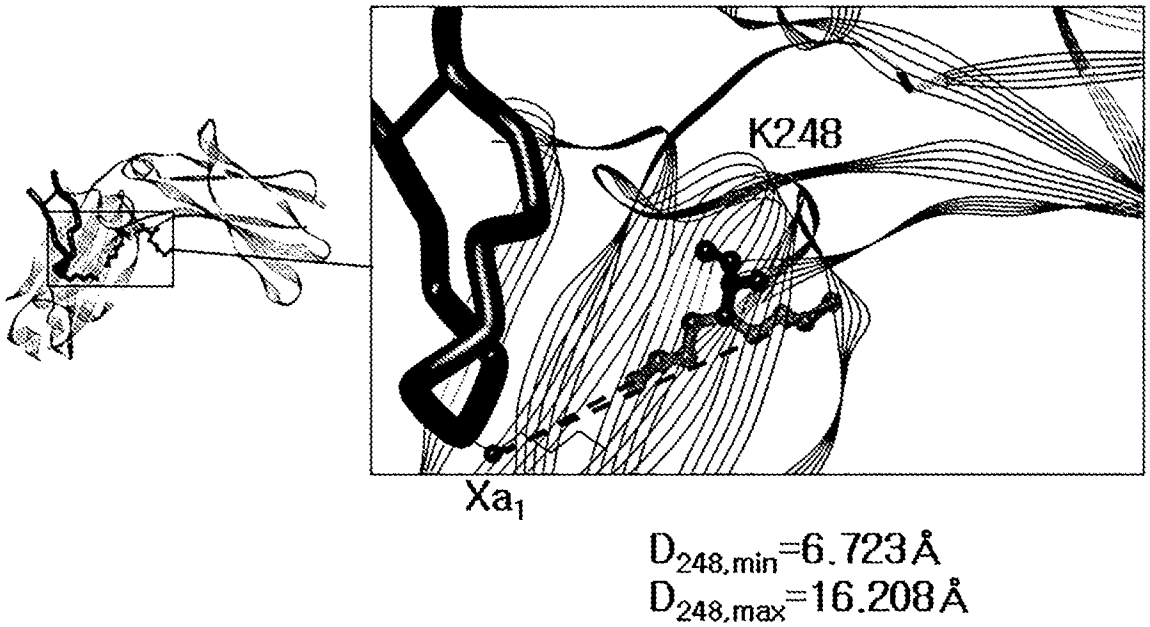


FIG. 7

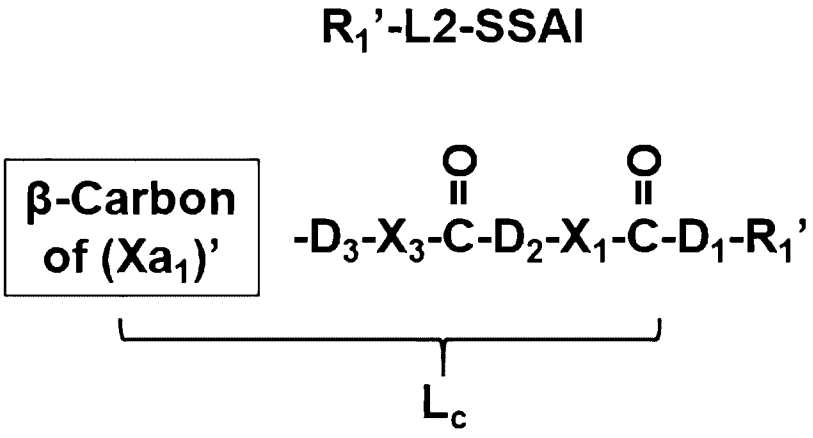


FIG. 8

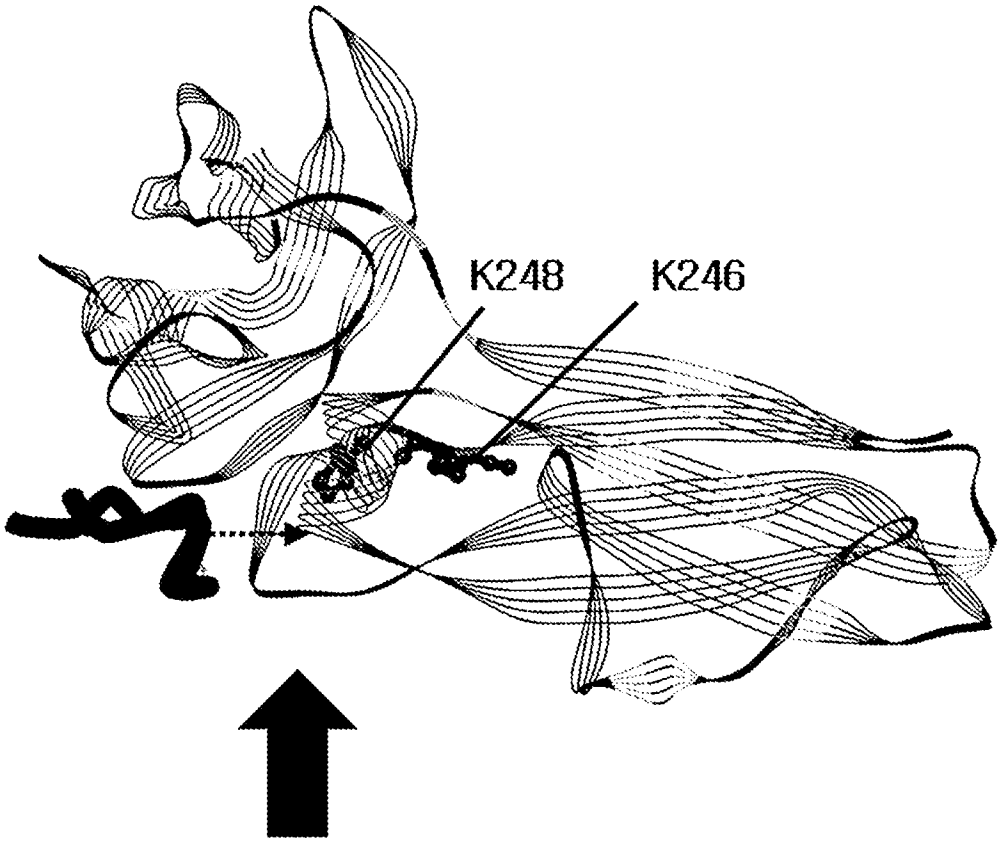


FIG. 9

If L_c is shorter than $D_{246,min}$

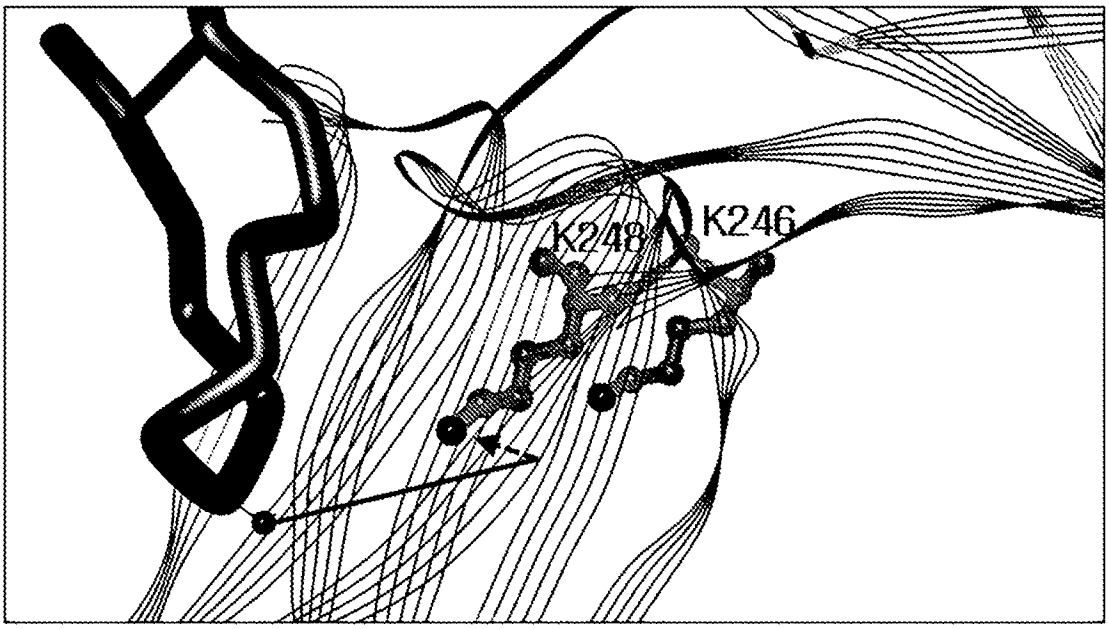


FIG. 10

If L_c is longer than $D_{248,max}$



FIG. 11

If L_c is between about $D_{246,min}$ and about $D_{248,max}$

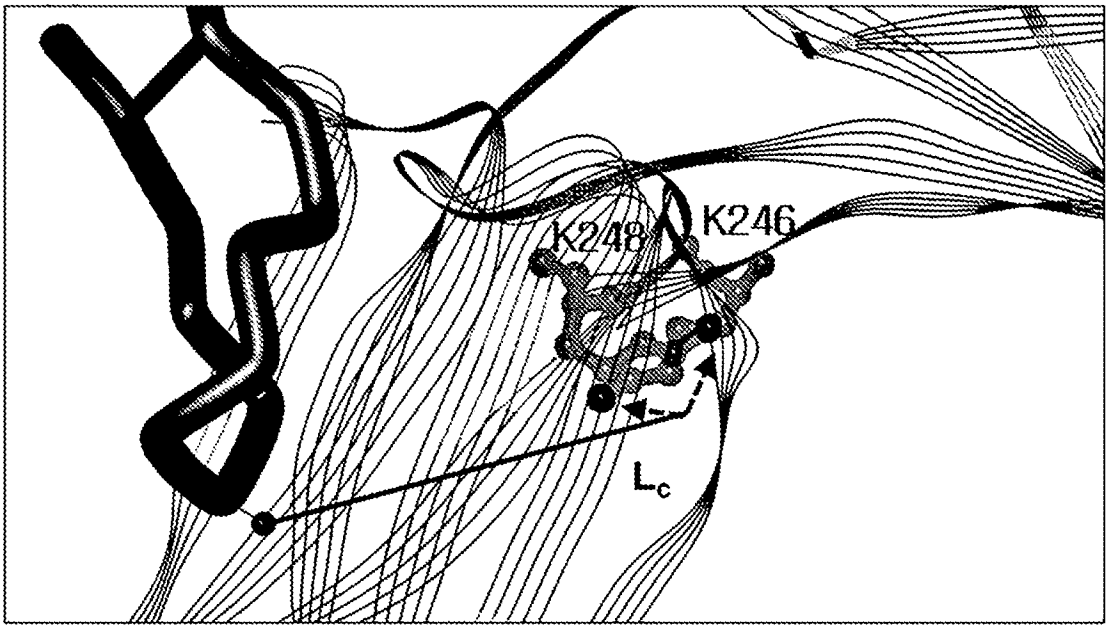


FIG. 12

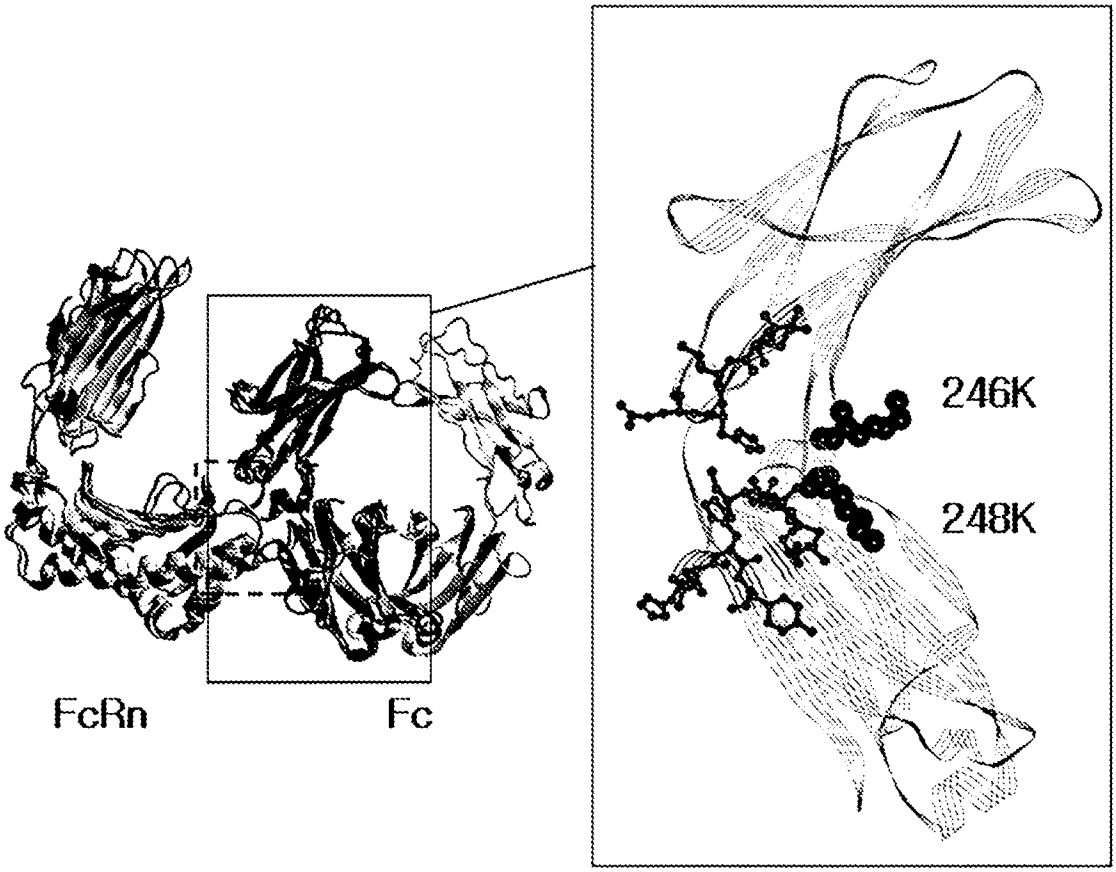


FIG. 13

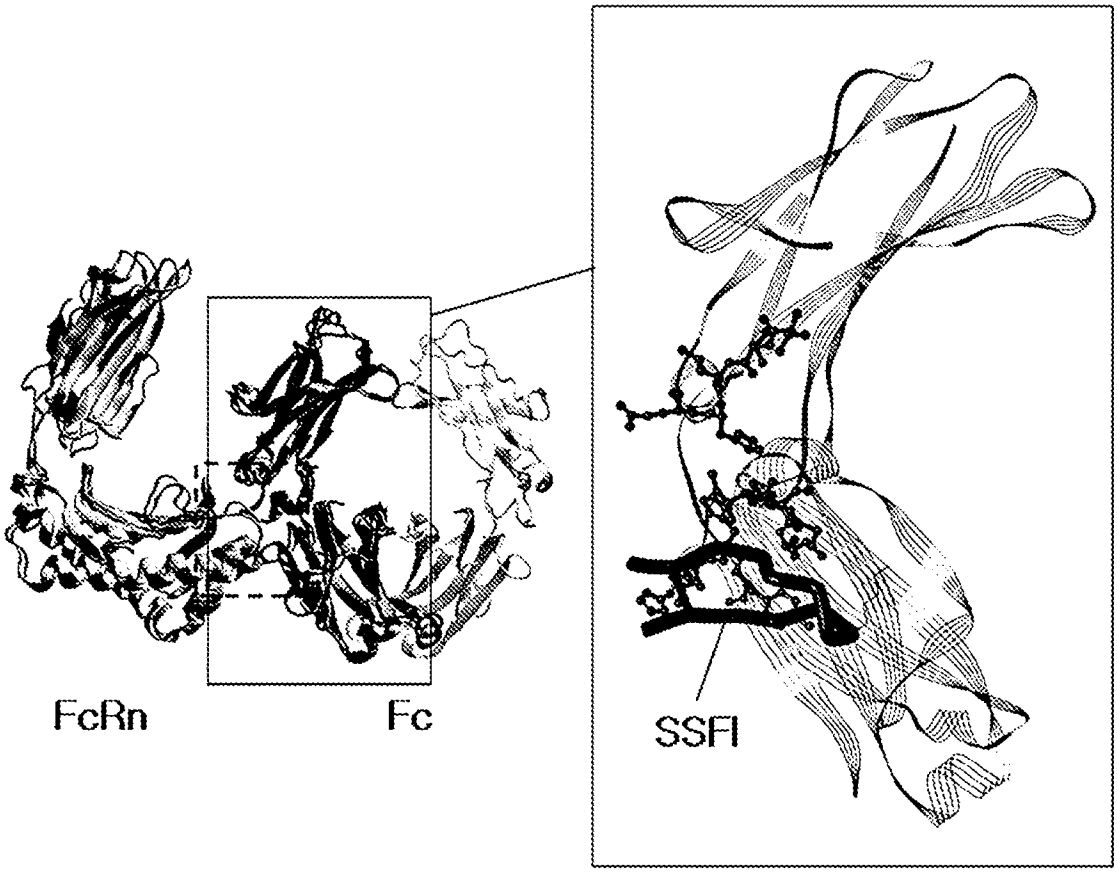


FIG. 14

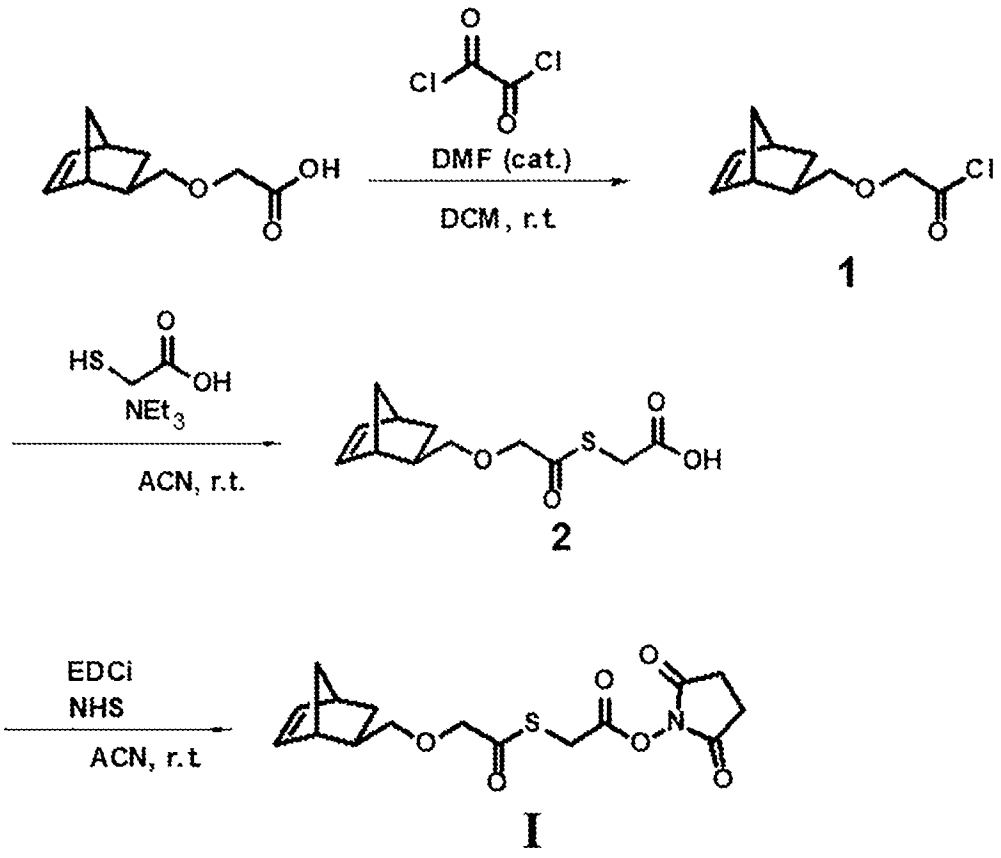


FIG. 15

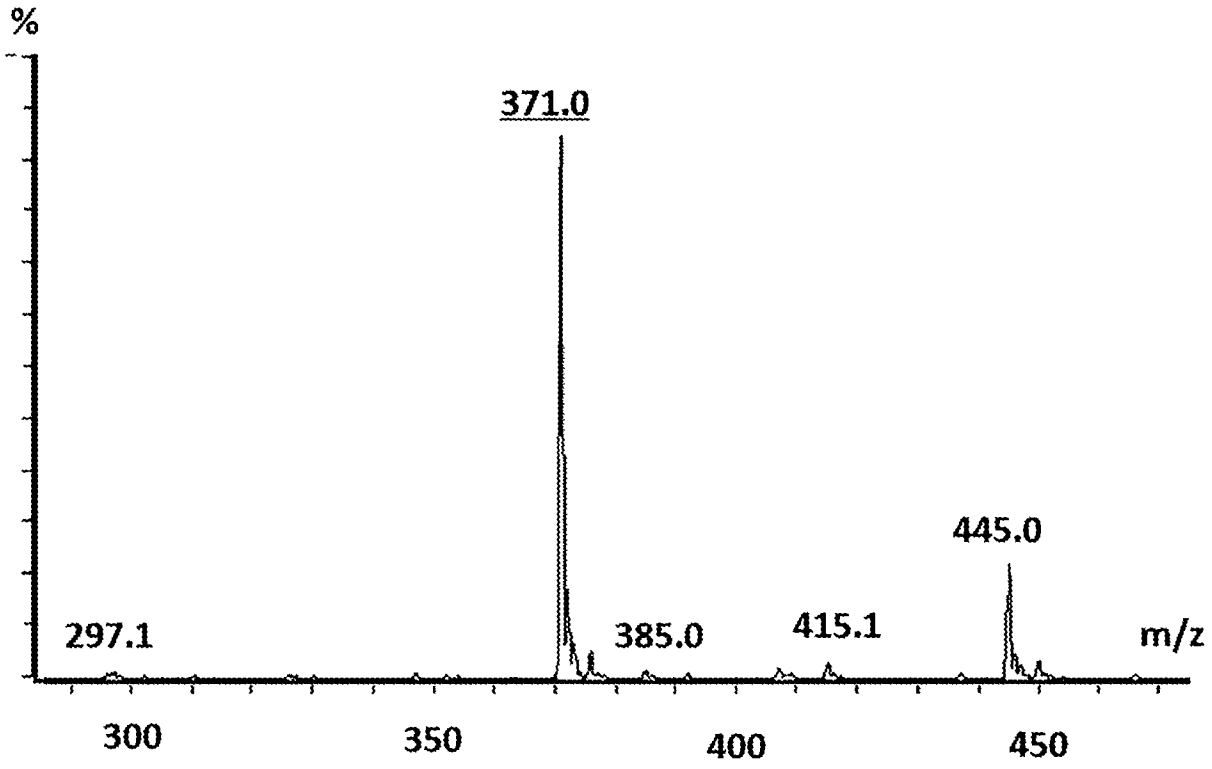


FIG. 16

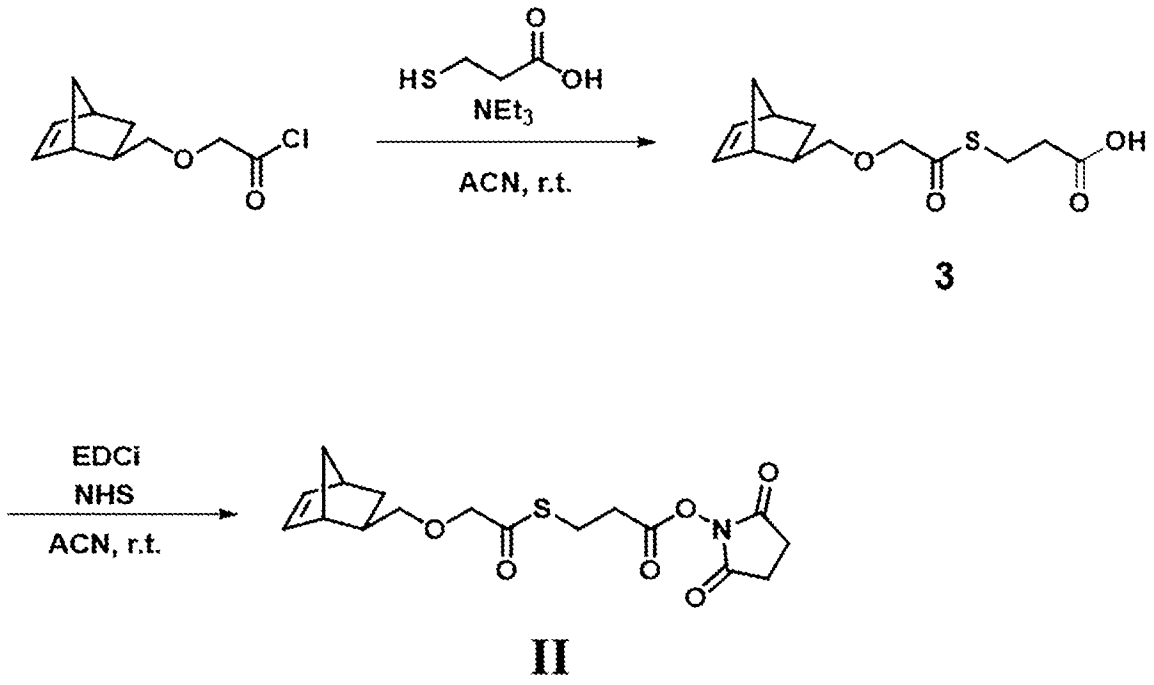


FIG. 17

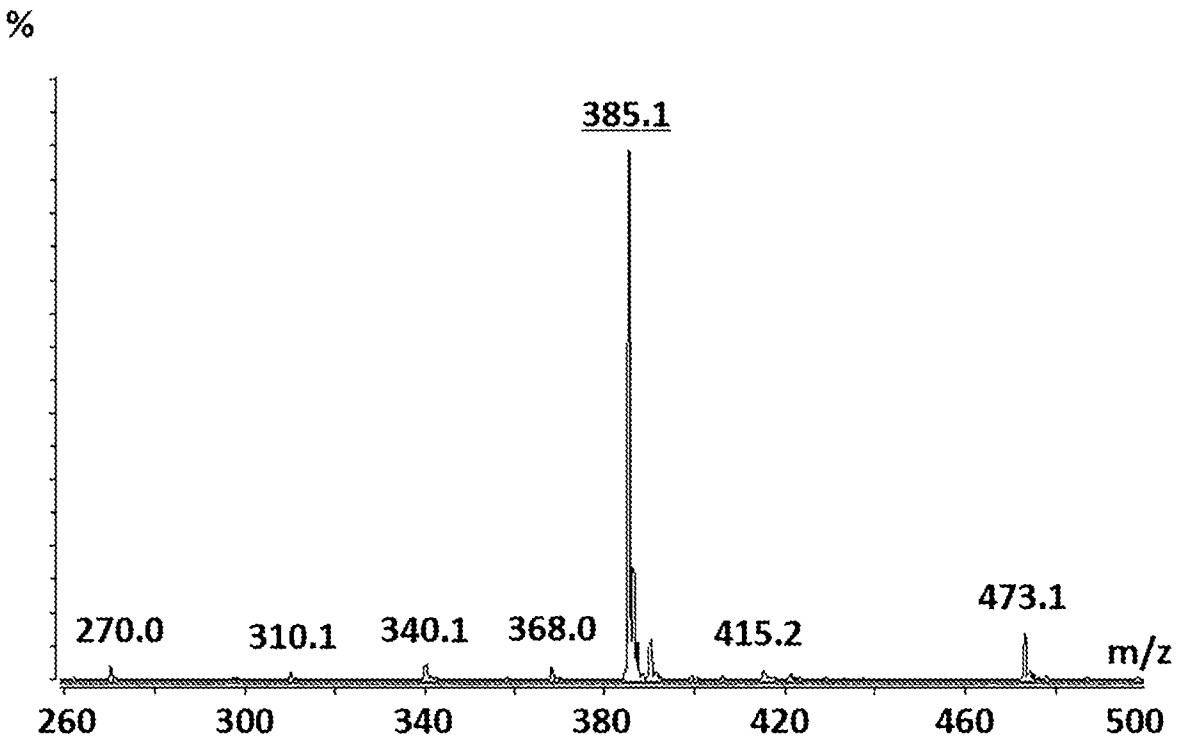

%
385.1
270.0
310.1
340.1
368.0
415.2
473.1
m/z
260
300
340
380
420
460
500

FIG. 18

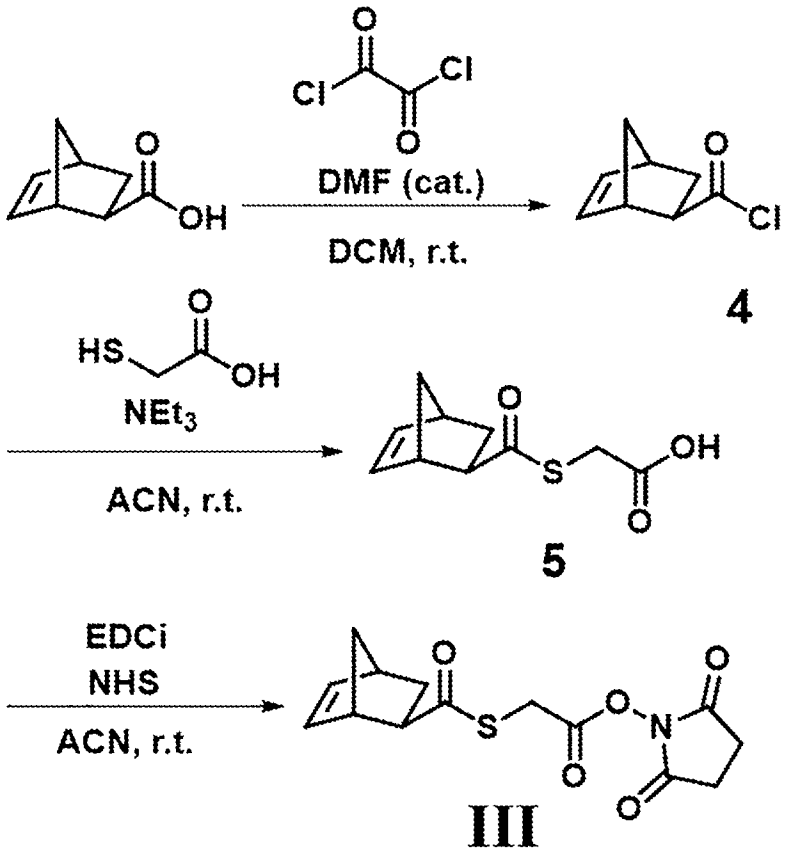


FIG. 19

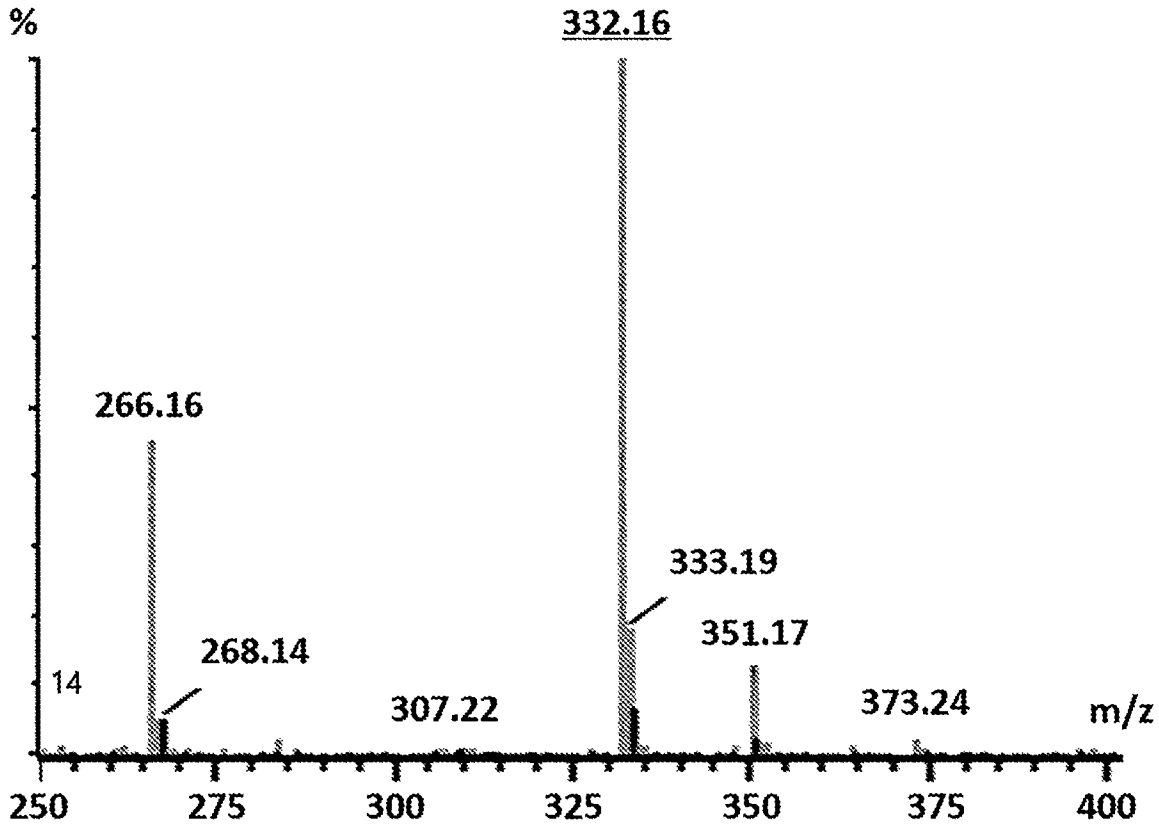

%
332.16
266.16
333.19
351.17
268.14
14
307.22
373.24
m/z
250
275
300
325
350
375
400

FIG. 20

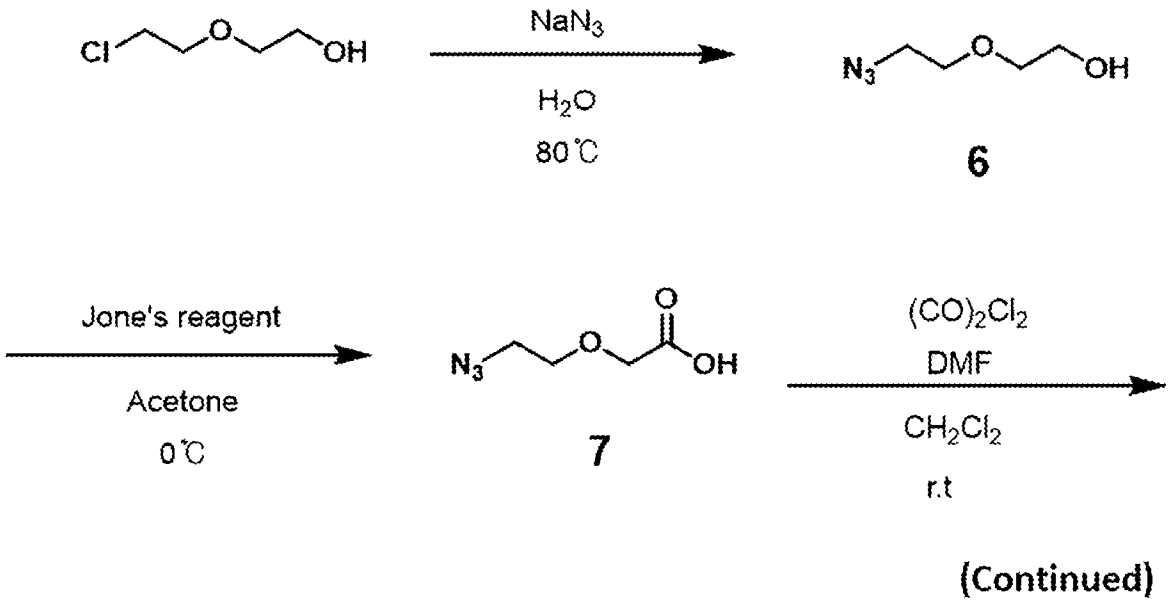

Cl
O
OH
NaN3
H2O
80℃
N3
O
OH
6
Jone's reagent
Acetone
0℃
N3
O
O
OH
7
(CO)2Cl2
DMF
CH2Cl2
r.t
(Continued)

FIG. 21

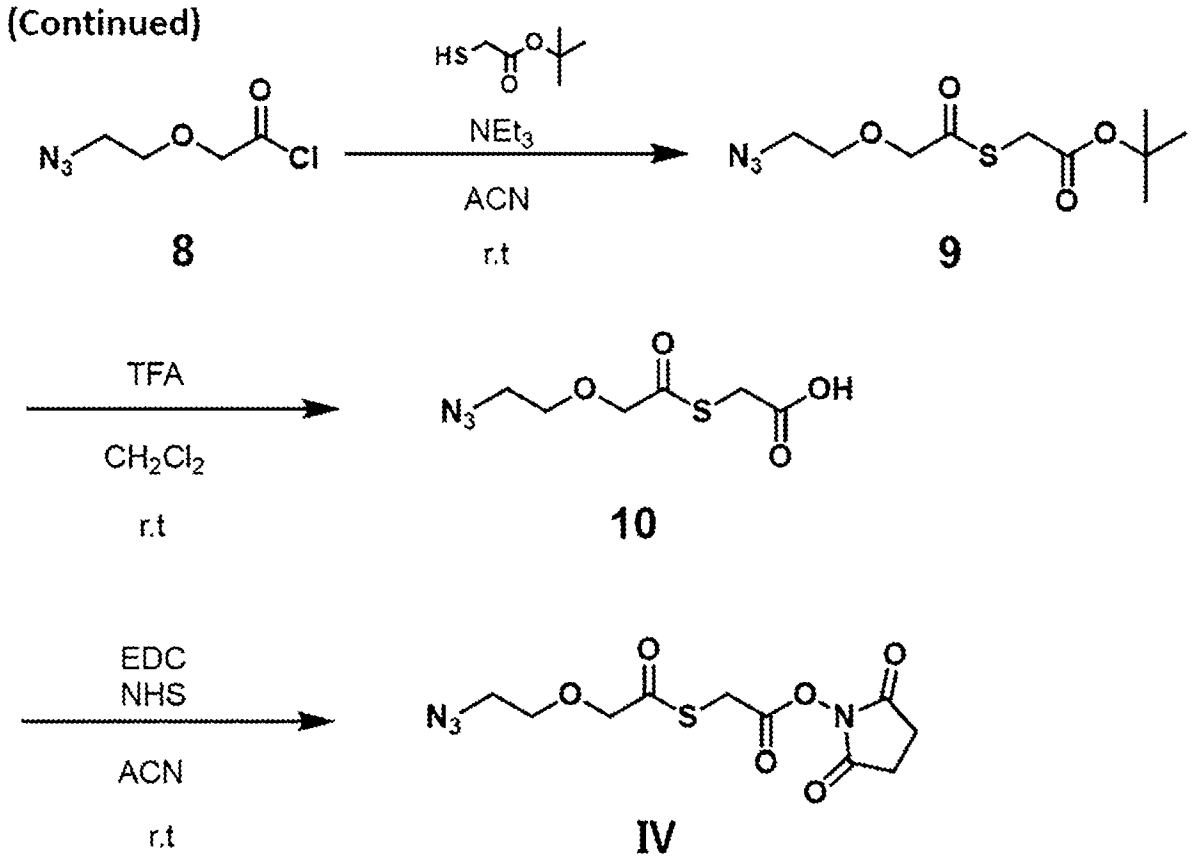

(Continued)
HS
O
O
O
NEt3
ACN
r.t
N3
O
O
Cl
8
N3
O
O
S
O
O
9
TFA
CH2Cl2
r.t
N3
O
O
S
OH
O
10
EDC
NHS
ACN
r.t
N3
O
O
S
O
O
O
N
O
O
IV

FIG. 22

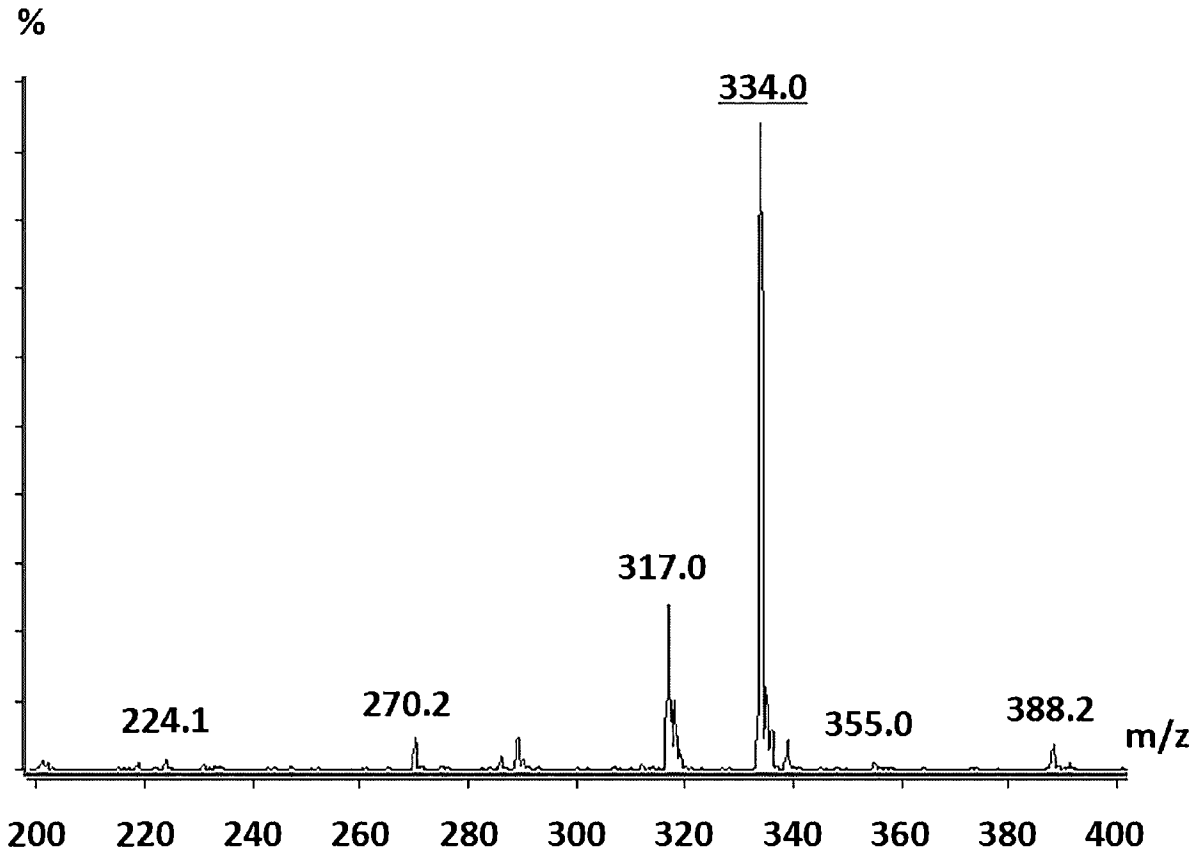

%
334.0
317.0
224.1
270.2
355.0
388.2
m/z
200
220
240
260
280
300
320
340
360
380
400

FIG. 23

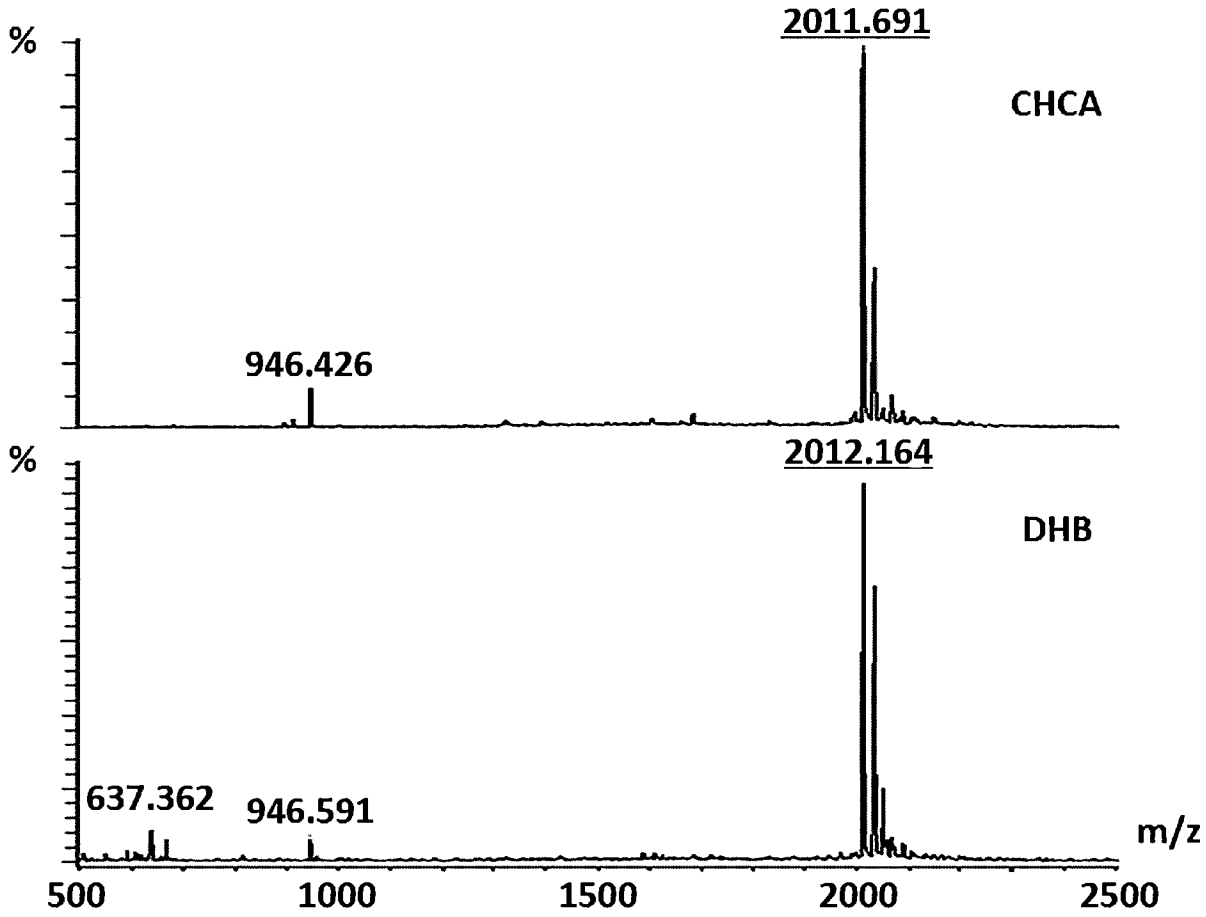

%
2011.691
CHCA
946.426
%
2012.164
DHB
637.362
946.591
m/z
500
1000
1500
2000
2500

FIG. 24

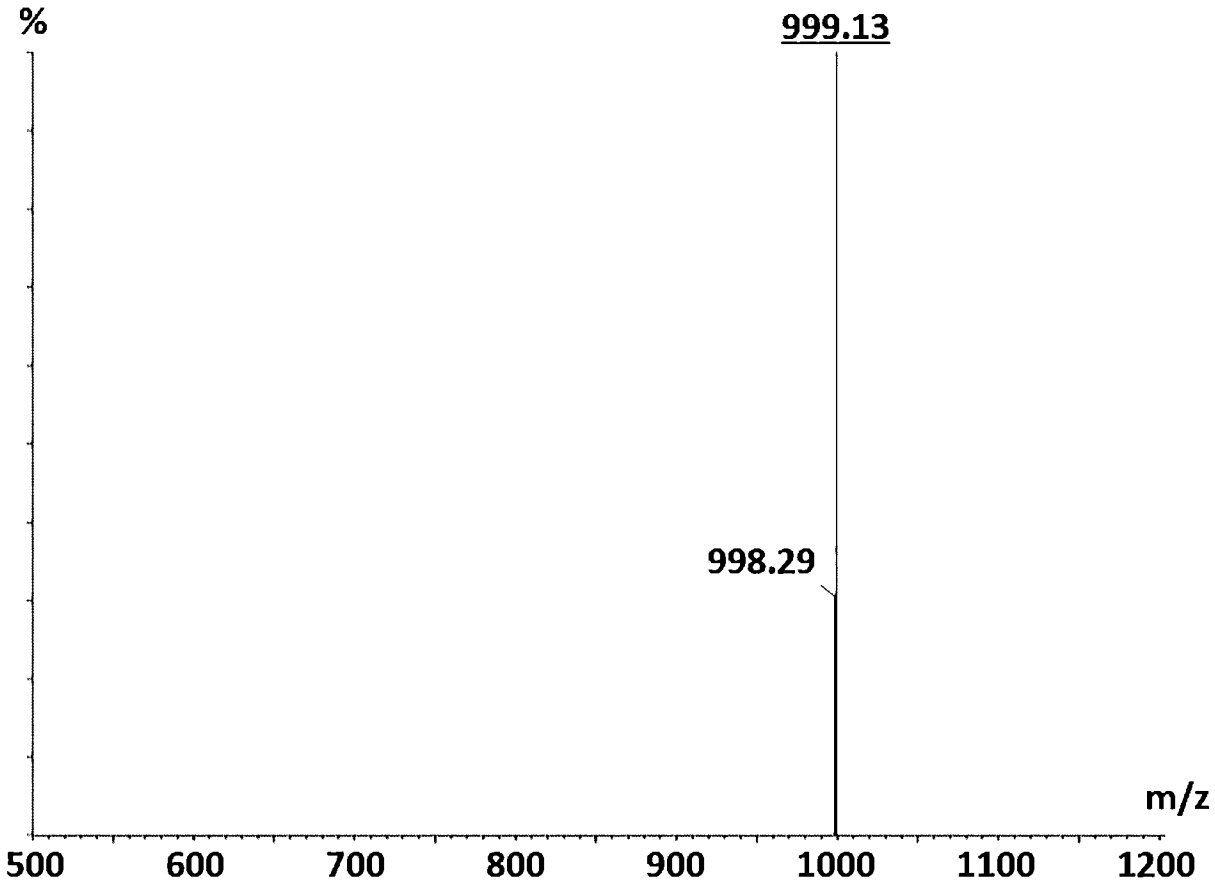


FIG. 25

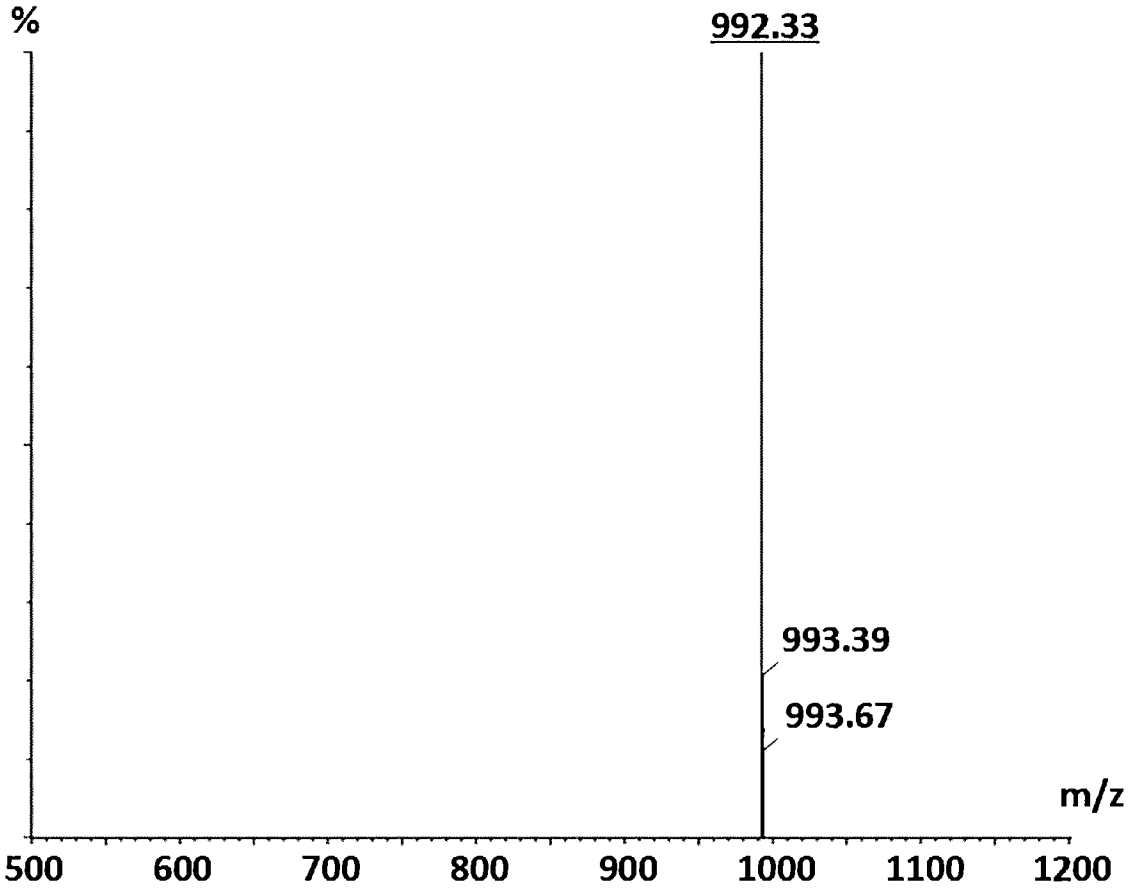


FIG. 26

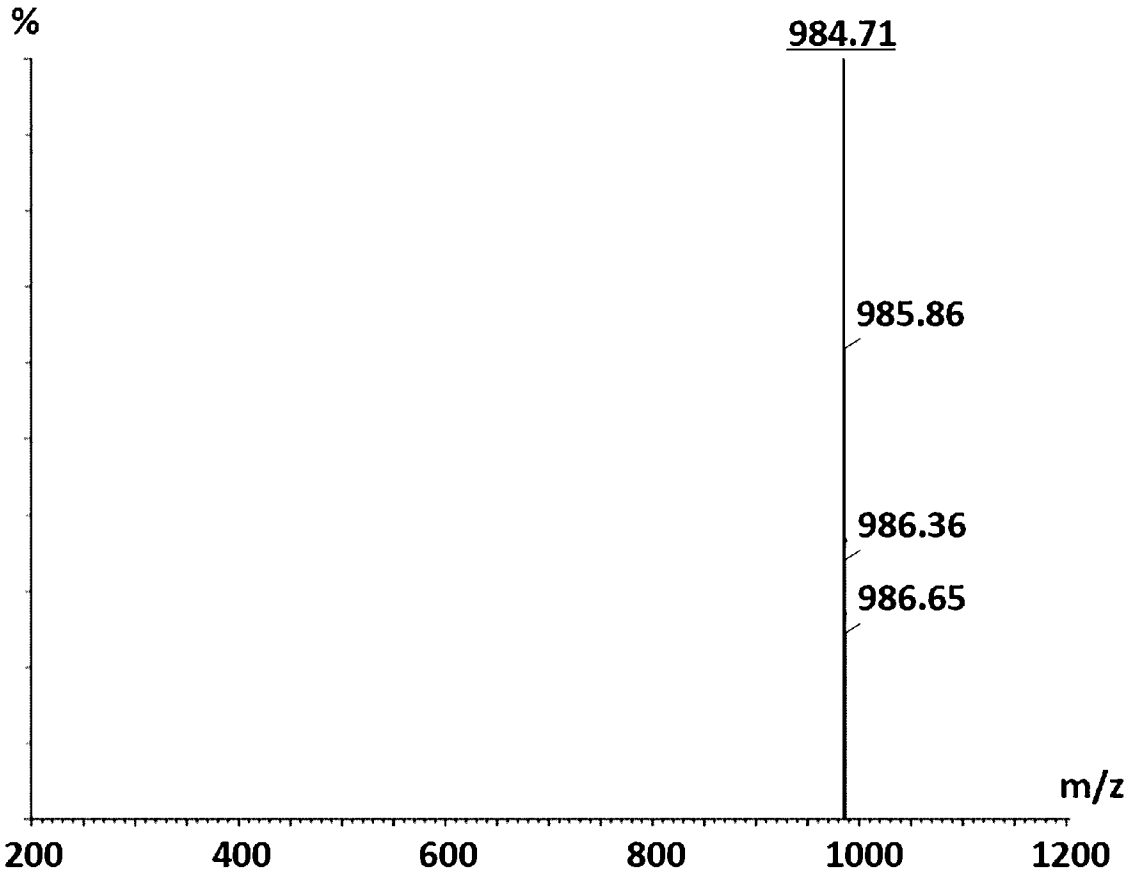


FIG. 27

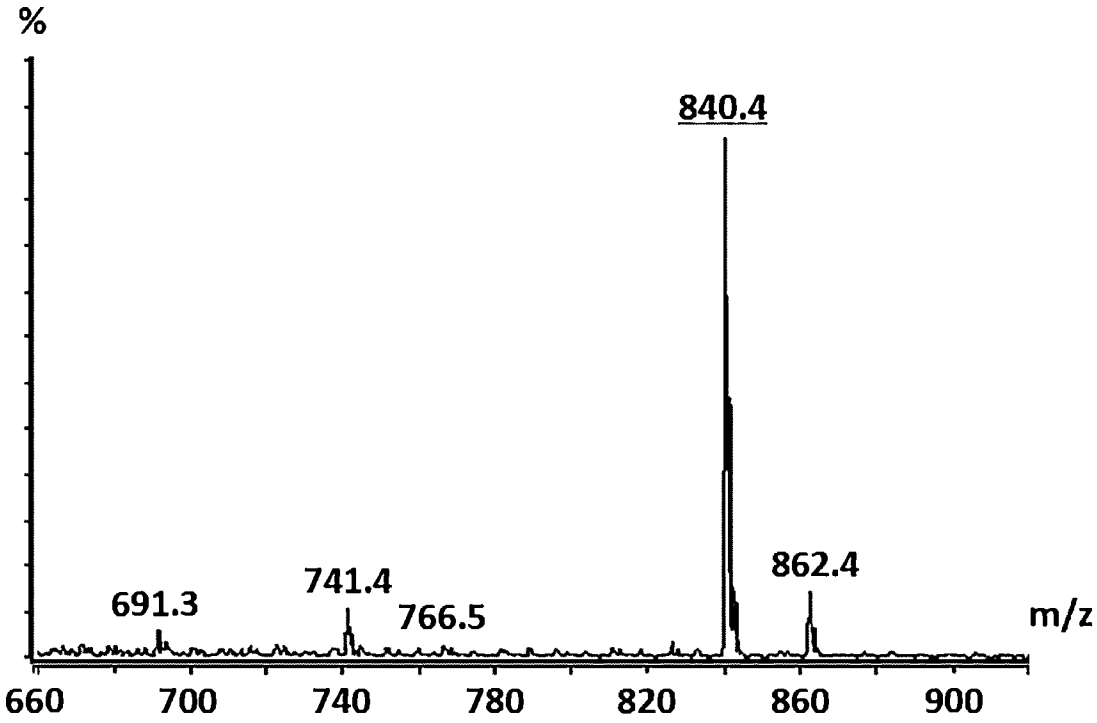


FIG. 28

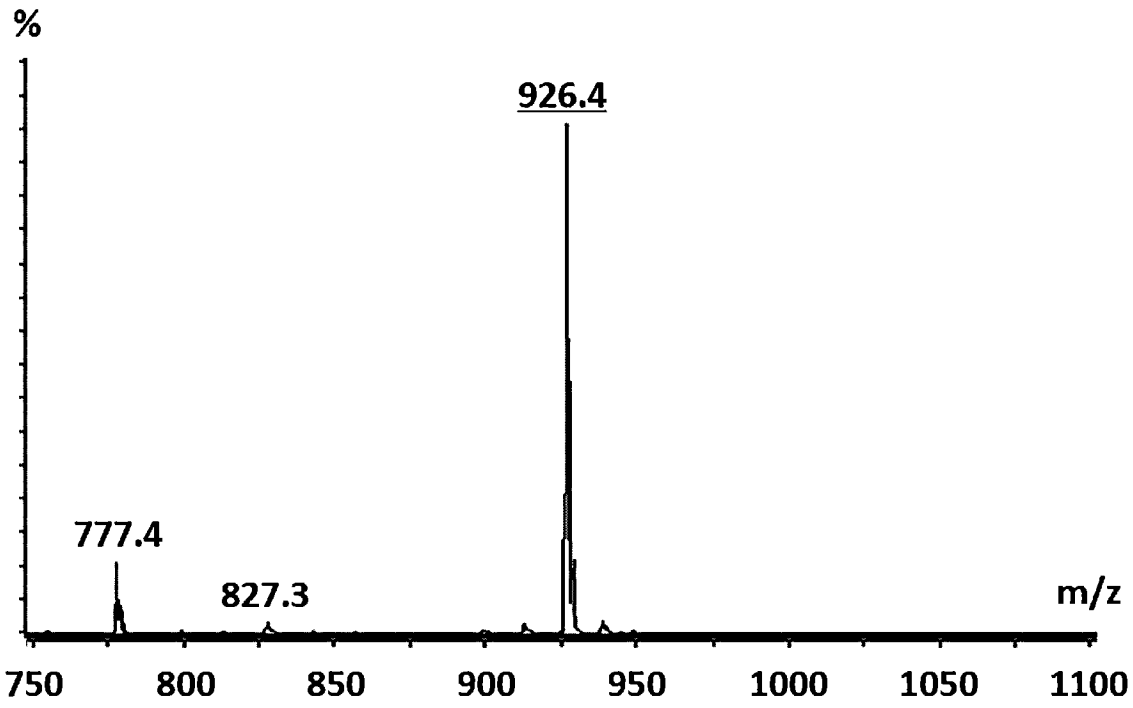


FIG. 29

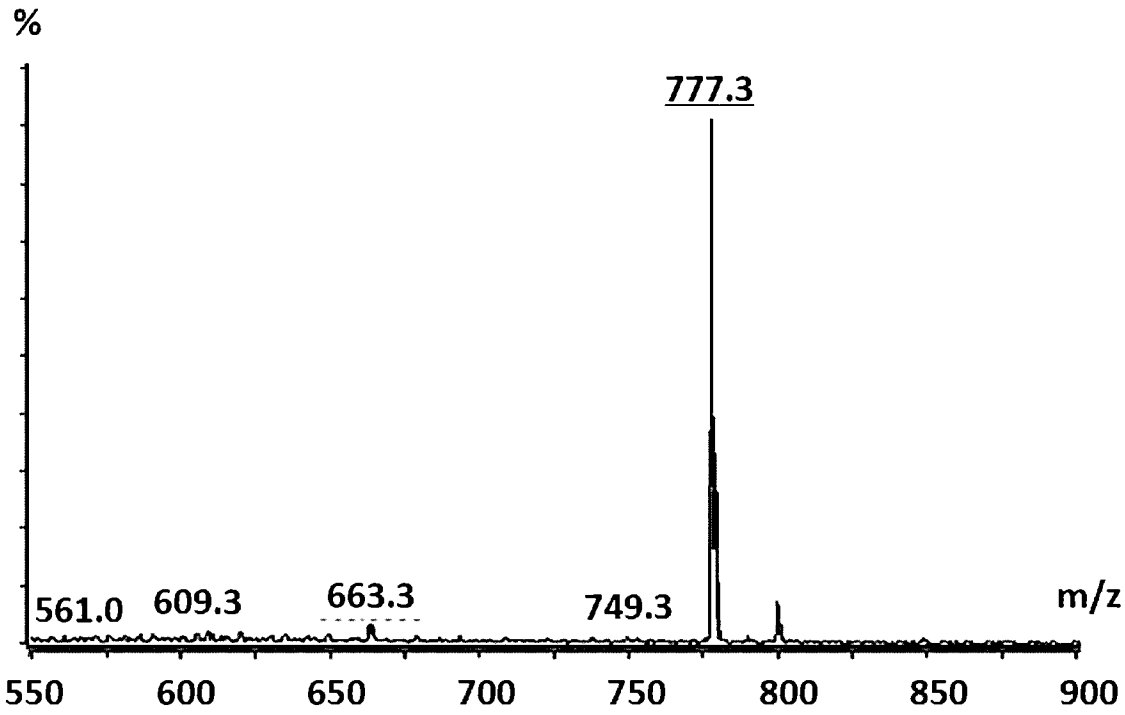


FIG. 30

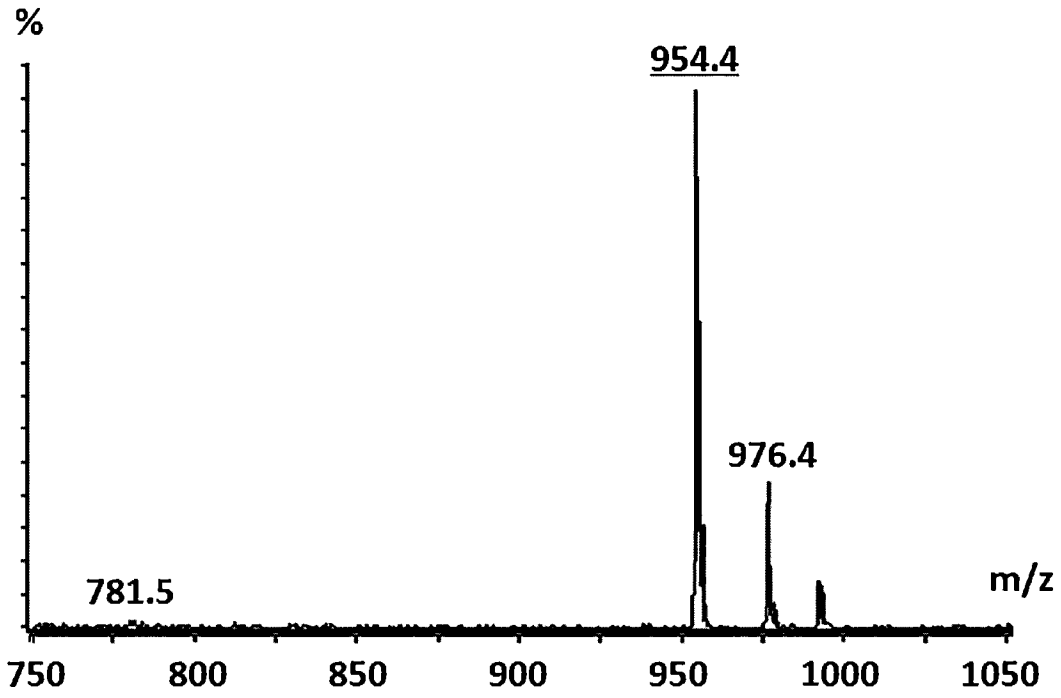


FIG. 31

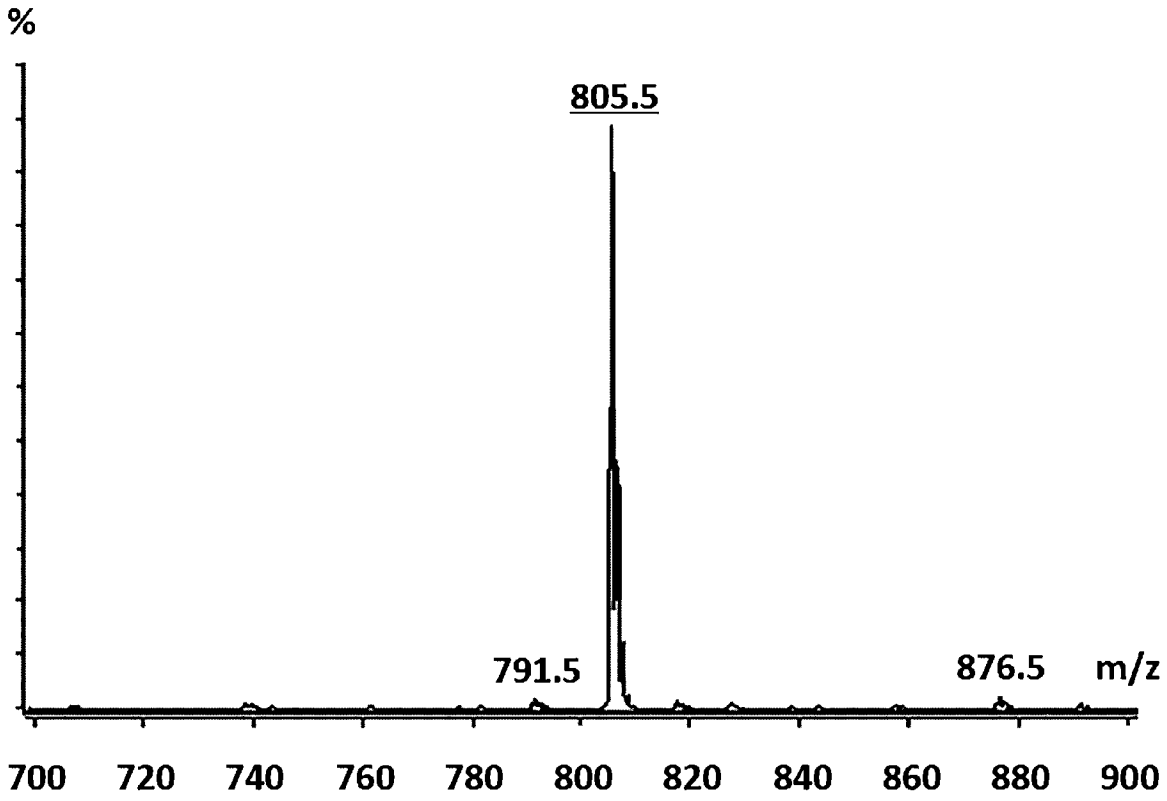


FIG. 32

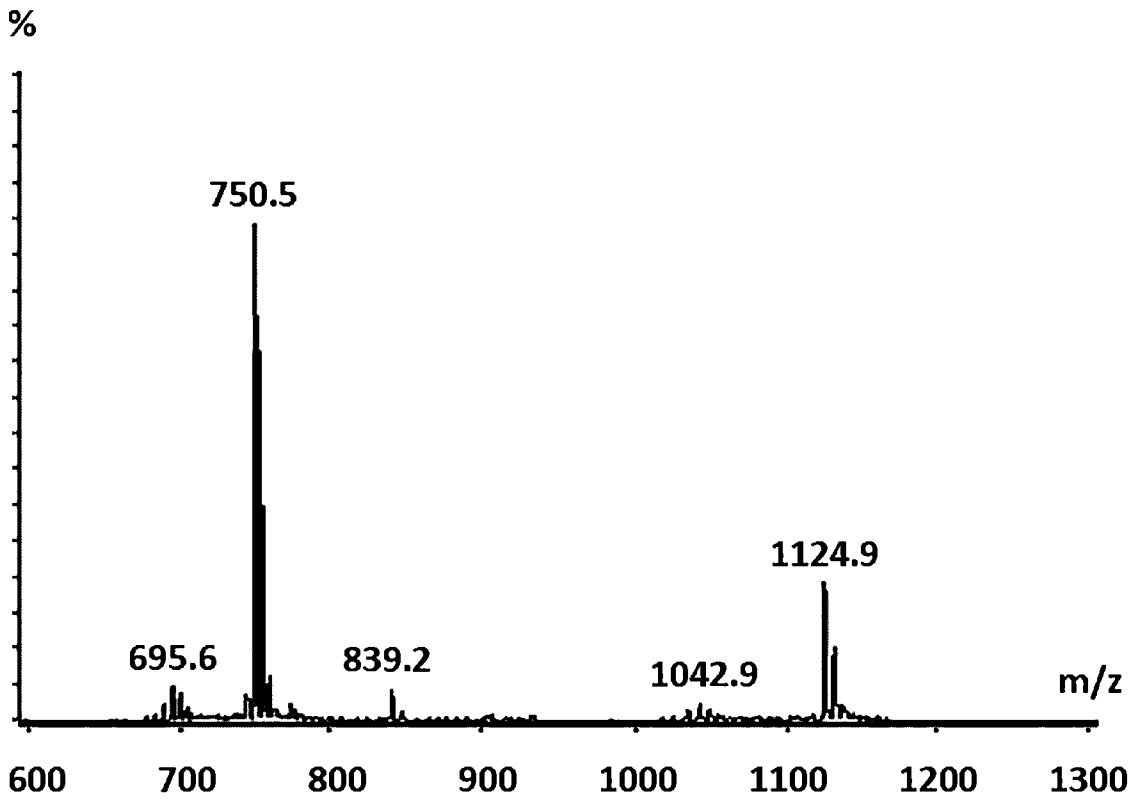

%
750.5
1124.9
695.6
839.2
1042.9
m/z
600
700
800
900
1000
1100
1200
1300

FIG. 33

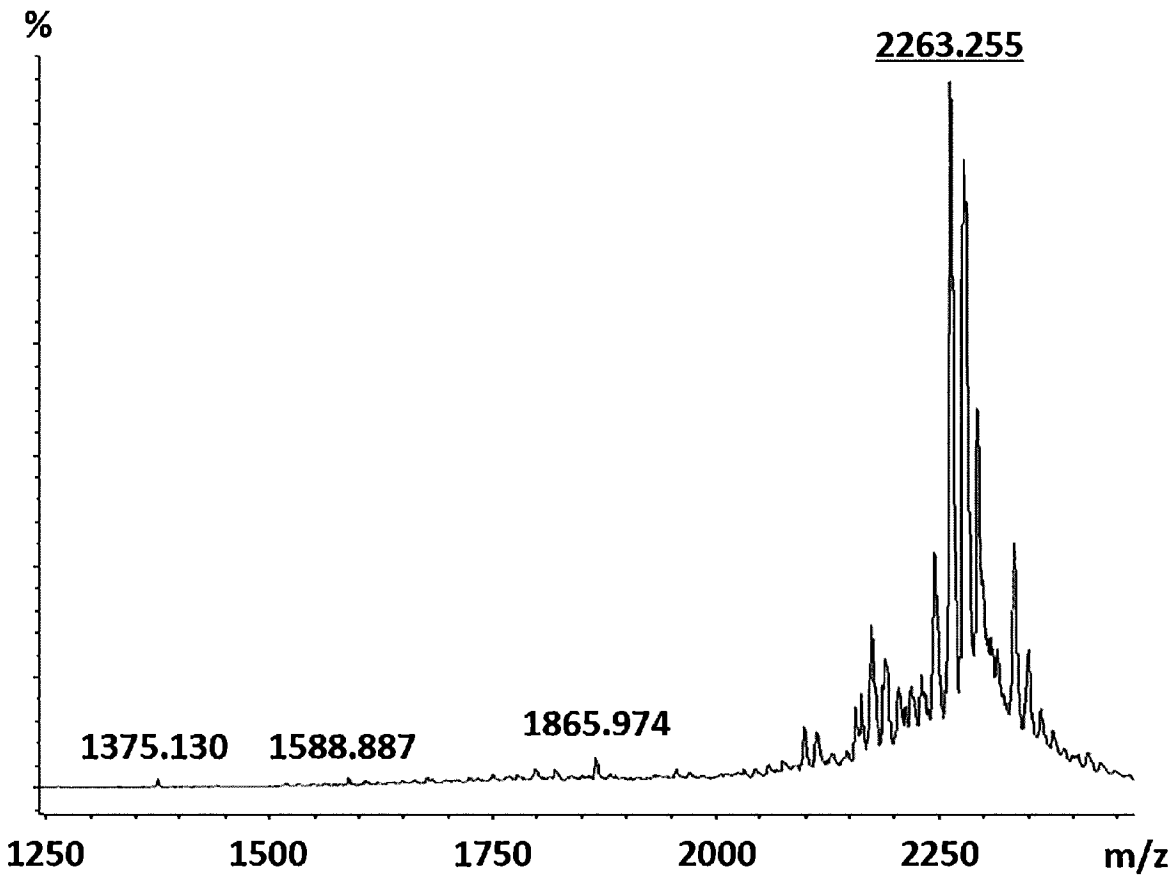

%
2263.255
1865.974
1375.130
1588.887
1250
1500
1750
2000
2250
m/z

FIG. 34

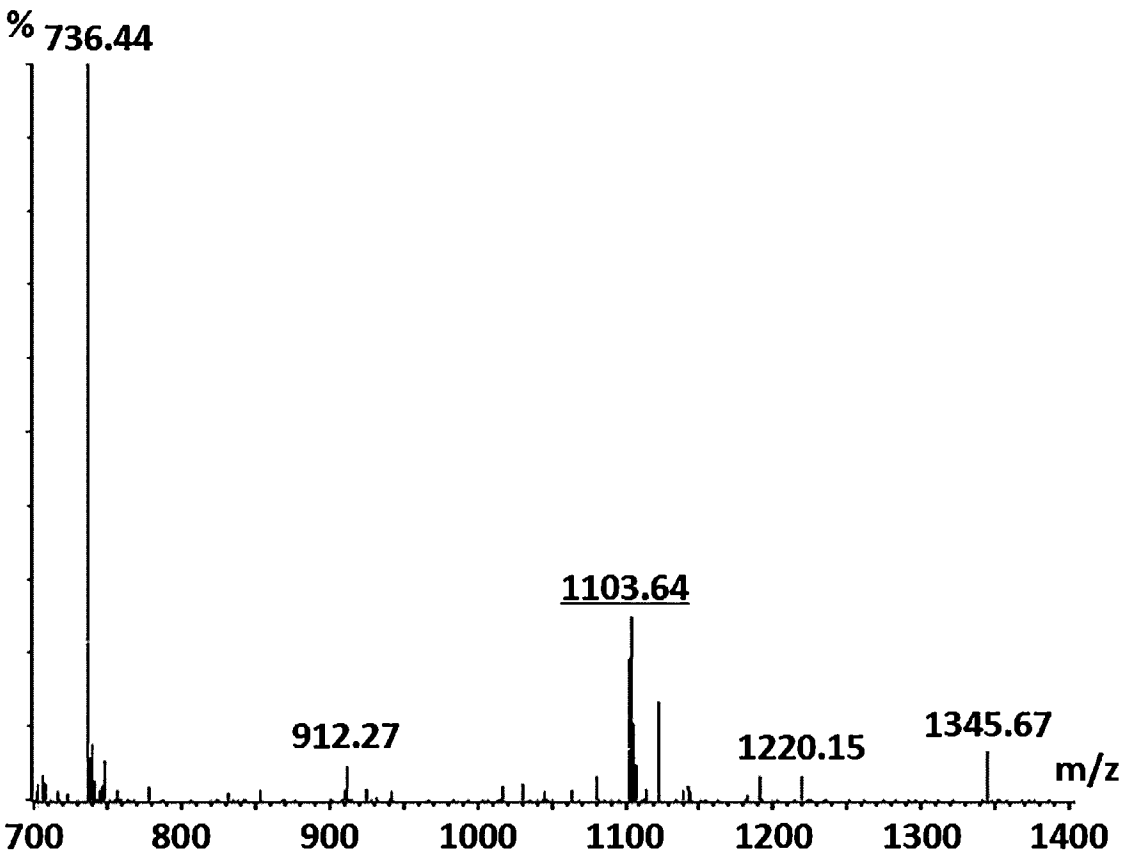

%
736.44
1103.64
912.27
1220.15
1345.67
m/z
700
800
900
1000
1100
1200
1300
1400

FIG. 35

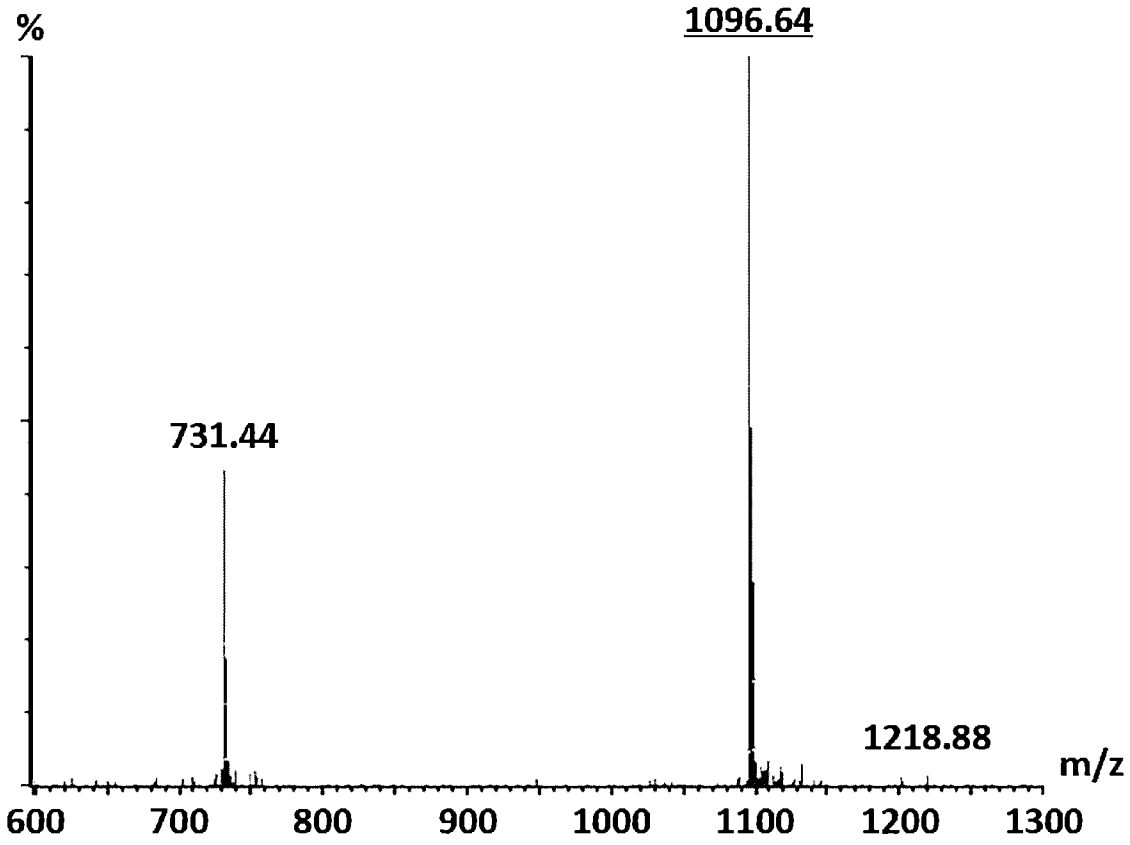


FIG. 36

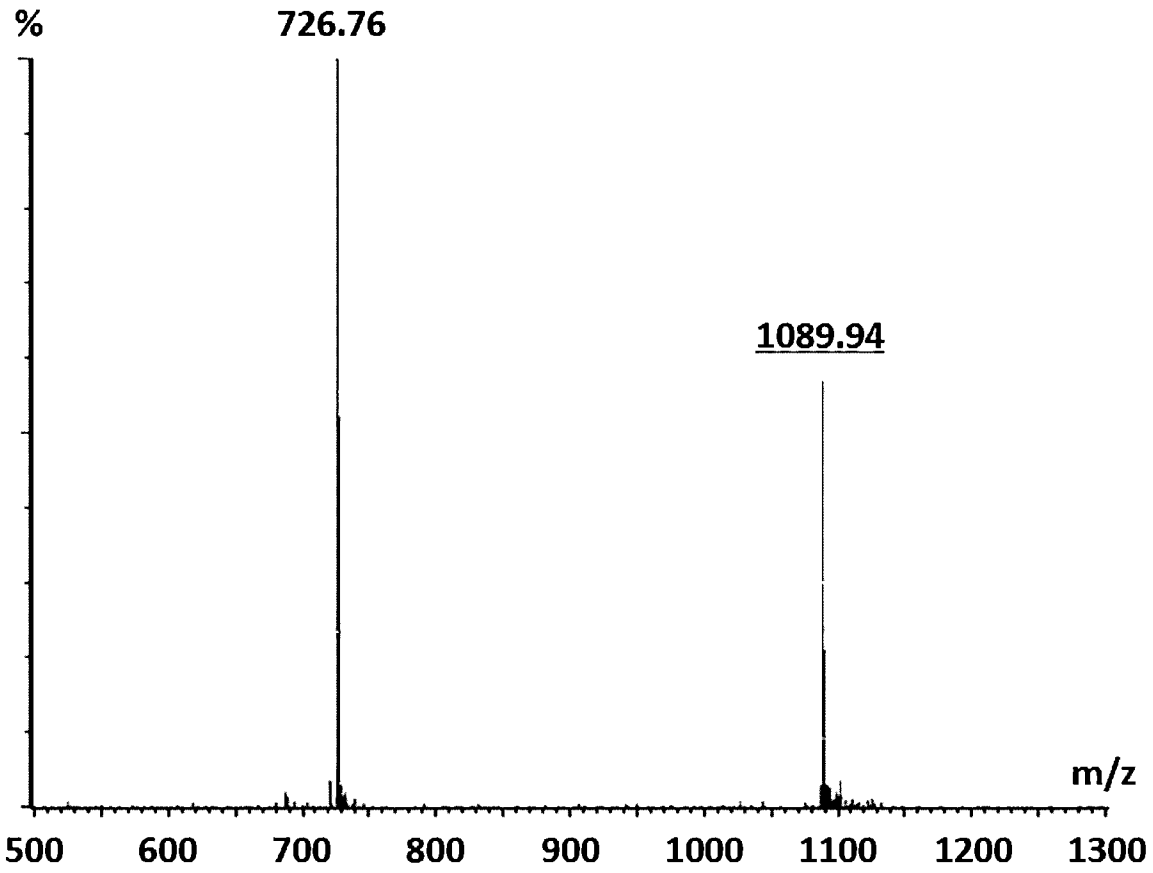


FIG. 37

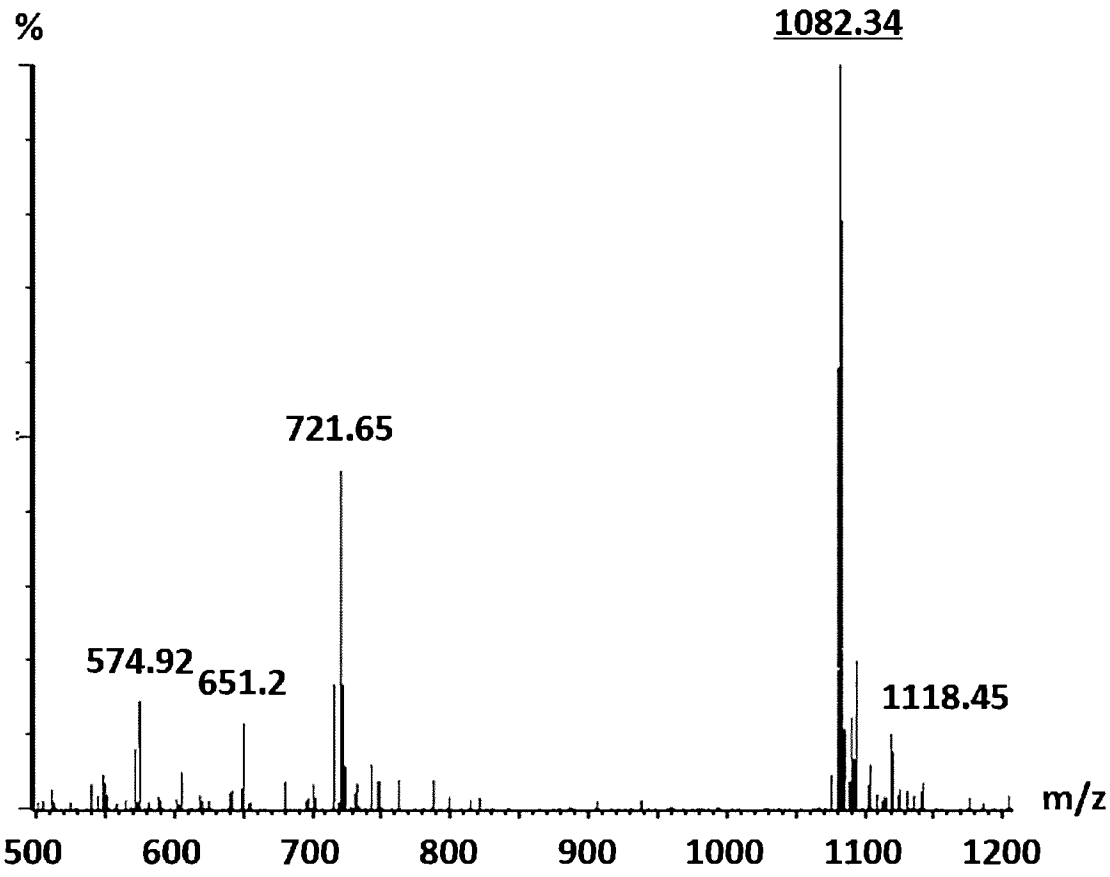

%
1082.34
721.65
574.92
651.2
1118.45
m/z
500
600
700
800
900
1000
1100
1200

FIG. 38

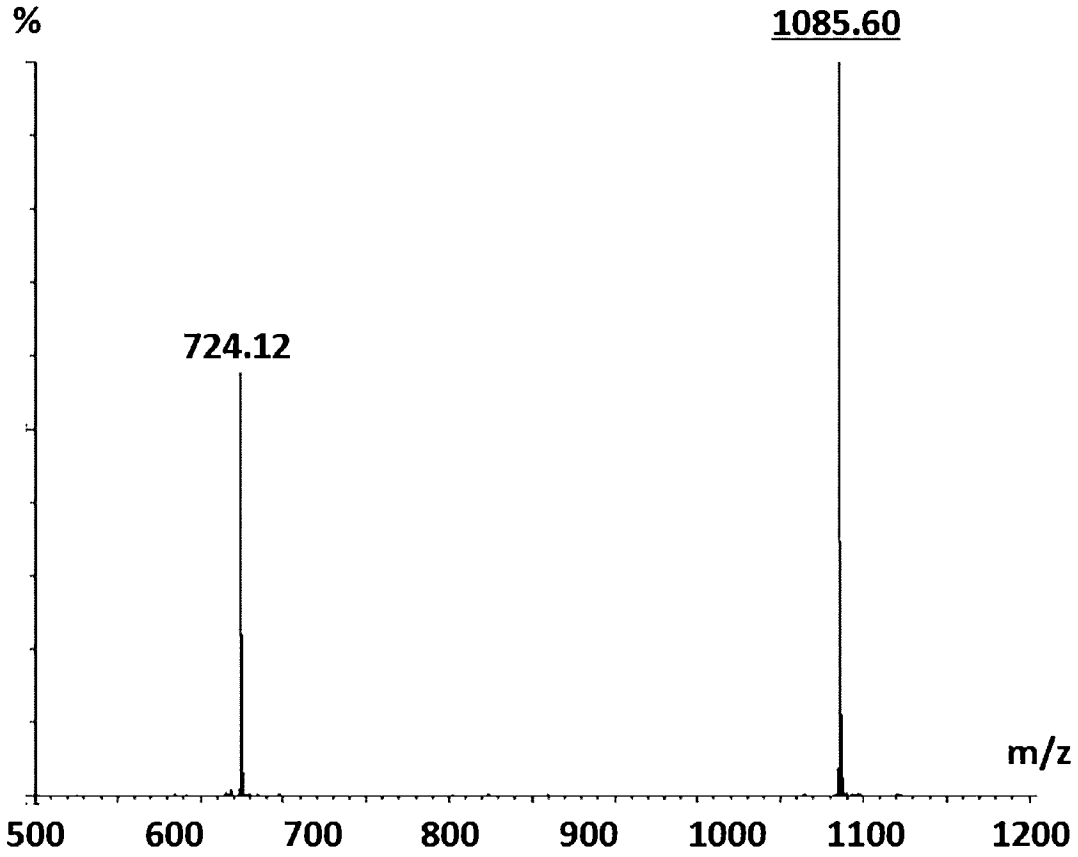


FIG. 39

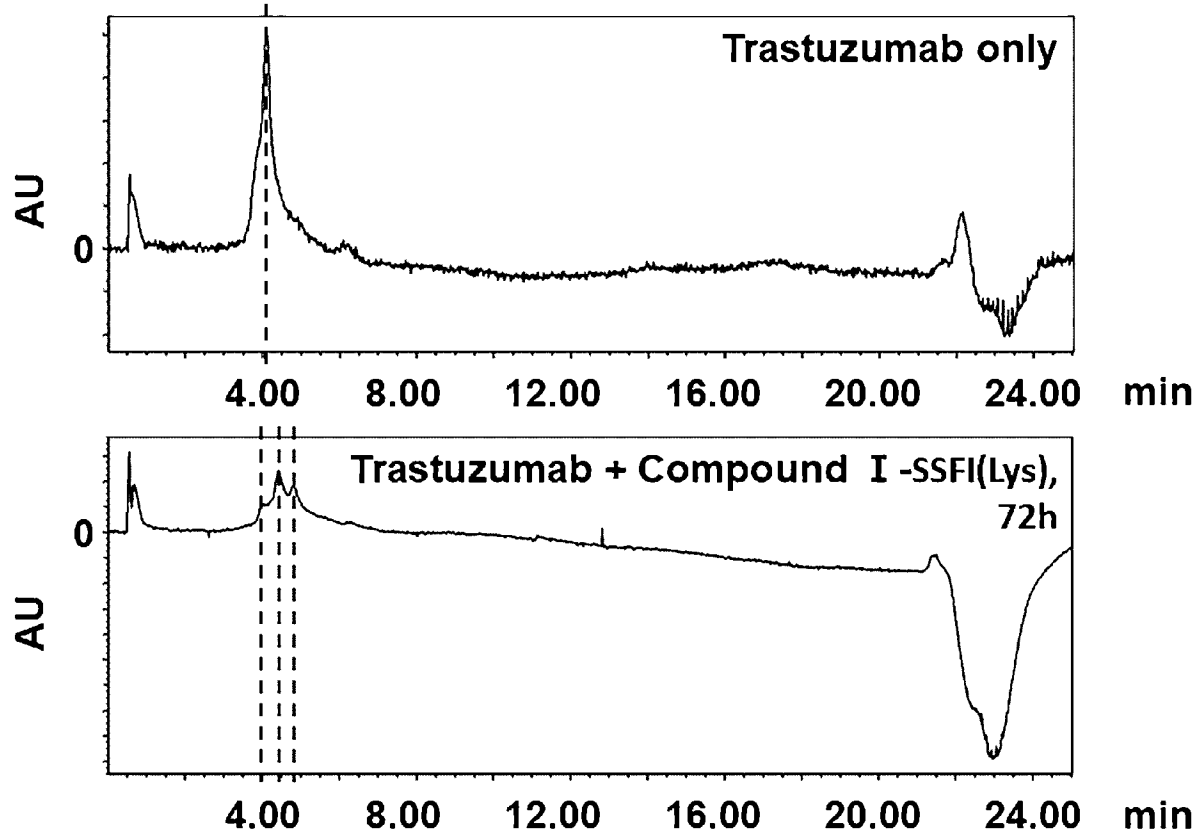

Trastuzumab only
AU
0
4.00
8.00
12.00
16.00
20.00
24.00
min
Trastuzumab + Compound I -SSFI(Lys), 72h
AU
0
4.00
8.00
12.00
16.00
20.00
24.00
min

FIG. 40

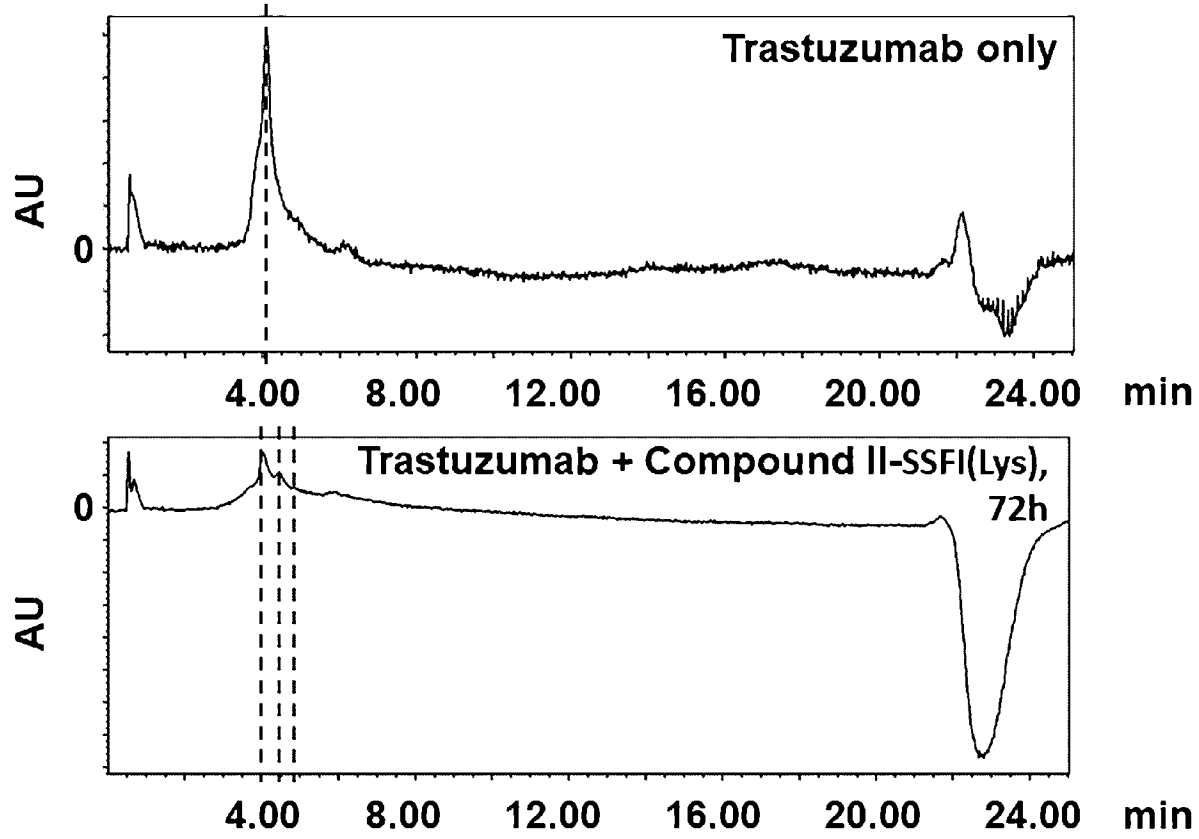
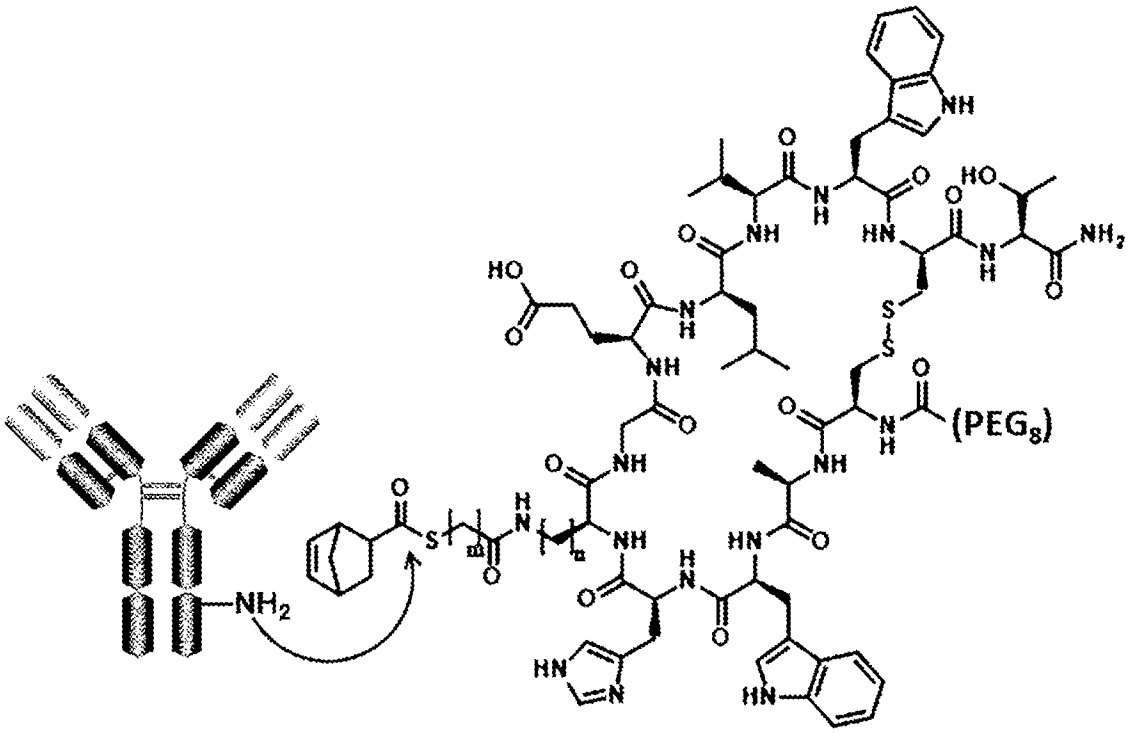

Trastuzumab only
AU
0
4.00
8.00
12.00
16.00
20.00
24.00
min
Trastuzumab + Compound II-SSFI(Lys), 72h
AU
0
4.00
8.00
12.00
16.00
20.00
24.00
min

FIG. 41



(chemical structure of formula 16 pegylated)

FIG. 42

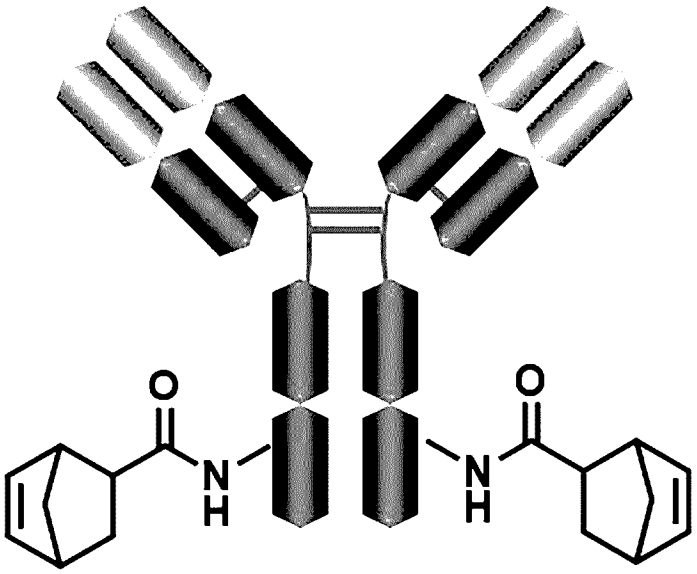


FIG. 43

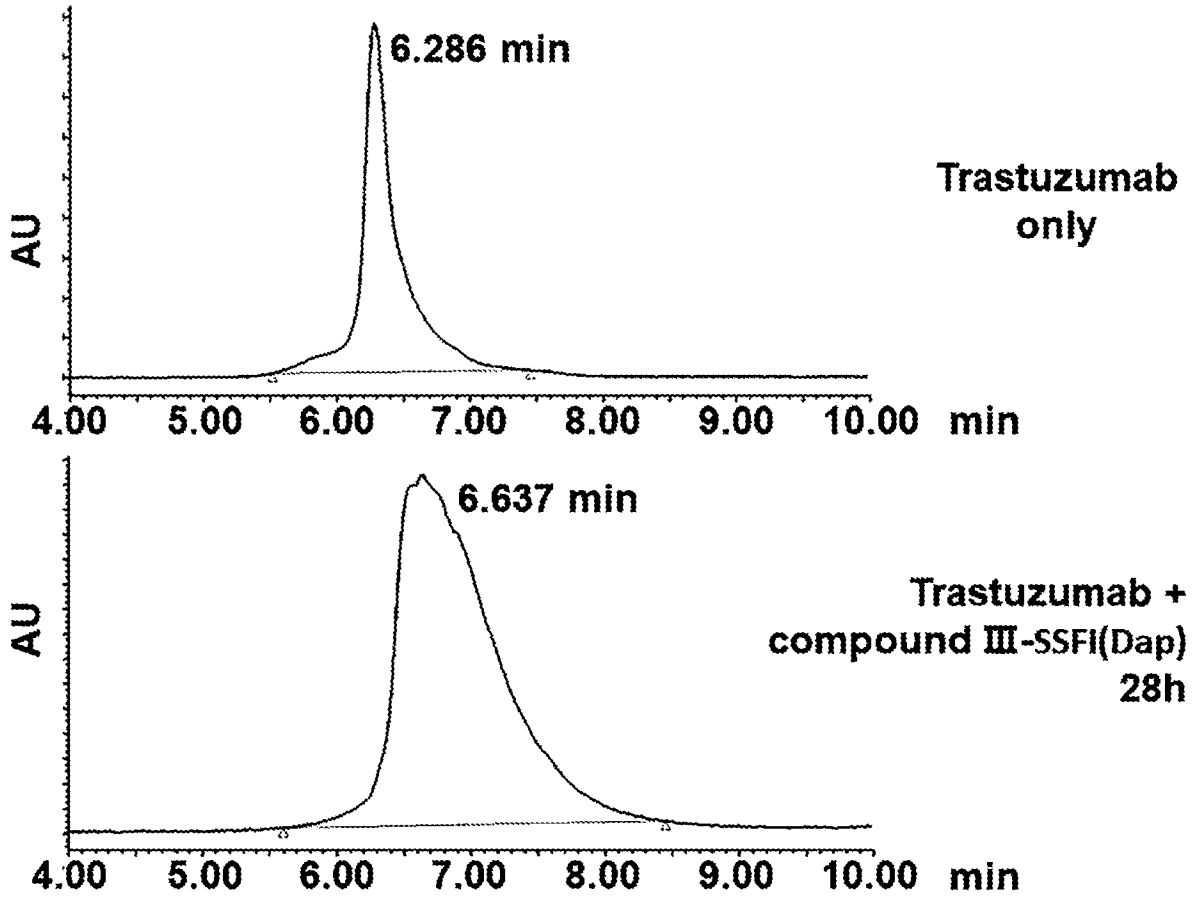

6.286 min
AU
Trastuzumab only
4.00
5.00
6.00
7.00
8.00
9.00
10.00
min
6.637 min
AU
Trastuzumab + compound Ⅲ-SSFI(Dap) 28h
4.00
5.00
6.00
7.00
8.00
9.00
10.00
min

FIG. 44

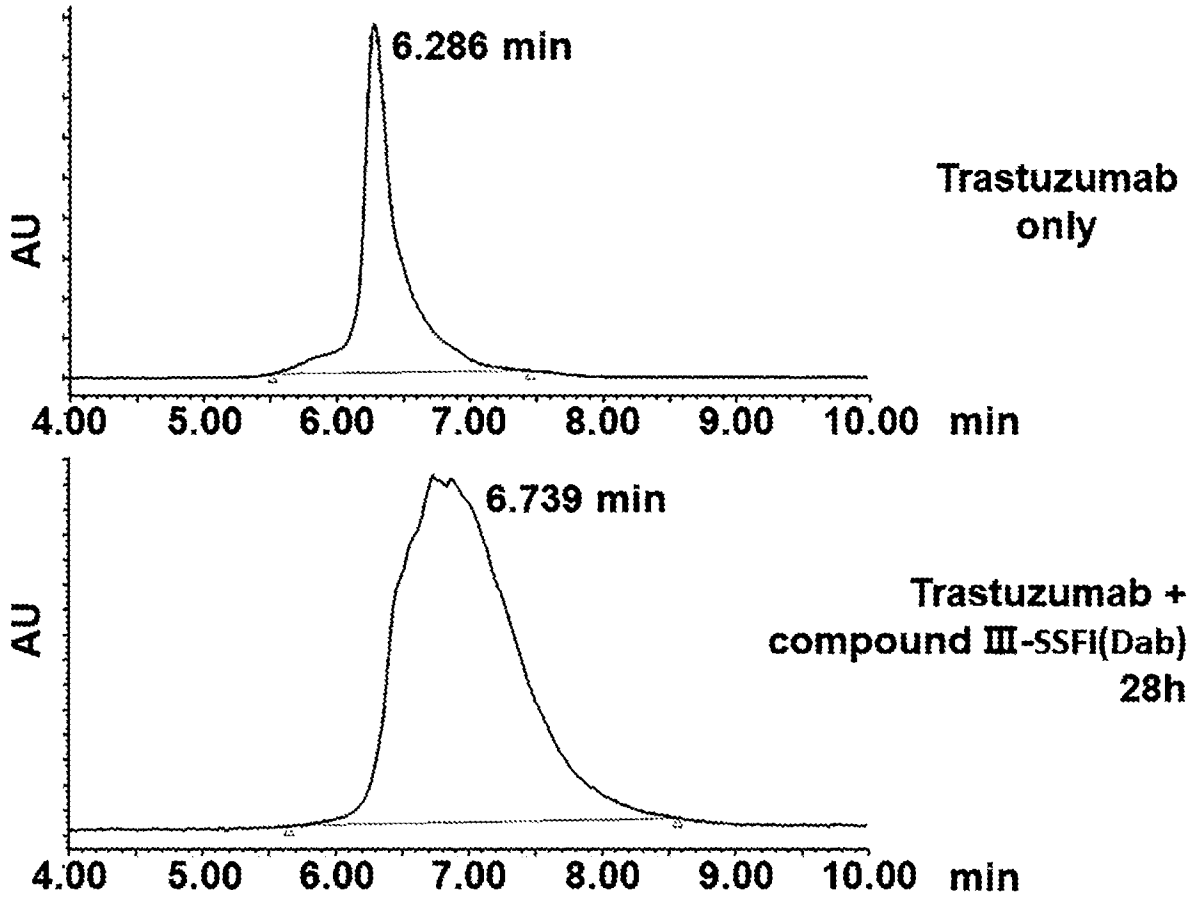

6.286 min
AU
Trastuzumab only
4.00
5.00
6.00
7.00
8.00
9.00
10.00
min
6.739 min
AU
Trastuzumab + compound Ⅲ-SSFI(Dab) 28h
4.00
5.00
6.00
7.00
8.00
9.00
10.00
min

FIG. 45

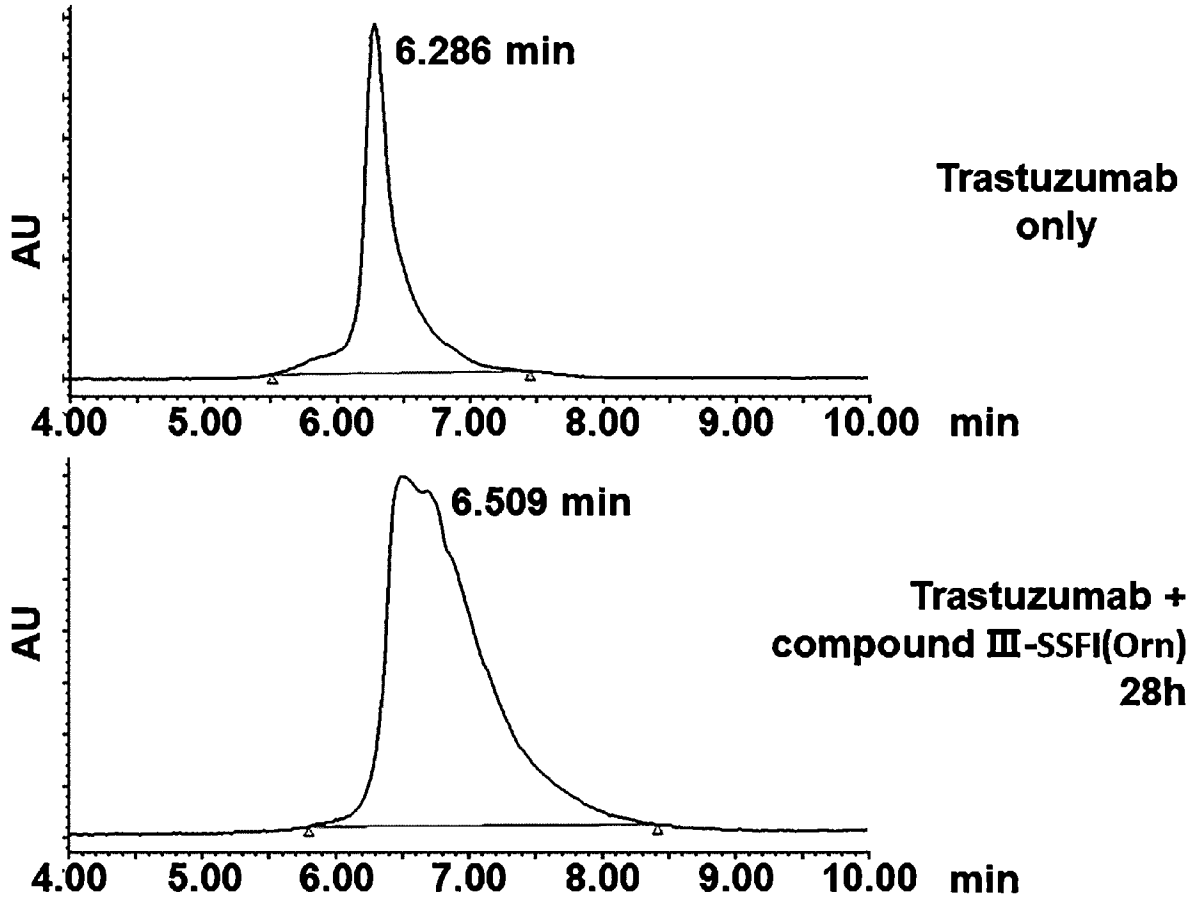

6.286 min
AU
Trastuzumab only
4.00
5.00
6.00
7.00
8.00
9.00
10.00
min
6.509 min
AU
Trastuzumab + compound Ⅲ-SSFI(Orn) 28h
4.00
5.00
6.00
7.00
8.00
9.00
10.00
min

FIG. 46

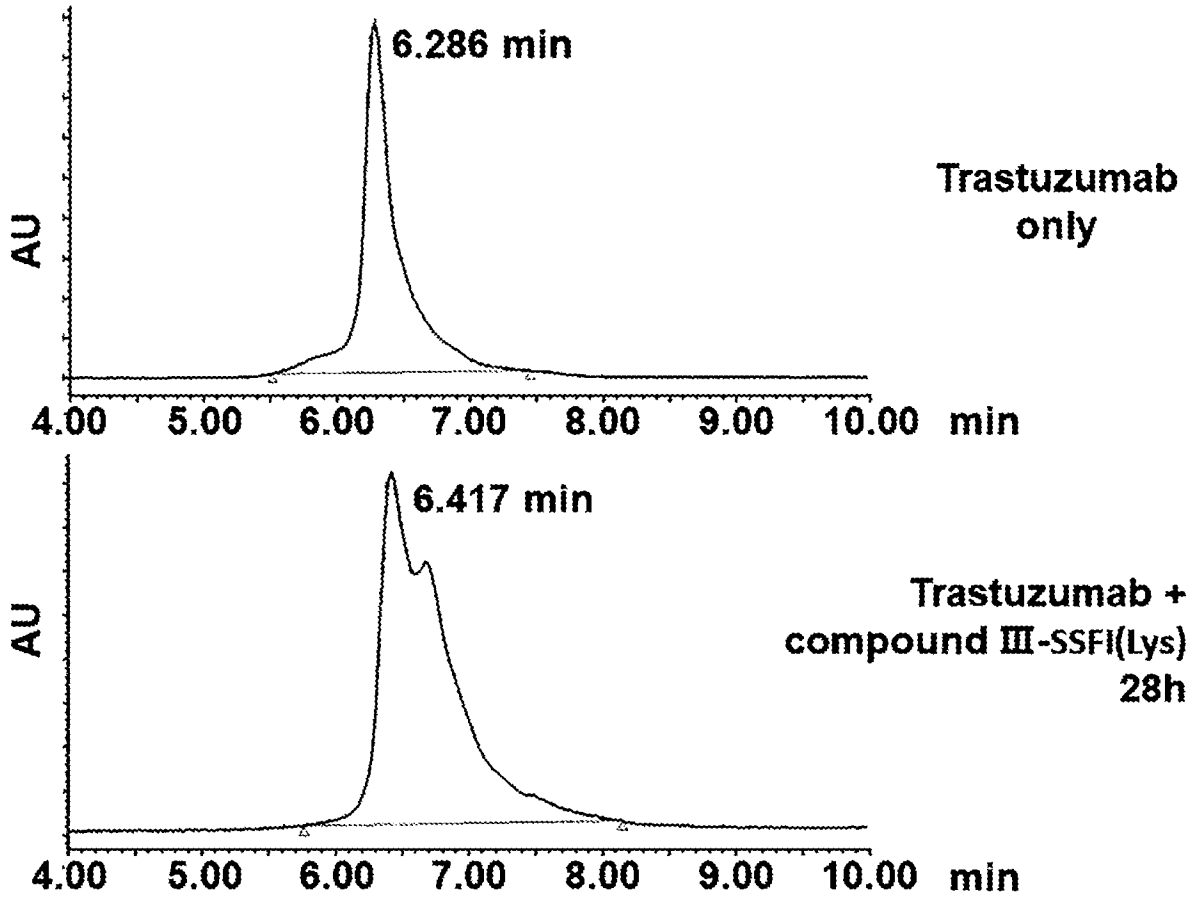

6.286 min
AU
Trastuzumab only
4.00
5.00
6.00
7.00
8.00
9.00
10.00
min
6.417 min
AU
Trastuzumab + compound Ⅲ-SSFI(Lys) 28h
4.00
5.00
6.00
7.00
8.00
9.00
10.00
min

FIG. 47

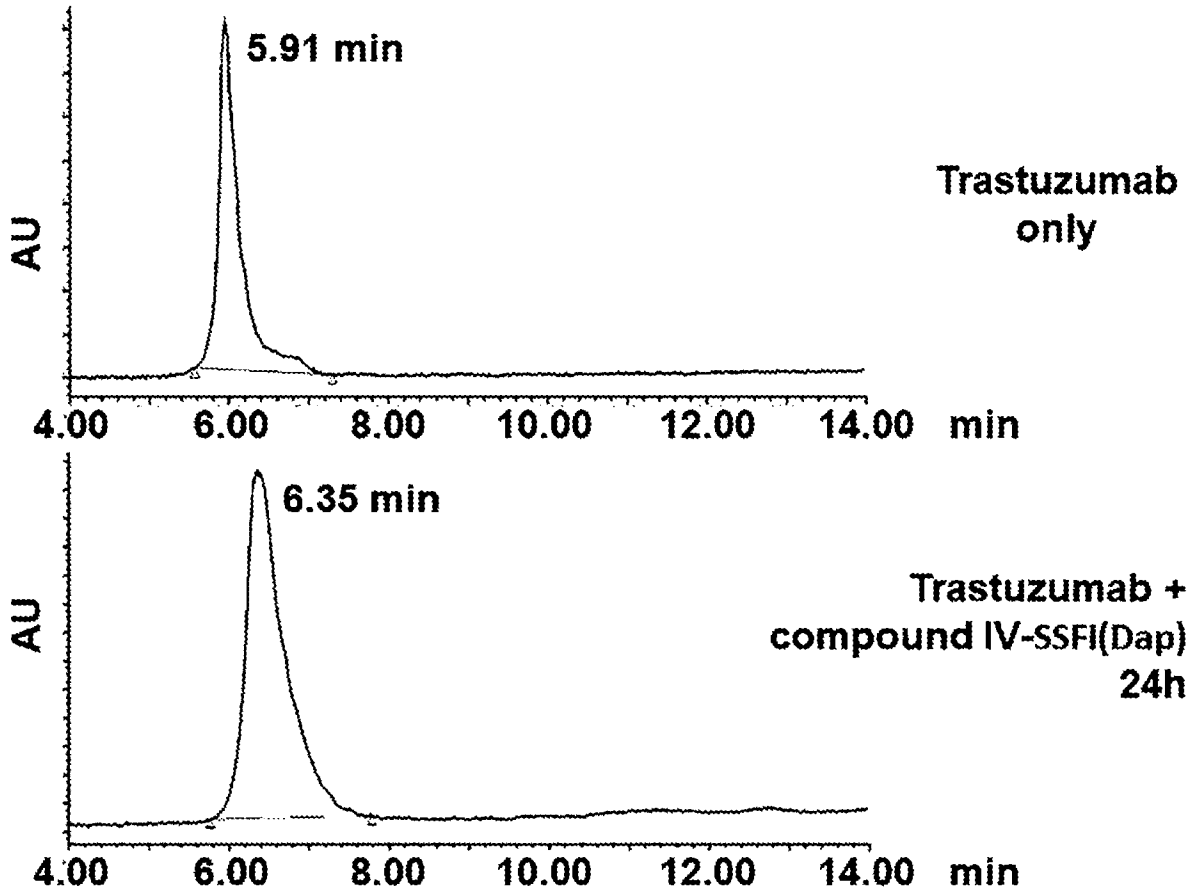

5.91 min
AU
Trastuzumab only
4.00
6.00
8.00
10.00
12.00
14.00
min
6.35 min
AU
Trastuzumab + compound IV-SSFI(Dap) 24h
4.00
6.00
8.00
10.00
12.00
14.00
min

FIG. 48

Trastuzumab only (Fc/2)

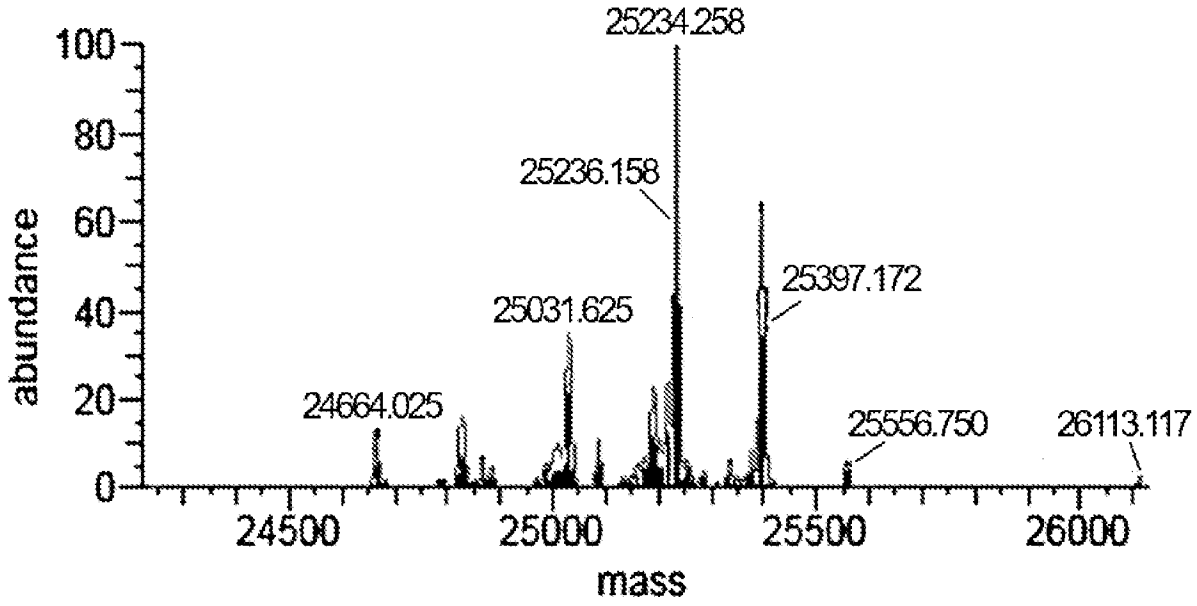


FIG. 49

Trastuzumab-norbornene conjugate (Fc/2)

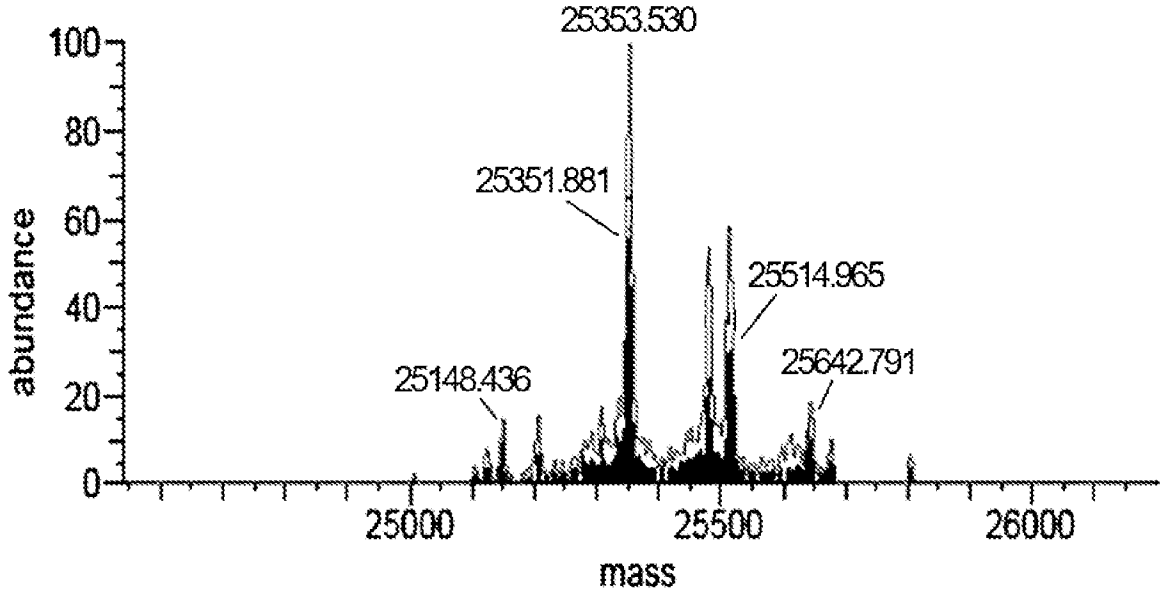


FIG. 50

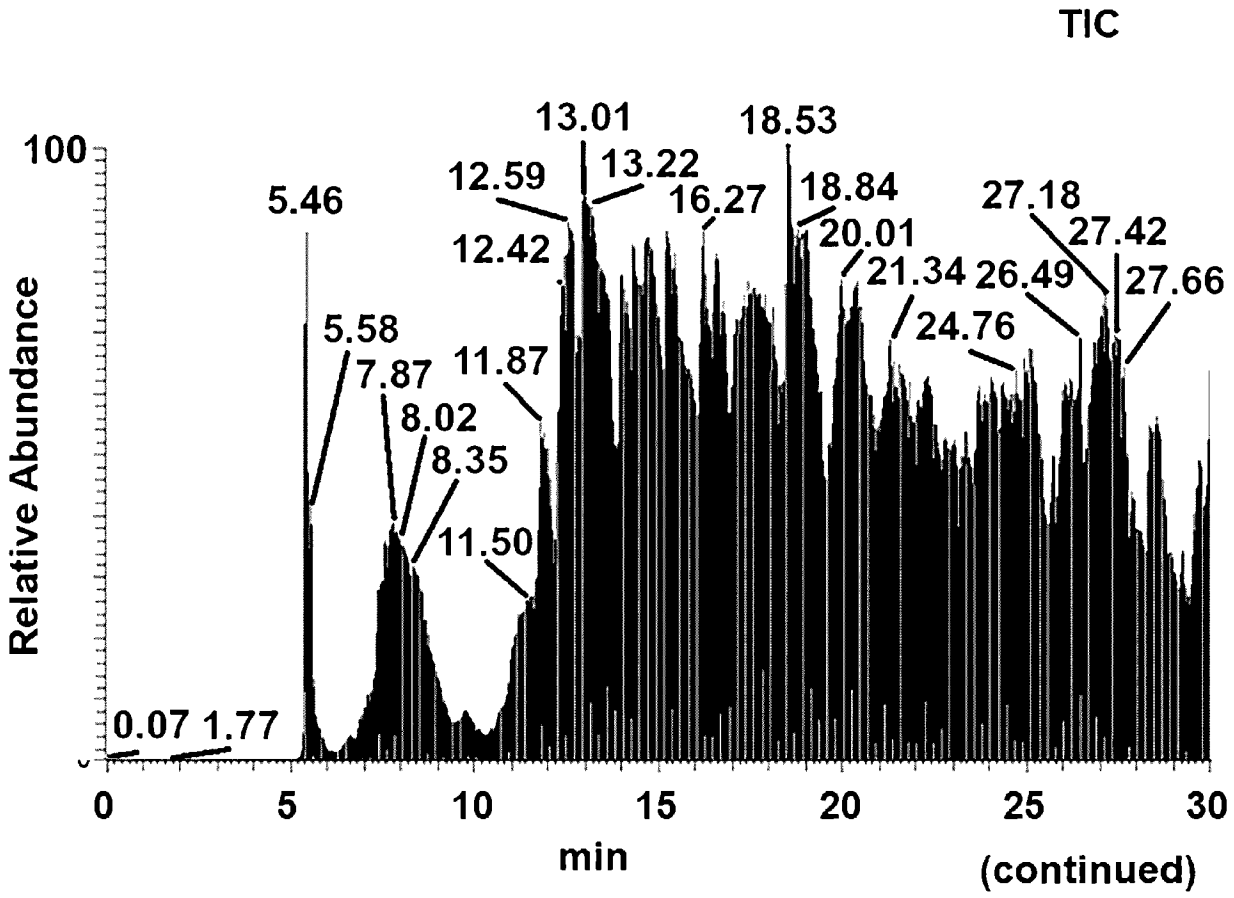

TIC
100
Relative Abundance
13.01
18.53
12.59
13.22
5.46
16.27
18.84
27.18
12.42
20.01
27.42
21.34
26.49
27.66
5.58
24.76
7.87
11.87
8.02
8.35
11.50
0.07
1.77
0
5
10
15
20
25
30
min
(continued)

FIG. 51

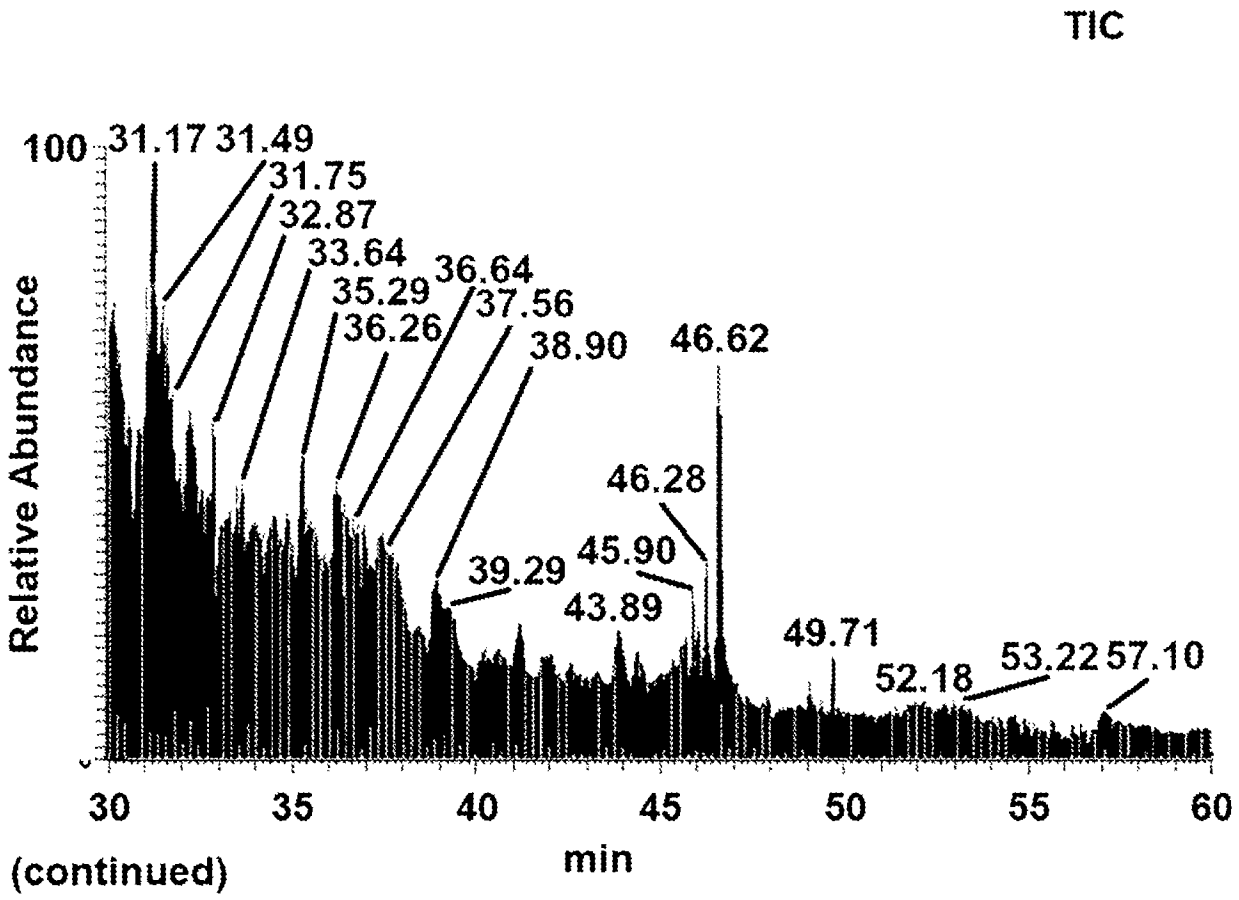

TIC
100
31.17
31.49
31.75
32.87
33.64
35.29
36.64
36.26
37.56
38.90
46.62
46.28
45.90
39.29
43.89
49.71
52.18
53.22
57.10
Relative Abundance
30
35
40
45
50
55
60
min
(continued)

FIG. 52

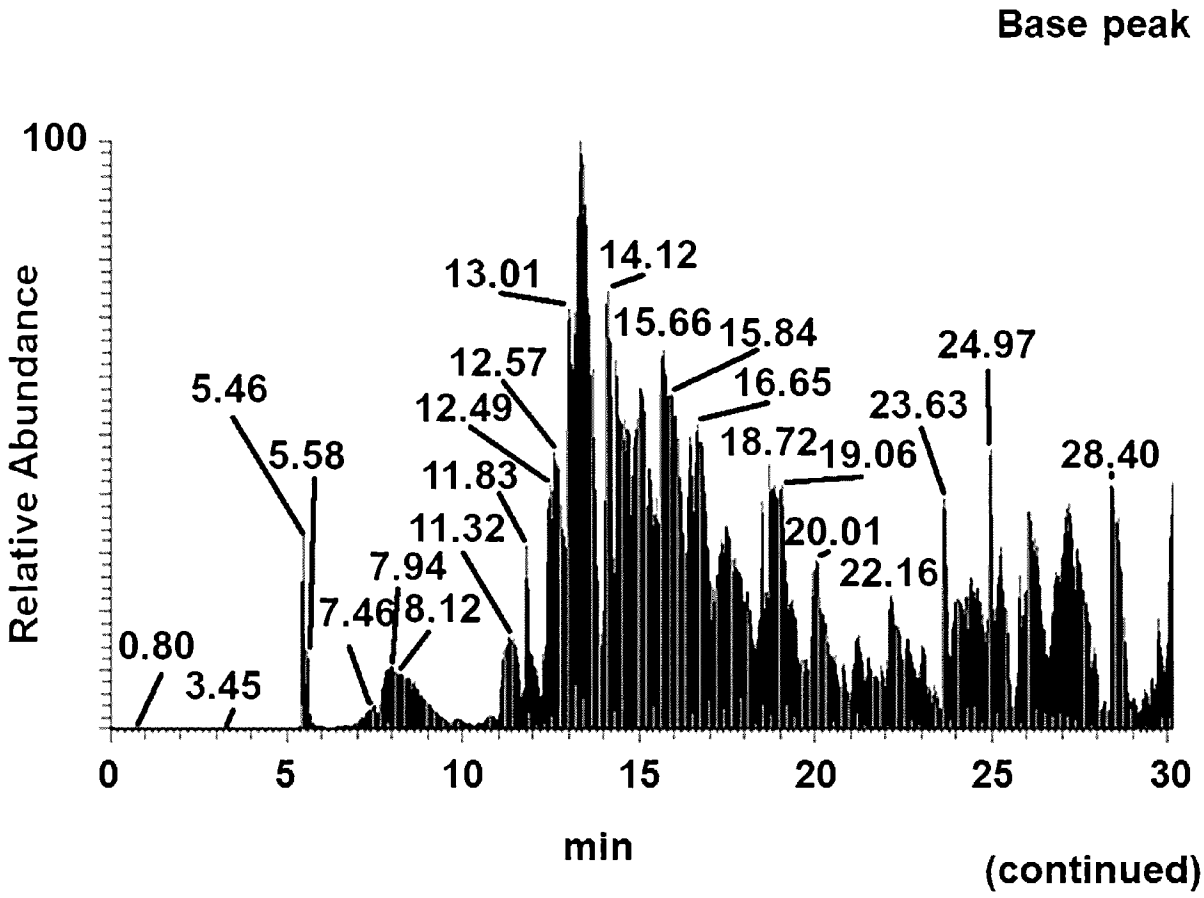

Base peak
100
Relative Abundance
13.01
14.12
15.66
15.84
24.97
12.57
5.46
16.65
12.49
23.63
5.58
18.72
19.06
28.40
11.83
11.32
20.01
7.94
22.16
7.46
8.12
0.80
3.45
0
5
10
15
20
25
30
min
(continued)

FIG. 53

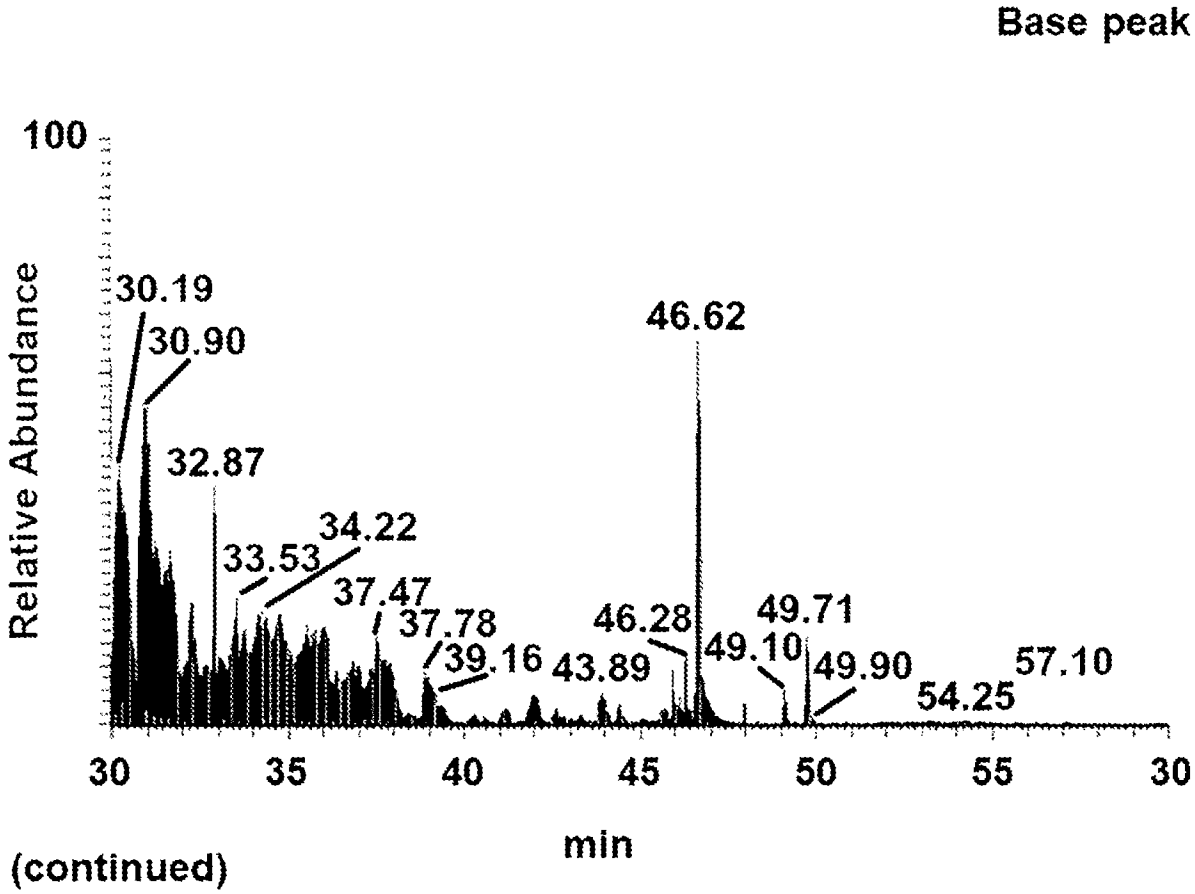

Base peak
100
Relative Abundance
30.19
30.90
32.87
33.53
34.22
37.47
37.78
39.16
43.89
46.28
46.62
49.10
49.71
49.90
54.25
57.10
30
35
40
45
50
55
30
min
(continued)

FIG. 54

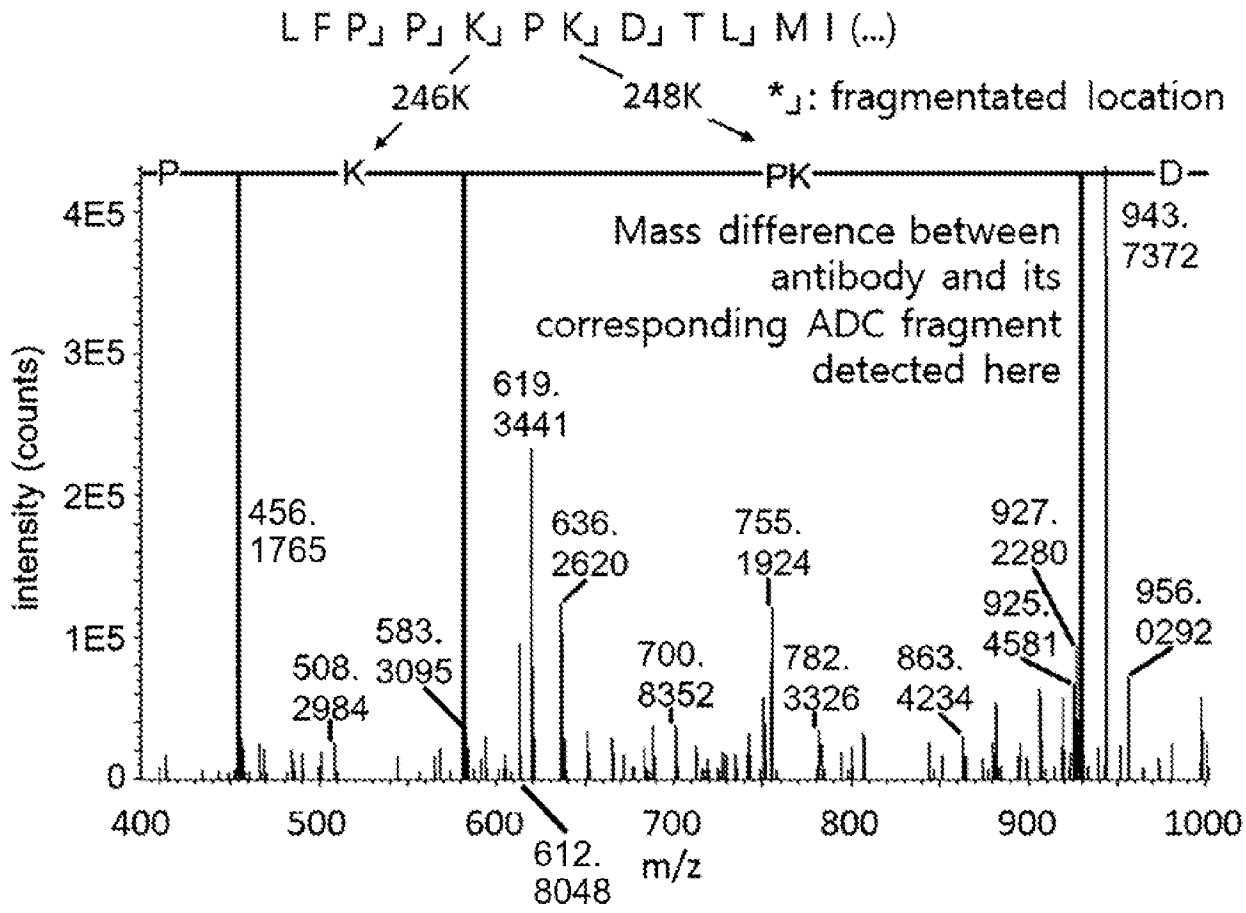

L F P」 P」 K」 P K」 D」 T L」 M I (...)
246K
248K
*」: fragmentated location
P
K
PK
D
Mass difference between antibody and its corresponding ADC fragment detected here
intensity (counts)
4E5
3E5
2E5
1E5
0
400
500
600
700
800
900
1000
m/z
456. 1765
508. 2984
583. 3095
612. 8048
619. 3441
636. 2620
700. 8352
755. 1924
782. 3326
863. 4234
925. 4581
927. 2280
943. 7372
956. 0292

FIG. 55

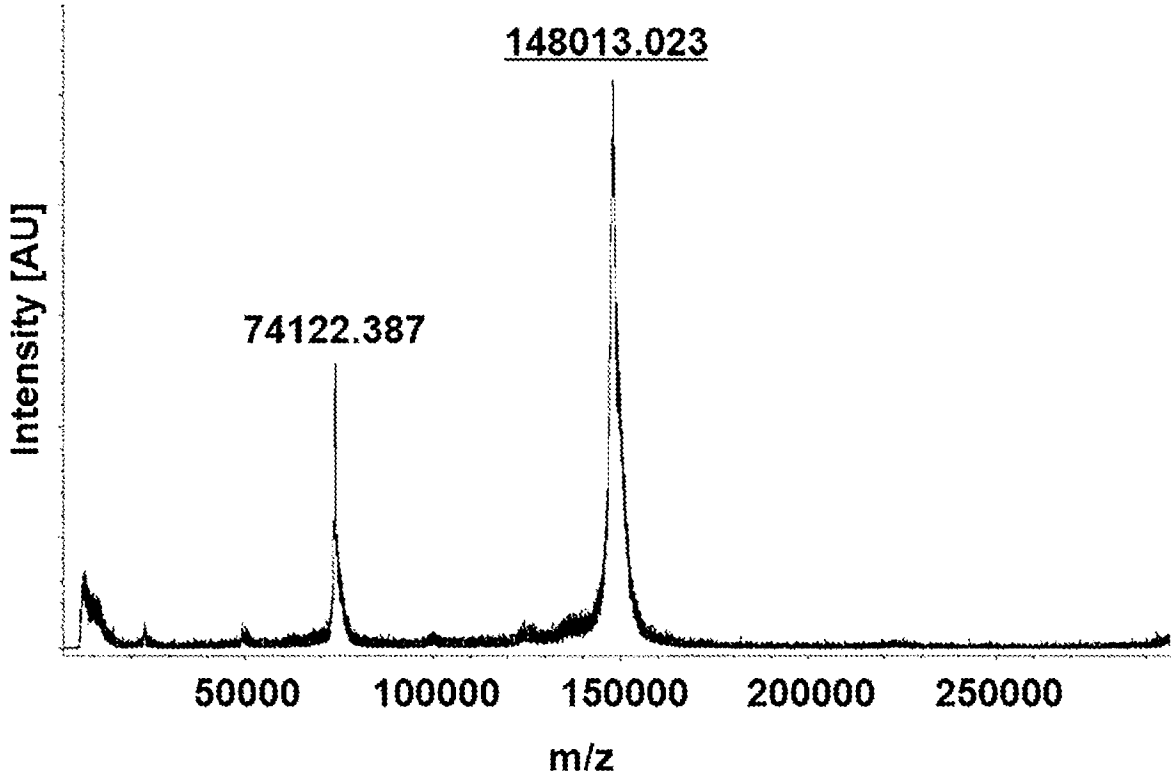

148013.023
74122.387
Intensity [AU]
50000
100000
150000
200000
250000
m/z

FIG. 56

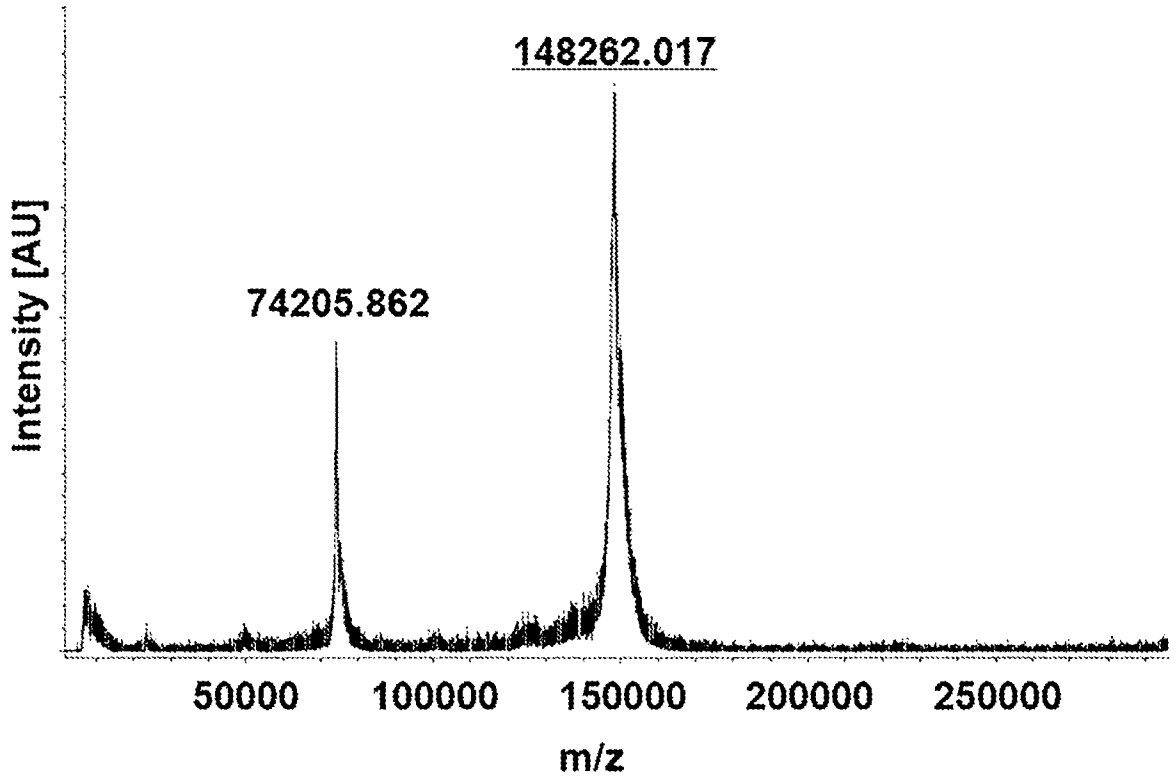

148262.017
74205.862
Intensity [AU]
50000
100000
150000
200000
250000
m/z

FIG. 57

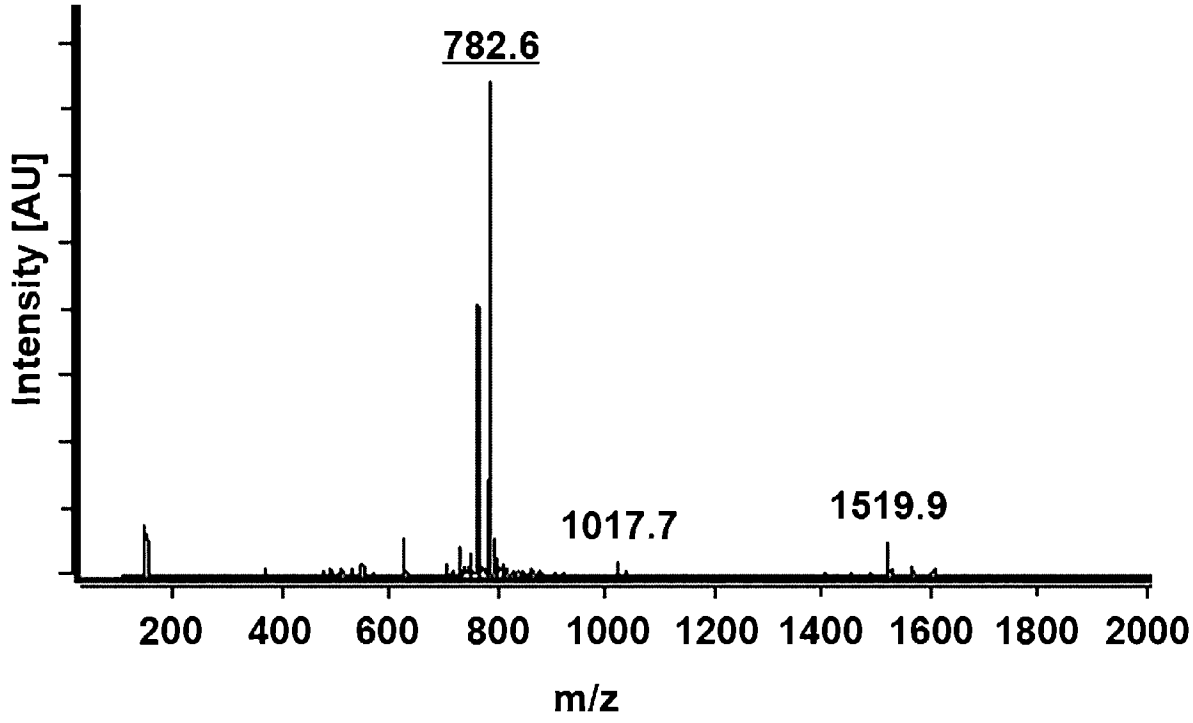

782.6
1017.7
1519.9
Intensity [AU]
200
400
600
800
1000
1200
1400
1600
1800
2000
m/z

FIG. 58

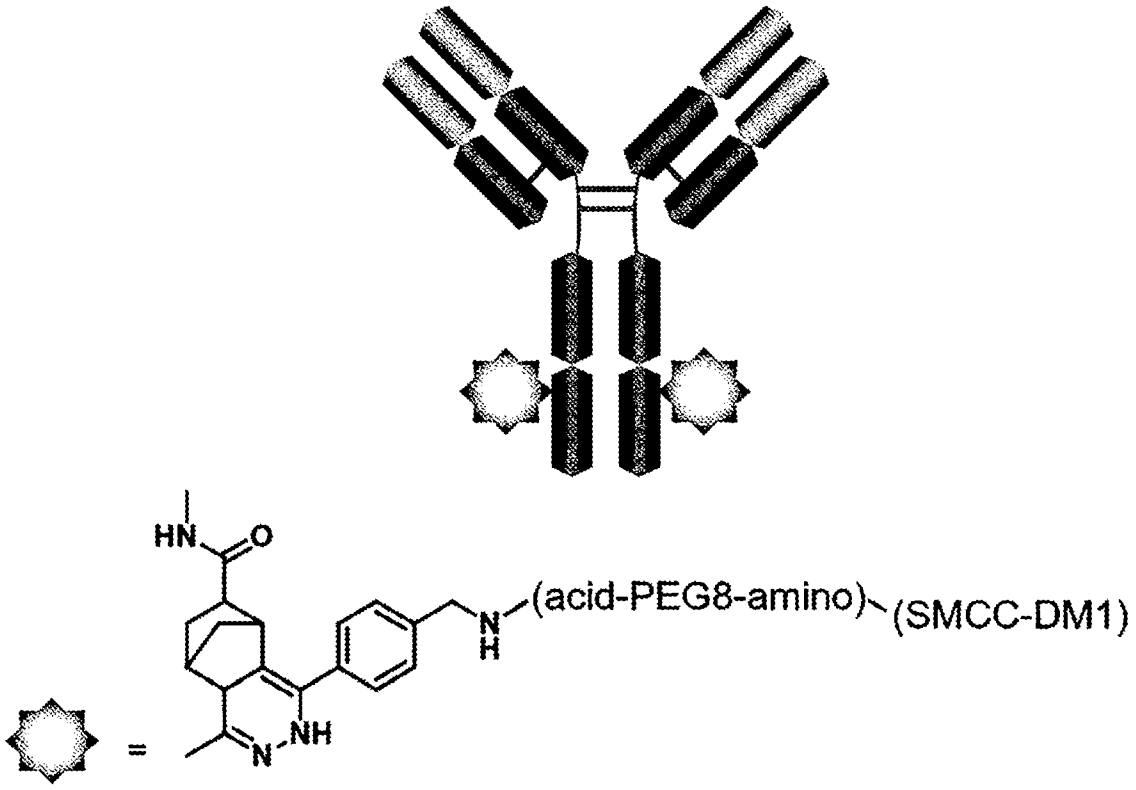


FIG. 59

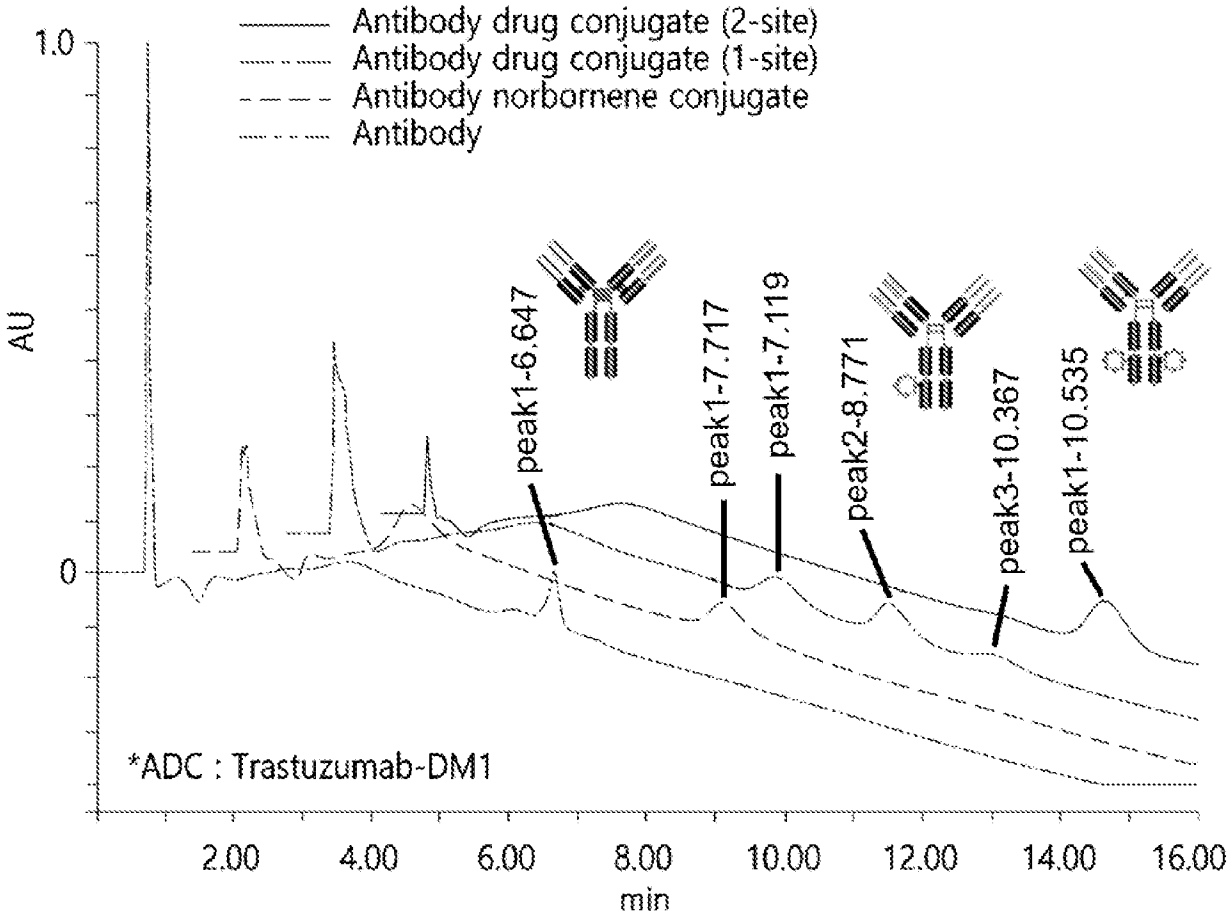

Antibody drug conjugate (2-site)
Antibody drug conjugate (1-site)
Antibody norbornene conjugate
Antibody
1.0
AU
0
peak1-6.647
peak1-7.717
peak1-7.119
peak2-8.771
peak3-10.367
peak1-10.535
*ADC : Trastuzumab-DM1
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
min

FIG. 60

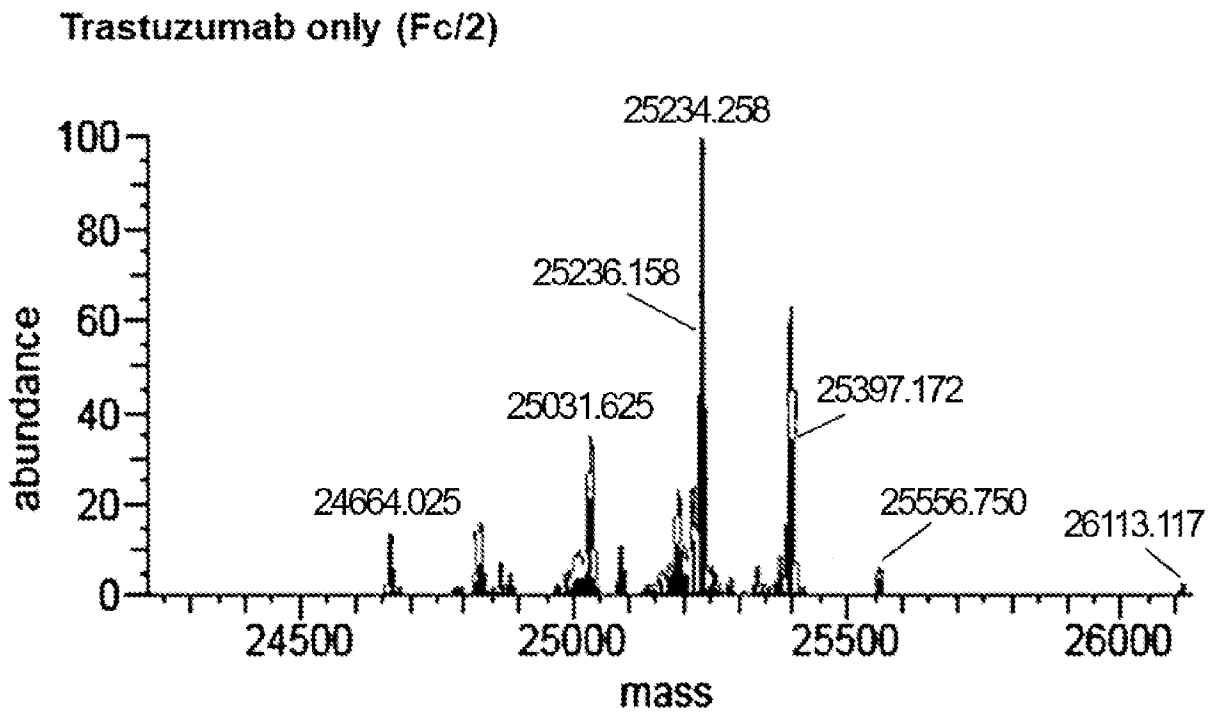

Trastuzumab only (Fc/2)
25234.258
25236.158
25397.172
25031.625
24664.025
25556.750
26113.117
100
80
60
40
20
0
abundance
24500
25000
25500
26000
mass

FIG. 61

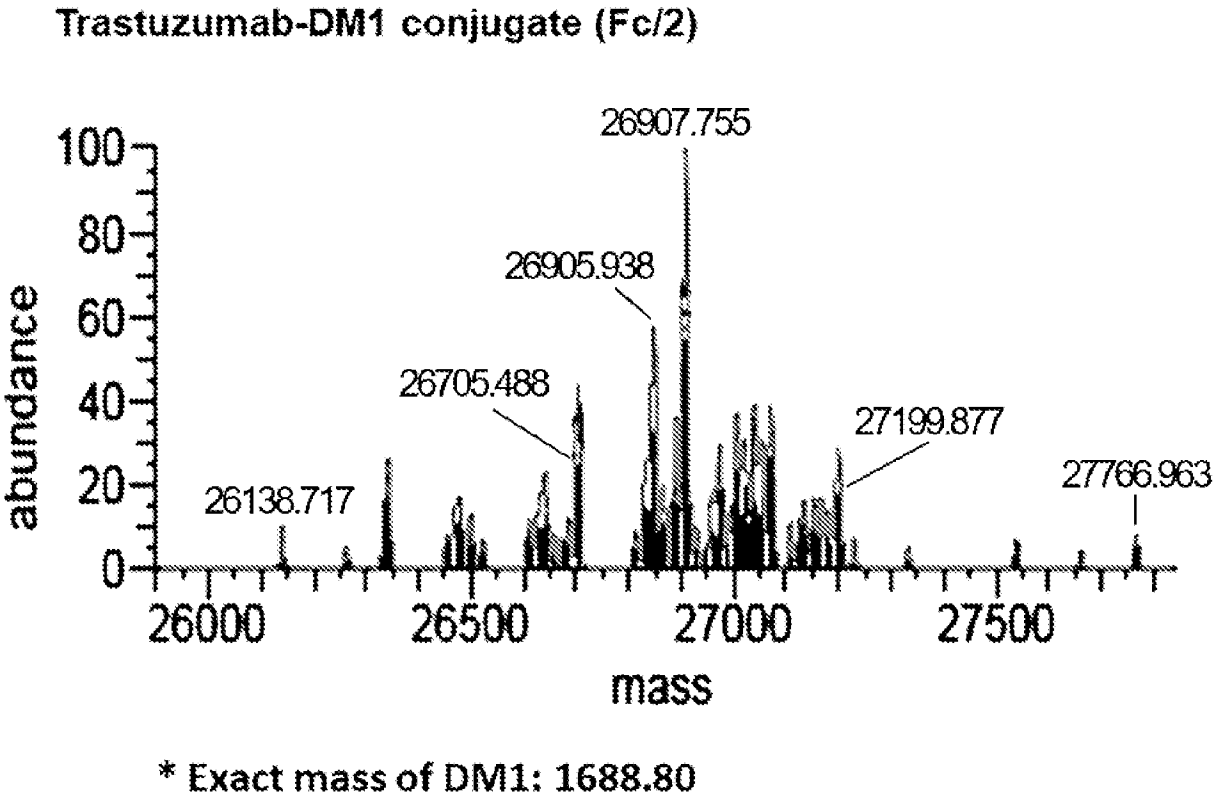
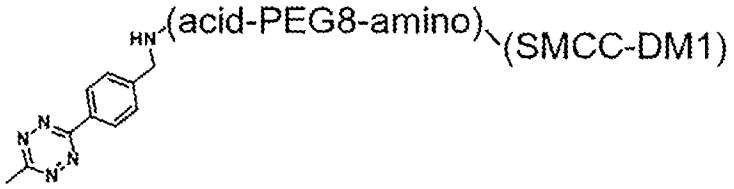
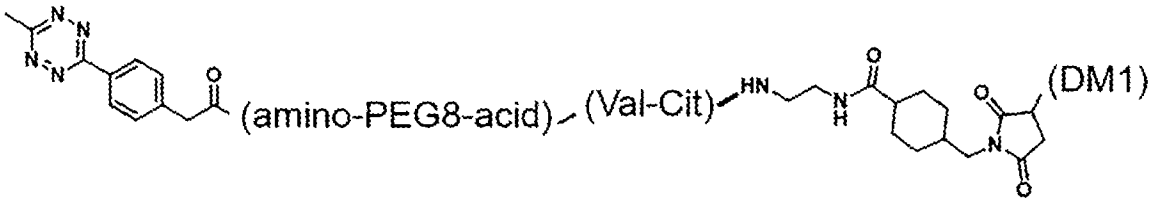

Trastuzumab-DM1 conjugate (Fc/2)
26907.755
26905.938
26705.488
27199.877
27766.963
26138.717
100
80
60
40
20
0
abundance
26000
26500
27000
27500
mass
* Exact mass of DM1: 1688.80

FIG. 62

1. NC-DM1 : non-cleavable DM1 (tetrazine-PEG8-DM1)



2. VC-DM1 : cleavable DM1 (tetrazine-PEG8-valine-citrulline-DM1)



3. VC-MMAE : cleavable DM1 (tetrazine-PEG8-valine-citrulline-PAB-MMAE)

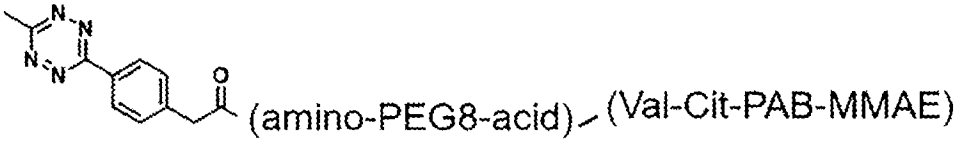


FIG. 63

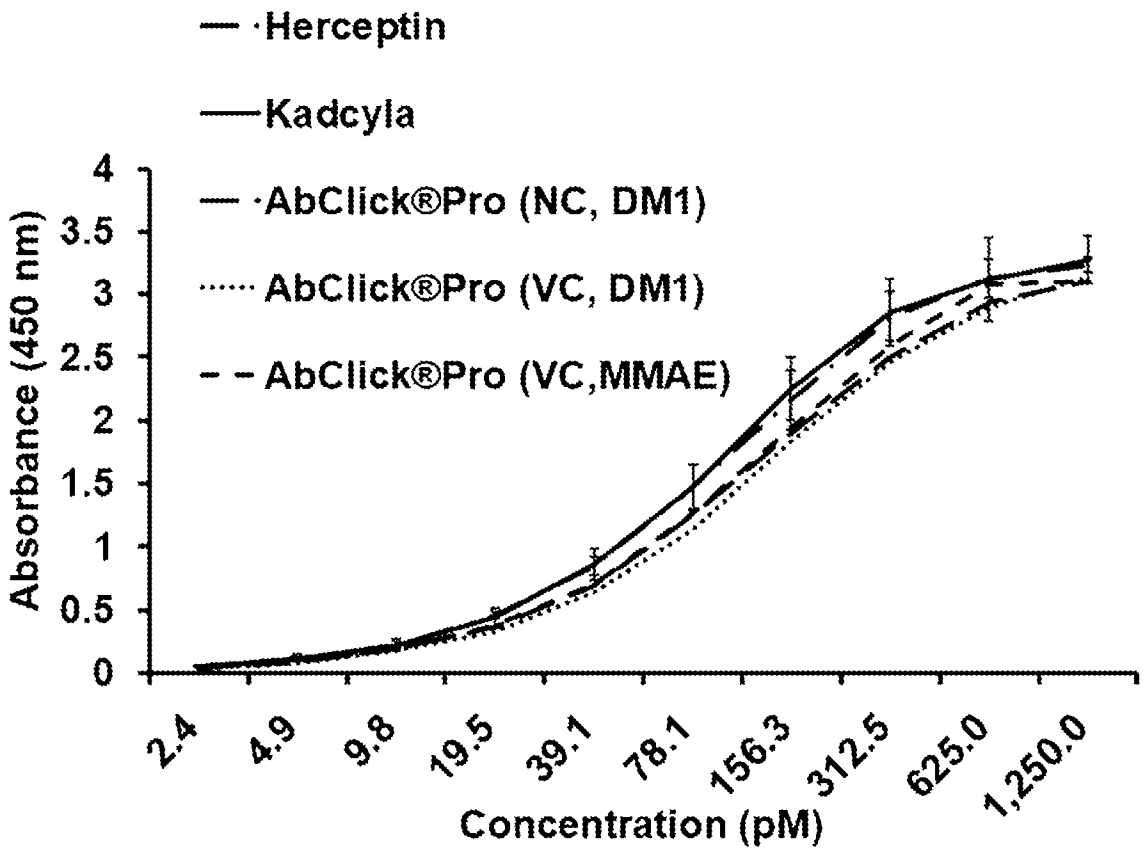

Herceptin
Kadcyla
AbClick®Pro (NC, DM1)
AbClick®Pro (VC, DM1)
AbClick®Pro (VC,MMAE)
Absorbance (450 nm)
0
0.5
1
1.5
2
2.5
3
3.5
4
2.4
4.9
9.8
19.5
39.1
78.1
156.3
312.5
625.0
1,250.0
Concentration (pM)

FIG. 64

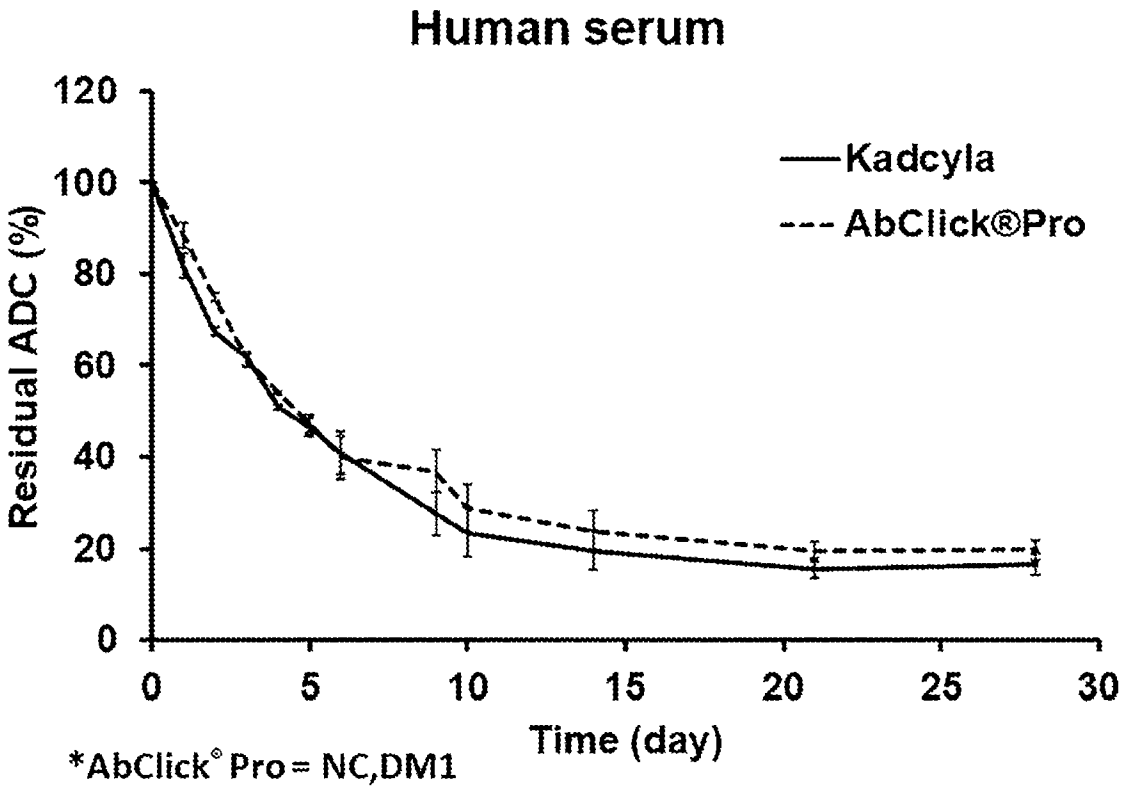

Human serum
Residual ADC (%)
120
100
80
60
40
20
0
0
5
10
15
20
25
30
Time (day)
Kadcyla
AbClick®Pro
*AbClick® Pro = NC,DM1

FIG. 65

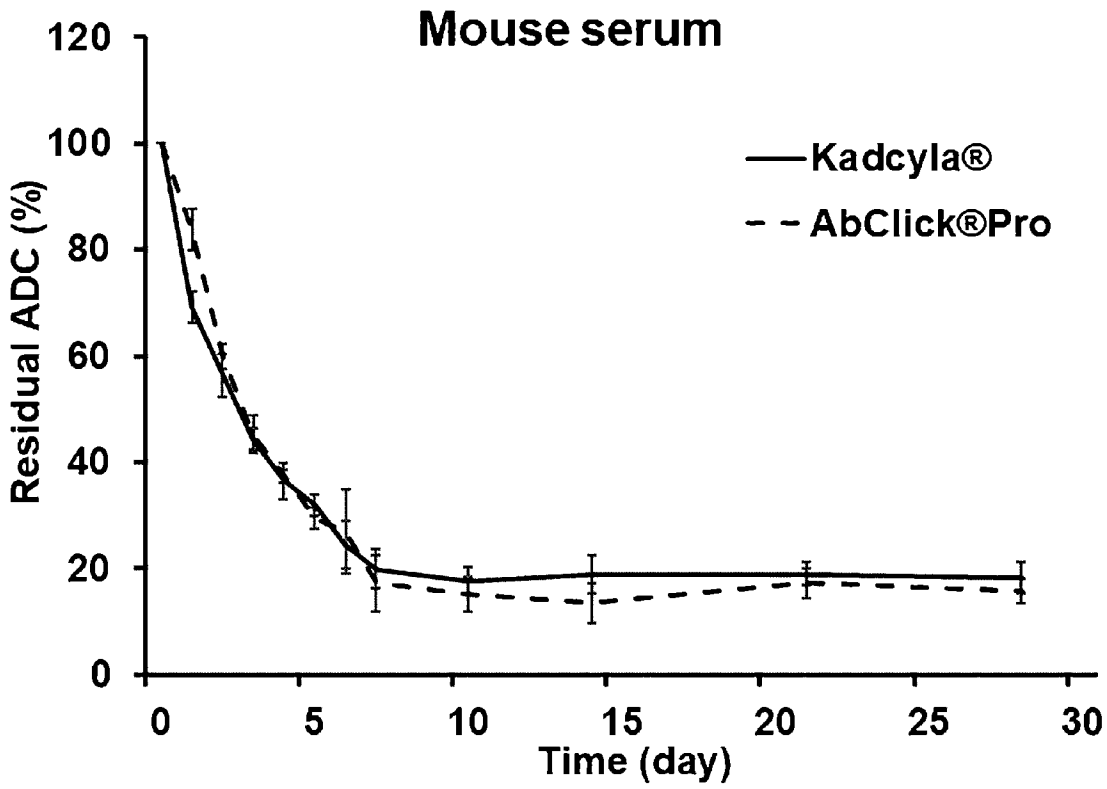

Mouse serum
Kadcyla®
AbClick®Pro
Residual ADC (%)
120
100
80
60
40
20
0
0
5
10
15
20
25
30
Time (day)

FIG. 66

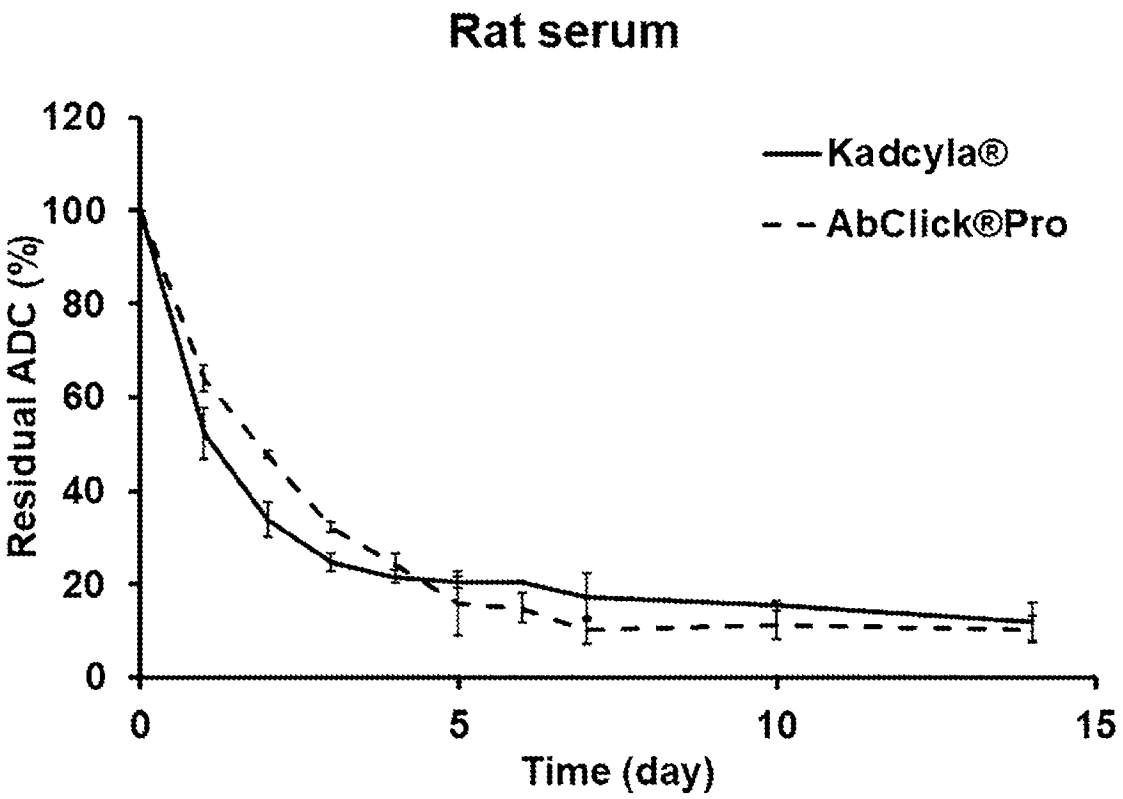

Rat serum
Kadcyla®
AbClick®Pro
Residual ADC (%)
120
100
80
60
40
20
0
0
5
10
15
Time (day)

FIG. 67

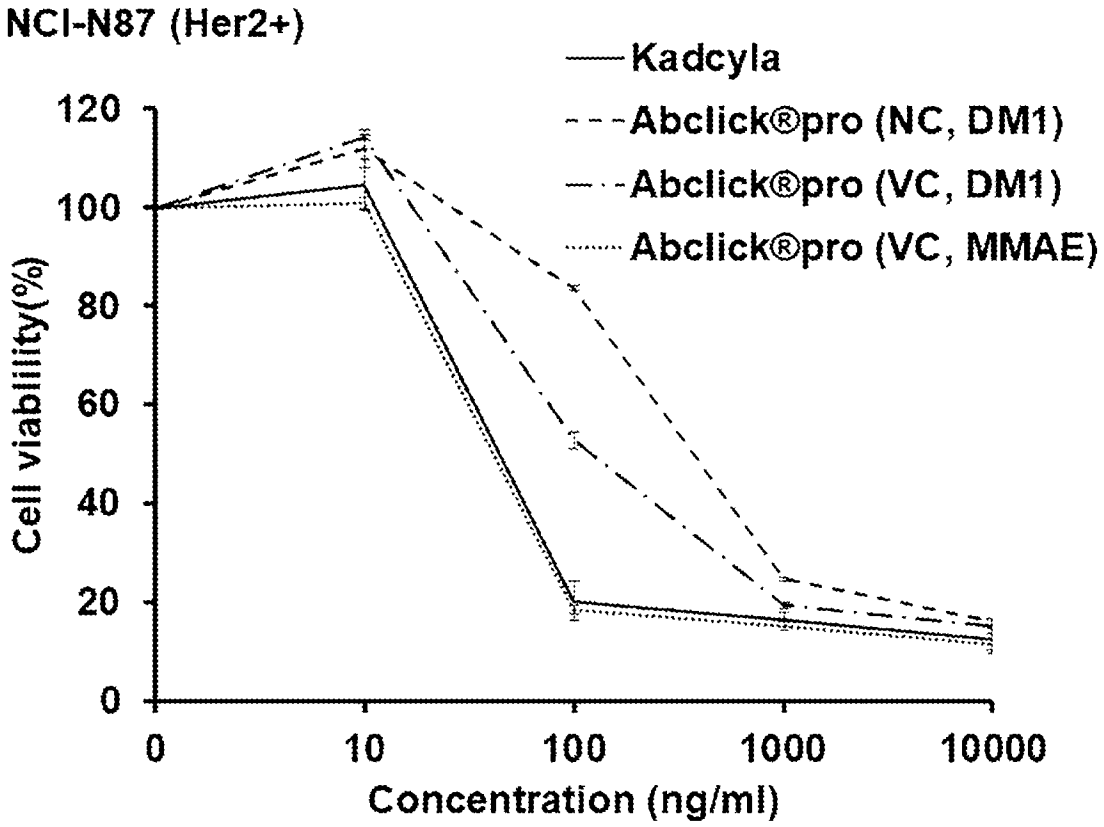

NCI-N87 (Her2+)
Kadcyla
Abclick®pro (NC, DM1)
Abclick®pro (VC, DM1)
Abclick®pro (VC, MMAE)
Cell viability(%)
120
100
80
60
40
20
0
0
10
100
1000
10000
Concentration (ng/ml)

FIG. 68

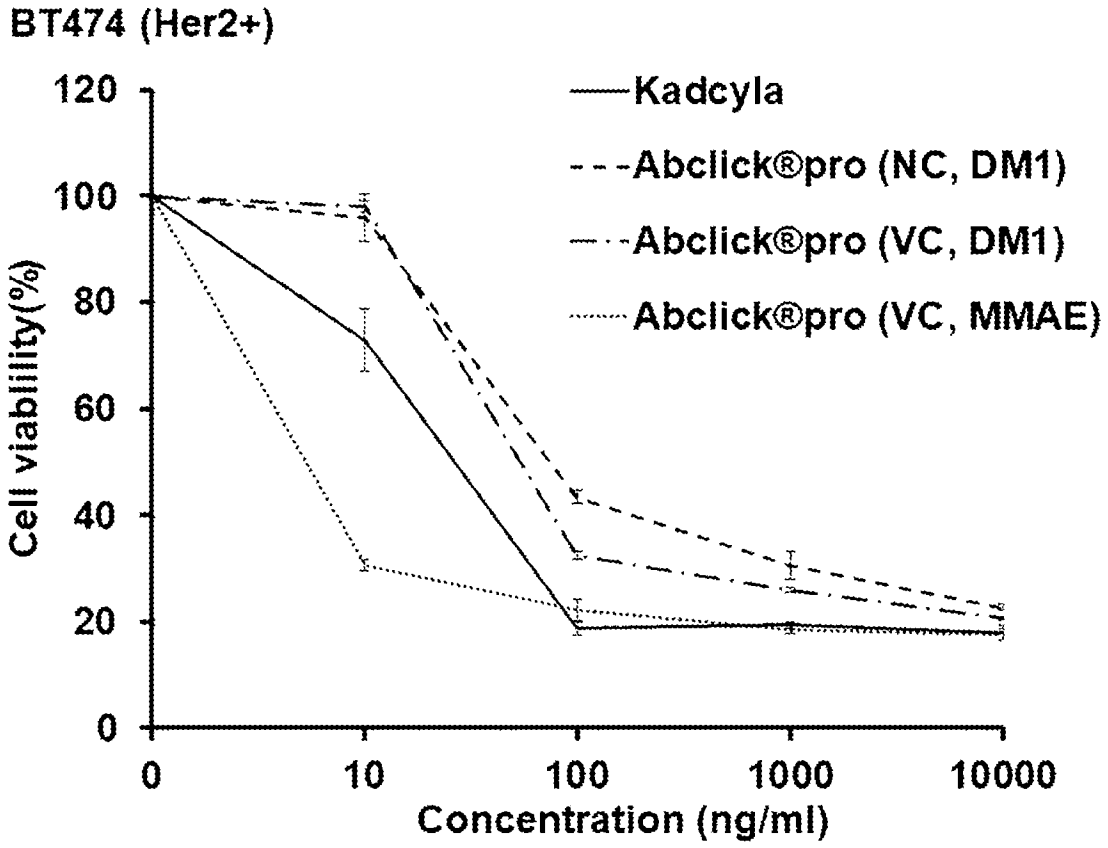

BT474 (Her2+)
Kadcyla
Abclick®pro (NC, DM1)
Abclick®pro (VC, DM1)
Abclick®pro (VC, MMAE)
Cell viability(%)
120
100
80
60
40
20
0
0
10
100
1000
10000
Concentration (ng/ml)

FIG. 69

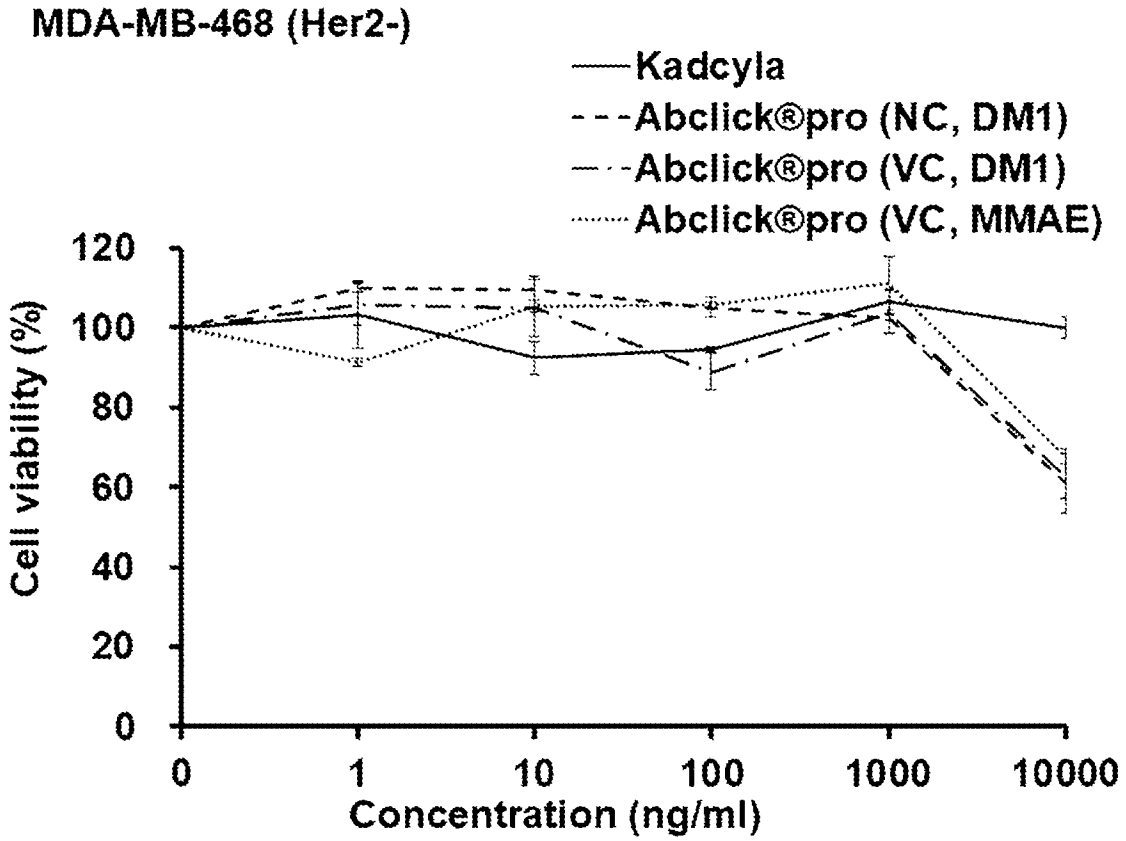

MDA-MB-468 (Her2-)
Kadcyla
Abclick®pro (NC, DM1)
Abclick®pro (VC, DM1)
Abclick®pro (VC, MMAE)
Cell viability (%)
120
100
80
60
40
20
0
0
1
10
100
1000
10000
Concentration (ng/ml)

FIG. 70

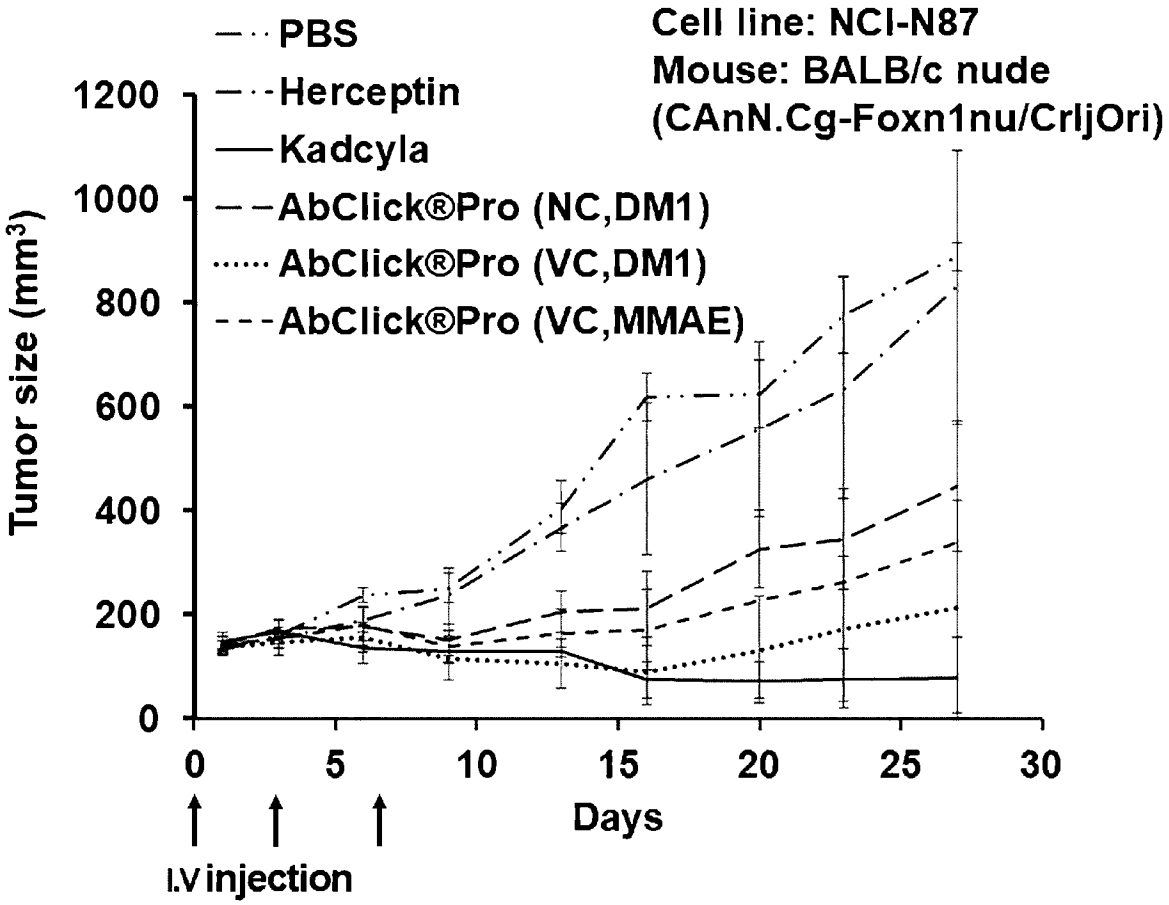

PBS
Herceptin
Kadcyla
AbClick®Pro (NC,DM1)
AbClick®Pro (VC,DM1)
AbClick®Pro (VC,MMAE)
Cell line: NCI-N87
Mouse: BALB/c nude
(CAnN.Cg-Foxn1nu/CrljOri)
Tumor size (mm3)
1200
1000
800
600
400
200
0
0
5
10
15
20
25
30
Days
I.V injection

FIG. 71

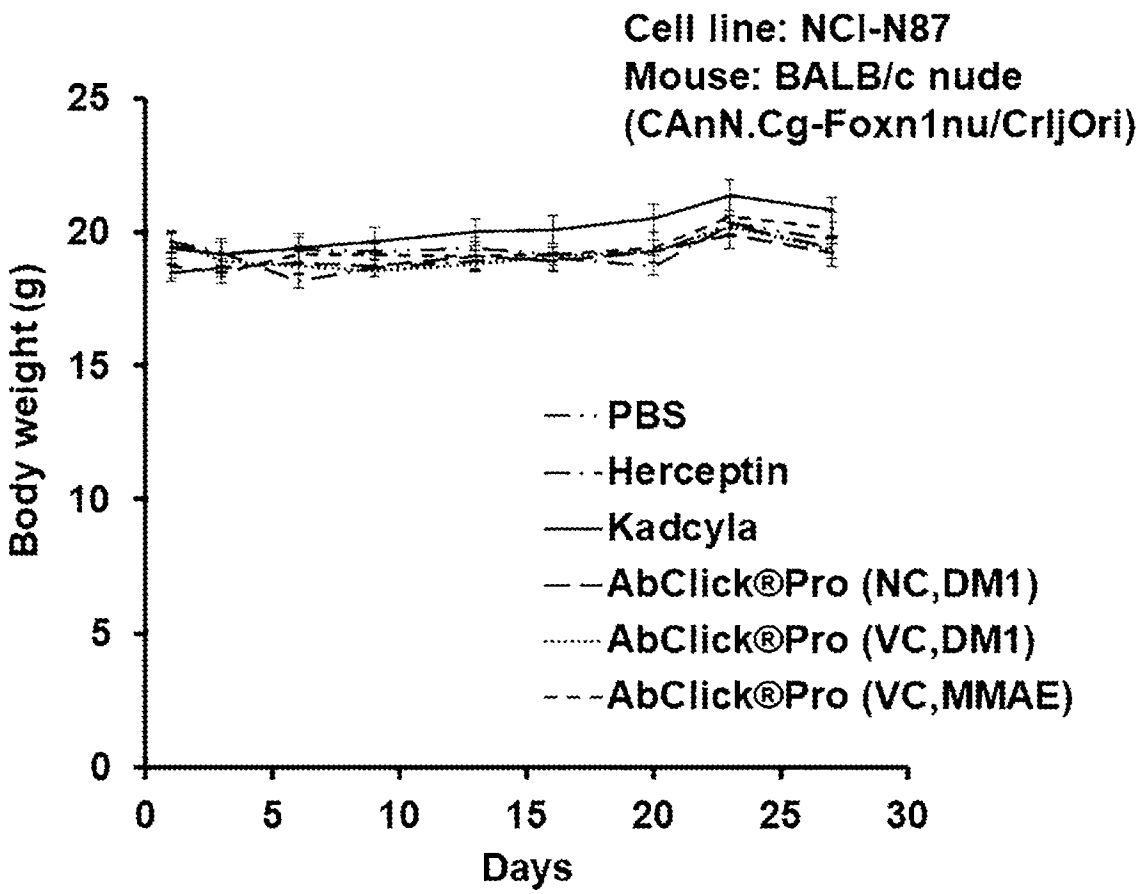

Cell line: NCI-N87
Mouse: BALB/c nude
(CAnN.Cg-Foxn1nu/CrljOri)
Body weight (g)
25
20
15
10
5
0
0
5
10
15
20
25
30
Days
PBS
Herceptin
Kadcyla
AbClick®Pro (NC,DM1)
AbClick®Pro (VC,DM1)
AbClick®Pro (VC,MMAE)

FIG. 72

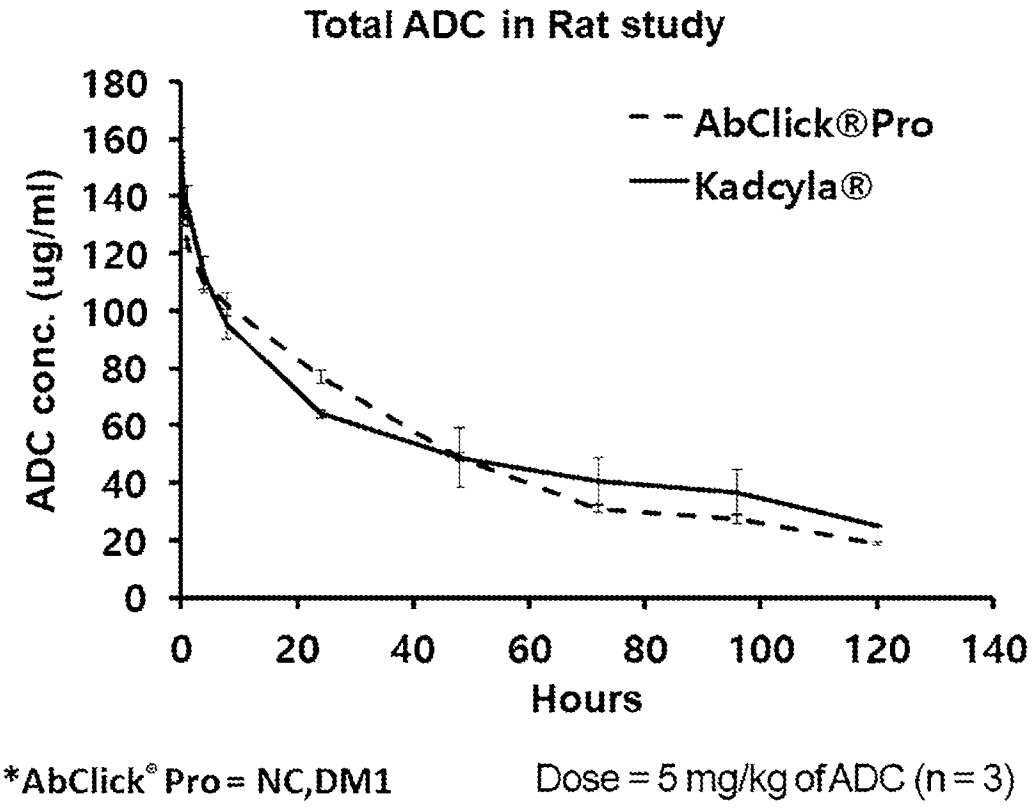

Total ADC in Rat study
ADC conc. (ug/ml)
180
160
140
120
100
80
60
40
20
0
0
20
40
60
80
100
120
140
Hours
AbClick®Pro
Kadcyla®
*AbClick® Pro = NC,DM1
Dose = 5 mg/kg of ADC (n = 3)

FIG. 73

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United States Patent and Trademark Office
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APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
18/154,998	01/16/2023		1288	18896-000072-US-COA	23	2

CONFIRMATION NO. 5143

28997
Harness Dickey (St. Louis)
7700 Bonhomme, Suite 400
St. Louis, MO 63105

FILING RECEIPT

OC00000055899934

Date Mailed: 04/12/2023

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Inventor(s)

Sang Jeon CHUNG, Gyeonggi-do, KOREA, REPUBLIC OF;
Ju Hwan KIM, Gyeonggi-do, KOREA, REPUBLIC OF;
Young Geun LEE, Gyeonggi-do, KOREA, REPUBLIC OF;
Tae Jin LEE, Gyeonggi-do, KOREA, REPUBLIC OF;
Jin Woo SEO, Gyeonggi-do, KOREA, REPUBLIC OF;

Applicant(s)

AbTis Co., Ltd., Gyeonggi-do, KOREA, REPUBLIC OF;

Power of Attorney: The patent practitioners associated with Customer Number 28997

Domestic Priority data as claimed by applicant

This application is a CON of 16/980,503 09/14/2020
which is a 371 of PCT/KR20/03282 03/09/2020
which claims benefit of 62/815,557 03/08/2019

Foreign Applications for which priority is claimed (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.) - None.
Foreign application information must be provided in an Application Data Sheet in order to constitute a claim to foreign priority. See 37 CFR 1.55 and 1.76.

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Permission to Access Search Results: Yes

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The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 18/154,998**
Projected Publication Date: PPUB
Non-Publication Request: No
Early Publication Request: No
**** SMALL ENTITY ****
Title

SITE-SPECIFIC ANTIBODY CONJUGATION AND ANTIBODY-DRUG CONJUGATE AS SPECIFIC EMBODIMENT THEREOF

Preliminary Class

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

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Title 37, Code of Federal Regulations, 5.11 & 5.15

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The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign AssetsControl, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

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The United States represents the largest, most dynamic marketplace in the world and is an unparalleled location for business investment, innovation, and commercialization of new technologies. The U.S. offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to promote and facilitate business investment. SelectUSA provides information assistance to the international investor community; serves as an ombudsman for existing and potential investors; advocates on behalf of U.S. cities, states, and regions competing for global investment; and counsels U.S. economic development organizations on investment attraction best practices. To learn more about why the United States is the best country in the world to develop

technology, manufacture products, deliver services, and grow your business, visit http://www.SelectUSA.gov or call +1-202-482-6800.

PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 18/154,998

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	64		N/A	
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	280		N/A	
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	320		N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	23 minus 20 =	* 3	x 40 =	120	OR		
INDEPENDENT CLAIMS (37 CFR 1.16(h))	2 minus 3 =	*	x =	0			
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			504			
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				0			
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	1168		TOTAL	

APPLICATION AS AMENDED - PART II

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT A	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE($)	ADDITIONAL FEE($)	OR	RATE($)	ADDITIONAL FEE($)
AMENDMENT B	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.

** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".

*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".

The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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P.O. Box 1450
Alexandria, Virginia 22313-1450
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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
18/154,998	01/16/2023	Sang Jeon CHUNG	18896-000072-US-COA

CONFIRMATION NO. 5143

28997
Harness Dickey (St. Louis)
7700 Bonhomme, Suite 400
St. Louis, MO 63105

FORMALITIES LETTER

OC00000055899936

Date Mailed: 04/12/2023

NOTICE TO FILE CORRECTED APPLICATION PAPERS

Filing Date Granted

An application number and filing date have been accorded to the above-identified nonprovisional application. The application is informal since it does not comply with the regulations for the reason(s) indicated below. Applicant is given **TWO MONTHS** from the date of this Notice within which to file all required items below to avoid abandonment. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

- **Drawing Items**

  The drawings submitted on 01/16/2023 are not acceptable because:

  - Figure(s) 49, 50, 55, 60 - 62 contain extraneous marks, smudging, or strike-through. The drawings must be reasonably free from erasures and must be free from alterations, overwriting, interlineations, folds, and copy marks. See 37 CFR 1.84(e). Replacement drawing sheets in compliance with 37 CFR 1.84 and 1.121(d) are required.

Replies must be received in the USPTO within the set time period or must include a proper Certificate of Mailing or Transmission under 37 CFR 1.8 with a mailing or transmission date within the set time period. For more information and a suggested format, see Form PTO/SB/92 and MPEP 512.

Replies should be addressed to:

Mail Stop Missing Parts
Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

REPLY VIA ELECTRONIC FILING

A reply may be submitted via electronic filing only by registered users of the appropriate USPTO patent electronic filing system and must include:

- A separate document having the document description, "**Applicant response to Pre-Exam Formalities Notice**"; and
- the item(s) for which a reply period is set forth above.

For more information about electronic filing, contact the Patent Electronic Business Center (EBC) 1-866-217-9197 or visit https://www.uspto.gov/ebc

NOTE: If the reply is not being submitted electronically, a copy of this notice must be included.

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at **(571) 272-4000** or **(571) 272-4200** or **1-888-786-0101**.

/ygizaw/



United States Patent and Trademark Office

Office of the Chief Financial Officer

Document Code:WFEE

User :Tram Vo

Sale Accounting Date:04/11/2023

Sale Item Reference Number	Effective Date
18154998	01/16/2023

Document Number	Fee Code	Fee Code Description	Amount Paid	Payment Method
I20234A048209501	2081	UTILITY APPL SIZE FEE PER 50 SHEETS >100	$168.00	Deposit Account

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application No.:	18/154,998
Filing Date:	16 January 2023
Applicant:	Sang Jeon CHUNG
Group Art Unit:	To Be Assigned
Confirmation No.:	5143
Examiner:	To Be Assigned
Title:	SITE-SPECIFIC ANTIBODY CONJUGATION AND ANTIBODY-DRUG CONJUGATE AS SPECIFIC EMBODIMENT THEREOF
Attorney Docket:	18896-000072-US-COA

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

16 February 2023

RESPONSE TO NOTICE OF DEFECTIVE SEQUENCE LISTING DATED 17 JANUARY 2023

This paper is in response to the Notice of Defective Sequence Listing dated 17 January 2023 in the above referenced application. This response is timely and no fee is believed due. If a fee is due, authorization is hereby provided to charge any fee due to Deposit Account No. **08-0750**.

* * *

AMENDMENTS to the specification begin on page 2 of this paper.

REMARKS on the present amendment begin on page 3 of this paper.

AMENDMENT TO THE SPECIFICATION

Please replace paragraph [1412] on page 172 with the following paragraph:

[1412] This application contains references to amino acid sequences and/or nucleic acid sequences which have been submitted concurrently herewith as the sequence listing entitled "000072uscoa_SequenceListing.XML", file size **60** **[[62]]** kilobytes (KB), created on **15 February** ~~**16 January**~~ 2023. The aforementioned sequence listing is hereby incorporated by reference in its entirety pursuant to 37 C.F.R. §1.52(e)(5).

RESPONSE TO OFFICE ACTION DATED 17 JANUARY 2023

1. Sequence listing

On 17 January 2023, the USPTO issued a Notice of Defective Sequence Listing for the sequence listing filed on 16 January 2023 contains errors could not be accepted due to errors. Applicant hereby submits a revised Sequence Listing .XML file addressing the errors. The revised sequence listing includes no new matter, as required by 37 CFR §§ 1.821(e), 1.821(f), 1.821(g), 1.824(a)-(d), 1.825(b), or 1.825(d). Applicant requests the revised sequence listing be entered into the application.

2. Conclusion

It is believed that the sequence listing complies with all relevant rules. It is believed that a full and complete response has been made to the outstanding Office Action and that the application is ready for examination. Thus, prompt and favorable consideration of this application is respectfully requested.

Respectfully submitted,

Dated: 16 February 2023

By: / Paul Zarek /
Paul Zarek, Reg. No. 75,190

HARNESS, DICKEY & PIERCE, P.L.C.
7700 Bonhomme, Suite 400
St. Louis, MO 63105
314-446-7668 (telephone)
314-726-7501 (facsimile)

UNITED STATES
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P.O. Box 1450
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ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
18/154,998	**02/16/2023 01:18:37 PM ET**	**18896-000072-US-COA**

Title of Invention

SITE-SPECIFIC ANTIBODY CONJUGATION AND ANTIBODY-DRUG CONJUGATE AS SPECIFIC EMBODIMENT THEREOF

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	5143	FILED BY	Erica Imergoot
PATENT CENTER #	61606905	FILING DATE	-
CUSTOMER #	28997	FIRST NAMED INVENTOR	Sang Jeon CHUNG
CORRESPONDENCE ADDRESS	-	AUTHORIZED BY	Paul Zarek

Documents

TOTAL DOCUMENTS: 4

DOCUMENT		PAGES	DESCRIPTION	SIZE (KB)
000072uscoa_ResponseToNoticeOfDefectiveSequenceListingDated17January2023.PDF		3	-	87 KB
000072uscoa_ResponseToNoticeOfDefectiveSequenceListingDated17January2023-PEFRSEQ.pdf	(1-1)	1	Response to Pre-Exam Sequence Notice	75 KB
000072uscoa_ResponseToNoticeOfDefectiveSequenceListingDated17January2023-SPEC.pdf	(2-2)	1	Specification	72 KB

000072uscoa_ResponseToNoticeOfDefectiveSequenceListingDated17January2023-REM.pdf	(3-3)	1	Applicant Arguments/Remarks Made in an Amendment	74 KB
000072uscoa_SequenceListing.XML		-	Sequence Listing (ST.26 XML File)	61 KB

Warning: Please note that ST.23 and ST.25 sequence listings are required for applications with a filing date before July 1, 2022.

Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)
000072uscoa_ResponseToNoticeOfDefectiveSequenceListingDated17January2023.PDF	28BD7F6B4CE9649CBB1661CDDFEFD7225D7B061C864821DDE3DEF4D9513F0CFFF21733C2B2ECB9537E0D3FC8CA65B12F7DE2BC334A144E9A1C4A4D654597D8C3
000072uscoa_ResponseToNoticeOfDefectiveSequenceListingDated17January2023-PEFRSEQ.pdf	73E3C931CCEFAABB26DF358E6C417B5259AE952524D3CEFBBF0178635FA33A1DF17E8EB15721DB04C00390700F87BE2402B013FCB219A9F33856E0DD1F2DA5E7
000072uscoa_ResponseToNoticeOfDefectiveSequenceListingDated17January2023-SPEC.pdf	5BAEE4512ACBC80378EFEF2BE84C2210CEC33BB2AF743753EBB86BD661D47CB986294A7D1EE2F53B38CE8C436C66D827AA881258703D2BCC8D68F8469C724792
000072uscoa_ResponseToNoticeOfDefectiveSequenceListingDated17January2023-REM.pdf	C5394261EBF52F1B48CE83971E9016715FBF24306A378D78814C543C397C5407CDD82D99B7CA68CBC332BE0F0F9A97F7D6489103E9D1AB9B5FA0918449CBE581
000072uscoa_SequenceListing.XML	B275FA81C8780AF64E35D8EF1328A423C61AAEB0A118C6CA987BFD28606C556CCC9000A932A7541BF8DD9A9FA5508D309FBDE88914F7D8F482D4CF2E5BE31F2E

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 filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Doc Code: TRACK1.REQ
Document Description: TrackOne Request

PTO/AIA/424 (04-14)

CERTIFICATION AND REQUEST FOR PRIORITIZED EXAMINATION UNDER 37 CFR 1.102(e) (Page 1 of 1)

First Named Inventor:	Sang Jeon CHUNG	Nonprovisional Application Number (if known):	Not Yet Assigned
Title of Invention:	SITE-SPECIFIC ANTIBODY CONJUGATION AND ANTIBODY-DRUG CONJUGATE AS SPECIFIC EMBODIMENT THEREOF		

APPLICANT HEREBY CERTIFIES THE FOLLOWING AND REQUESTS PRIORITIZED EXAMINATION FOR THE ABOVE-IDENTIFIED APPLICATION.

1. The processing fee set forth in 37 CFR 1.17(i)(1) and the prioritized examination fee set forth in 37 CFR 1.17(c) have been filed with the request. The publication fee requirement is met because that fee, set forth in 37 CFR 1.18(d), is currently $0. The basic filing fee, search fee, and examination fee are filed with the request or have been already been paid. I understand that any required excess claims fees or application size fee must be paid for the application.
2. I understand that the application may not contain, or be amended to contain, more than four independent claims, more than thirty total claims, or any multiple dependent claims, and that any request for an extension of time will cause an outstanding Track I request to be dismissed.
3. The applicable box is checked below:

I. [x] **Original Application (Track One) - Prioritized Examination under § 1.102(e)(1)**

i. (a) The application is an original nonprovisional utility application filed under 35 U.S.C. 111(a). This certification and request is being filed with the utility application via EFS-Web.

---OR---

(b) The application is an original nonprovisional plant application filed under 35 U.S.C. 111(a). This certification and request is being filed with the plant application in paper.

ii. An executed inventor's oath or declaration under 37 CFR 1.63 or 37 CFR 1.64 for each inventor, **or** the application data sheet meeting the conditions specified in 37 CFR 1.53(f)(3)(i) is filed with the application.

II. [ ] **Request for Continued Examination - Prioritized Examination under § 1.102(e)(2)**

i. A request for continued examination has been filed with, or prior to, this form.
ii. If the application is a utility application, this certification and request is being filed via EFS-Web.
iii. The application is an original nonprovisional utility application filed under 35 U.S.C. 111(a), or is a national stage entry under 35 U.S.C. 371.
iv. This certification and request is being filed prior to the mailing of a first Office action responsive to the request for continued examination.
v. No prior request for continued examination has been granted prioritized examination status under 37 CFR 1.102(e)(2).

Signature	/Kisuk Lee/	Date	January 16, 2023
Name (Print/Typed)	Kisuk Lee	Practitioner Registration Number	66,861

Note: *This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4(d) for signature requirements and certifications. Submit multiple forms if more than one signature is required.**

[ ] *Total of 1 forms are submitted.

PTO/AIA/15 (10-17)
Approved for use through 05/31/2024. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Attorney Docket No.	18896-000072-US-COA
First Named Inventor	Sang Jeon CHUNG
Title	SITE-SPECIFIC ANTIBODY CONJUGATION AND ANTIBODY-DRUG CONJUGATE AS SPECIFIC
Priority Mail Express® Label No.	

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

1. [ ] **Fee Transmittal Form** (PTO/SB/17 or equivalent)
2. [X] **Applicant asserts small entity status.** See 37 CFR 1.27
3. [ ] **Applicant certifies micro entity status.** See 37 CFR 1.29. Applicant must attach form PTO/SB/15A or B or equivalent.
4. [X] **Specification** [*Total Pages* 190]
   Both the claims and abstract must start on a new page.
   (See MPEP § 608.01(a) for information on the preferred arrangement)
5. [X] **Drawing(s)** (35 U.S.C. 113) [*Total Sheets* 73]
6. **Inventor's Oath or Declaration** [*Total Pages* 7]
   (including substitute statements under 37 CFR 1.64 and assignments serving as an oath or declaration under 37 CFR 1.63(e))
   a. [ ] Newly executed (original or copy)
   b. [X] A copy from a prior application (37 CFR 1.63(d))
7. [X] **Application Data Sheet** ** See note below.*
   See 37 CFR 1.76 (PTO/AIA/14 or equivalent)
8. **CD-ROM or CD-R** in duplicate, large table, or Computer Program (*Appendix*)
   [ ] Landscape Table on CD
9. **Nucleotide and/or Amino Acid Sequence Submission** (*if applicable, items a. – c. are required*)
   a. [X] Computer Readable Form (CRF)
   b. [ ] Specification Sequence Listing on:
      i. [ ] CD-ROM or CD-R (2 copies); or
      ii. [ ] Paper
   c. [ ] Statements verifying identity of above copies

ADDRESS TO:

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

ACCOMPANYING APPLICATION PAPERS

10. [X] **Assignment Papers** (cover sheet & document(s))
    Name of Assignee: AbTis Co., Ltd.
11. [X] **37 CFR 3.73(c) Statement** (*when there is an assignee*) [X] **Power of Attorney**
12. [ ] **English Translation Document** (*if applicable*)
13. [X] **Information Disclosure Statement** (PTO/SB/08 or PTO-1449)
    [X] Copies of citations attached
14. [X] **Preliminary Amendment**
15. [ ] **Return Receipt Postcard** (*MPEP § 503*) (*Should be specifically itemized*)
16. [ ] **Certified Copy of Priority Document(s)** (*if foreign priority is claimed*)
17. [ ] **Nonpublication Request** Under 35 U.S.C. 122(b)(2)(B)(i). Applicant must attach form PTO/SB/35 or equivalent.
18. [ ] **Other:**

***Note:** (1) Benefit claims under 37 CFR 1.78 and foreign priority claims under 1.55 **must** be included in an Application Data Sheet (ADS).
(2) For applications filed under 35 U.S.C. 111, the application must contain an ADS specifying the applicant if the applicant is an assignee, person to whom the inventor is under an obligation to assign, or person who otherwise shows sufficient proprietary interest in the matter. See 37 CFR 1.46(b).

19. CORRESPONDENCE ADDRESS

[X] The address associated with Customer Number: 28997 **OR** [ ] Correspondence address below

Name					
Address					
City		State		Zip Code	
Country		Telephone		Email	

Signature	/Kisuk Lee/	Date	January 16, 2023
Name (Print/Type)	Kisuk Lee	Registration No. (Attorney/Agent)	66,861

KL/ei

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	18896-000072-US-COA
	Application Number	
Title of Invention	SITE-SPECIFIC ANTIBODY CONJUGATION AND ANTIBODY-DRUG CONJUGATE AS SPECIFIC EMBODIMENT THEREOF	

The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.
This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.

Secrecy Order 37 CFR 5.2:

☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

Inventor Information:

Inventor 1 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Sang Jeon		CHUNG	

Residence Information (Select One) US Residency ● Non US Residency Active US Military Service

City	Gyeonggi-do	Country of Residence i	KR

Mailing Address of Inventor:

Address 1	60-4, Siheung-daero 145-gil, Geumcheon-gu
Address 2	
City	Seoul
State/Province	
Postal Code	08532
Country i	KR

Inventor 2 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Ju Hwan		KIM	

Residence Information (Select One) US Residency ◉ Non US Residency Active US Military Service

City	Gyeonggi-do	Country of Residence i	KR

Mailing Address of Inventor:

Address 1	#503, 1, Sangtap-ro, Gwonseon-gu, Suwon-si
Address 2	
City	Gyeonggi-do
State/Province	
Postal Code	16603
Country i	KR

Inventor 3 Remove

Legal Name

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	18896-000072-US-COA
	Application Number	
Title of Invention	SITE-SPECIFIC ANTIBODY CONJUGATION AND ANTIBODY-DRUG CONJUGATE AS SPECIFIC EMBODIMENT THEREOF	

Prefix	Given Name	Middle Name	Family Name	Suffix
	Young Geun		LEE	

Residence Information (Select One) US Residency ◉ Non US Residency Active US Military Service

City	Gyeonggi-do	Country of Residence [i]	KR

Mailing Address of Inventor:

Address 1	#202, 66-6, Sangtap-ro, Gwonseon-gu, Suwon-si		
Address 2			
City	Gyeonggi-do	State/Province	
Postal Code	16607	Country [i]	KR

Inventor 4 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Tae Jin		LEE	

Residence Information (Select One) US Residency ◉ Non US Residency Active US Military Service

City	Gyeonggi-do	Country of Residence [i]	KR

Mailing Address of Inventor:

Address 1	#104-501, 2065, Seobu-ro, Jangan-gu, Suwon-si		
Address 2			
City	Gyeonggi-do	State/Province	
Postal Code	16363	Country [i]	KR

Inventor 5 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Jin Woo		SEO	

Residence Information (Select One) US Residency ◉ Non US Residency Active US Military Service

City	Gyeonggi-do	Country of Residence [i]	KR

Mailing Address of Inventor:

Address 1	#204 ,91-7, Guun-ro 45beon-gil, Gwonseon-gu,		
Address 2	Suwon-si		
City	Gyeonggi-do	State/Province	
Postal Code	16408	Country [i]	KR

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	18896-000072-US-COA
	Application Number	
Title of Invention	SITE-SPECIFIC ANTIBODY CONJUGATION AND ANTIBODY-DRUG CONJUGATE AS SPECIFIC EMBODIMENT THEREOF	

All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the **Add** button. Add

Correspondence Information:

Enter either Customer Number or complete the Correspondence Information section below.
For further information see 37 CFR 1.33(a).

☐ **An Address is being provided for the correspondence Information of this application.**

Customer Number	28997		
Email Address	stlmailroom@hdp.com	Add Email	Remove Email

Application Information:

Title of the Invention	SITE-SPECIFIC ANTIBODY CONJUGATION AND ANTIBODY-DRUG CONJUGATE AS SPECIFIC EMBODIMENT THEREOF		
Attorney Docket Number	18896-000072-US-COA	**Small Entity Status Claimed**	☒
Application Type	Nonprovisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	73	**Suggested Figure for Publication (if any)**	

Filing By Reference:

Only complete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., "Domestic Benefit/National Stage Information" and "Foreign Priority Information").

For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

Application number of the previously filed application	Filing date (YYYY-MM-DD)	Intellectual Property Authority or Country

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

☐ **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32).
Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	18896-000072-US-COA
	Application Number	
Title of Invention	SITE-SPECIFIC ANTIBODY CONJUGATION AND ANTIBODY-DRUG CONJUGATE AS SPECIFIC EMBODIMENT THEREOF	

Please Select One:	● Customer Number	US Patent Practitioner	◯ Limited Recognition (37 CFR 11.9)
Customer Number	28997		

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, 365(c), or 386(c) or indicate National Stage entry from a PCT application. Providing benefit claim information in the Application Data Sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.
When referring to the current application, please leave the "Application Number" field blank.

Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
	Continuation of	16/980503	2020-09-14
Prior Application Status			Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
16/980503	a 371 of international	PCT/KR2020/003282	2020-03-09
Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
PCT/KR2020/003282	Claims benefit of provisional	62/815557	2019-03-08

Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the **Add** button. Add

Foreign Priority Information:

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55. When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX)[i] the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(i)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

Remove

Application Number	Country[i]	Filing Date (YYYY-MM-DD)	Access Code[i] (if applicable)

Additional Foreign Priority Data may be generated within this form by selecting the **Add** button. Add

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	18896-000072-US-COA
	Application Number	
Title of Invention	SITE-SPECIFIC ANTIBODY CONJUGATION AND ANTIBODY-DRUG CONJUGATE AS SPECIFIC EMBODIMENT THEREOF	

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.
NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	18896-000072-US-COA
	Application Number	
Title of Invention	SITE-SPECIFIC ANTIBODY CONJUGATION AND ANTIBODY-DRUG CONJUGATE AS SPECIFIC EMBODIMENT THEREOF	

Authorization or Opt-Out of Authorization to Permit Access:

When this Application Data Sheet is properly signed and filed with the application, applicant has provided written authority to permit a participating foreign intellectual property (IP) office access to the instant application-as-filed (see paragraph A in subsection 1 below) and the European Patent Office (EPO) access to any search results from the instant application (see paragraph B in subsection 1 below).

Should applicant choose not to provide an authorization identified in subsection 1 below, applicant **must opt-out** of the authorization by checking the corresponding box A or B or both in subsection 2 below.

NOTE: This section of the Application Data Sheet is **ONLY** reviewed and processed with the **INITIAL** filing of an application. After the initial filing of an application, an Application Data Sheet cannot be used to provide or rescind authorization for access by a foreign IP office(s). Instead, Form PTO/SB/39 or PTO/SB/69 must be used as appropriate.

1. Authorization to Permit Access by a Foreign Intellectual Property Office(s)

A. Priority Document Exchange (PDX) - Unless box A in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the State Intellectual Property Office of the People's Republic of China (SIPO), the World Intellectual Property Organization (WIPO), and any other foreign intellectual property office participating with the USPTO in a bilateral or multilateral priority document exchange agreement in which a foreign application claiming priority to the instant patent application is filed, access to: (1) the instant patent application-as-filed and its related bibliographic data, (2) any foreign or domestic application to which priority or benefit is claimed by the instant application and its related bibliographic data, and (3) the date of filing of this Authorization. See 37 CFR 1.14(h)(1).

B. Search Results from U.S. Application to EPO - Unless box B in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the EPO access to the bibliographic data and search results from the instant patent application when a European patent application claiming priority to the instant patent application is filed. See 37 CFR 1.14(h)(2).

The applicant is reminded that the EPO's Rule 141(1) EPC (European Patent Convention) requires applicants to submit a copy of search results from the instant application without delay in a European patent application that claims priority to the instant application.

2. Opt-Out of Authorizations to Permit Access by a Foreign Intellectual Property Office(s)

☐ A. Applicant **DOES NOT** authorize the USPTO to permit a participating foreign IP office access to the instant application-as-filed. If this box is checked, the USPTO will not be providing a participating foreign IP office with any documents and information identified in subsection 1A above.

☐ B. Applicant **DOES NOT** authorize the USPTO to transmit to the EPO any search results from the instant patent application. If this box is checked, the USPTO will not be providing the EPO with search results from the instant application.

NOTE: Once the application has published or is otherwise publicly available, the USPTO may provide access to the application in accordance with 37 CFR 1.14.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	18896-000072-US-COA
	Application Number	
Title of Invention	SITE-SPECIFIC ANTIBODY CONJUGATION AND ANTIBODY-DRUG CONJUGATE AS SPECIFIC EMBODIMENT THEREOF	

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Applicant 1 — Remove

If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.

Clear

● Assignee	Legal Representative under 35 U.S.C. 117	Joint Inventor
Person to whom the inventor is obligated to assign.	Person who shows sufficient proprietary interest	

If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:

Name of the Deceased or Legally Incapacitated Inventor:

If the Applicant is an Organization check here. ☒

Organization Name	AbTis Co., Ltd.		
Mailing Address Information For Applicant:			
Address 1	#A-815, 142-10, Saneop-ro 156beon-gil,		
Address 2	Gwonseon-gu, Suwon-si,		
City	Gyeonggi-do	**State/Province**	
Country	KR	Postal Code	16648
Phone Number		Fax Number	
Email Address			

Additional Applicant Data may be generated within this form by selecting the Add button. Add

Assignee Information including Non-Applicant Assignee Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	18896-000072-US-COA
	Application Number	
Title of Invention	SITE-SPECIFIC ANTIBODY CONJUGATION AND ANTIBODY-DRUG CONJUGATE AS SPECIFIC EMBODIMENT THEREOF	

Assignee 1

Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication. An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.

Remove

If the Assignee or Non-Applicant Assignee is an Organization check here. ☐

Prefix	**Given Name**	Middle Name	**Family Name**	Suffix

Mailing Address Information For Assignee including Non-Applicant Assignee:

Address 1			
Address 2			
City		**State/Province**	
Country i		Postal Code	
Phone Number		Fax Number	
Email Address			

Additional Assignee or Non-Applicant Assignee Data may be generated within this form by selecting the Add button. Add

Signature:

Remove

NOTE: This Application Data Sheet must be signed in accordance with 37 CFR 1.33(b). **However, if this Application Data Sheet is submitted with the INITIAL filing of the application and either box A or B is not checked in subsection 2 of the "Authorization or Opt-Out of Authorization to Permit Access" section, then this form must also be signed in accordance with 37 CFR 1.14(c).**

This Application Data Sheet **must** be signed by a patent practitioner if one or more of the applicants is a **juristic entity** (e.g., corporation or association). If the applicant is two or more joint inventors, this form must be signed by a patent practitioner, **all** joint inventors who are the applicant, or one or more joint inventor-applicants who have been given power of attorney (e.g., see USPTO Form PTO/AIA/81) on behalf of **all** joint inventor-applicants.

See 37 CFR 1.4(d) for the manner of making signatures and certifications.

Signature	/Kisuk Lee/			Date (YYYY-MM-DD)	2023-01-16
First Name	Kisuk	Last Name	Lee	Registration Number	66,861

Additional Signature may be generated within this form by selecting the Add button. Add

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	18896-000072-US-COA
	Application Number	
Title of Invention	SITE-SPECIFIC ANTIBODY CONJUGATION AND ANTIBODY-DRUG CONJUGATE AS SPECIFIC EMBODIMENT THEREOF	

This collection of information is required by 37 CFR 1.76. 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 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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 the Freedom of Information Act requires disclosure of these records.

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 CooperationTreaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

PTO/AIA/96 (08-12)
Approved for use through 11/30/2020. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

STATEMENT UNDER 37 CFR 3.73(c)

Applicant/Patent Owner: AbTis Co., Ltd.

Application No./Patent No.: To Be Assigned Filed/Issue Date: To Be Assigned

Titled: SITE-SPECIFIC ANTIBODY CONJUGATION AND ANTIBODY-DRUG CONJUGATE AS SPECIFIC EMBODIMENT THEREOF

AbTis Co., Ltd. (Name of Assignee) , a corporation (Type of Assignee, e.g., corporation, partnership, university, government agency, etc.)

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

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

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

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

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

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

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

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

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

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

A. [ ] 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 _____, Frame _____, or for which a copy thereof is attached.

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

1. From: Inventors To: ATBIS CO., LTD. & RESEARCH & BUSINESS FOUNDATION
   The document was recorded in the United States Patent and Trademark Office at Reel 53758, Frame 0520, or for which a copy thereof is attached.
2. From: RESEARCH & BUSINESS FOUNDATION SUNGKYUNKWAN To: ATBIS CO., LTD.
   The document was recorded in the United States Patent and Trademark Office at Reel 58254, Frame 0109, or for which a copy thereof is attached.

KL/ei

PTO/AIA/96 (08-12)
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STATEMENT UNDER 37 CFR 3.73(c)

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

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

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

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

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

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

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

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

/Kisuk Lee/	January 16, 2023
Signature	Date
Kisuk Lee	66,861
Printed or Typed Name	Title or Registration Number

Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet	1	of	1

Complete if Known	
Application Number	Not Yet Assigned
Filing Date	Concurrently Herewith
First Named Inventor	Sang Jeon CHUNG
Art Unit	Not Yet Assigned
Examiner Name	Not Yet Assigned
Attorney Docket Number	18896-000072-US-COA

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (*if known*)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	AA	US-2012-0172575-A1	07-05-2012	BOONS et al.	
	AB	US-2018-0141976-A1	05-24-2018	ITO	Corresponding to KR 10-2018-0002734 A
	AC	US-2020-0190165-A1	06-18-2020	Yamada et al.	Corresponding to WO 2018-199337 A1

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]-Number[4]-Kind Code[5] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
	BA**	CN-107496978-A	12-22-2017	Liu, Yunhui		√
	BB**	EP-3 299 383-A1	03-28-2018	Univ Kagoshima		
	BC**	EP-3617235-A1	03-04-2020	Ajinomoto Co., Inc		
	BD**	KR-10-2018-0002734-A	01-08-2018	Univ Kagoshima		√
	BE**	WO-2018-199337-A1	11-01-2018	Ajinomoto KK		

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. * CITE NO.: Those application(s) which are marked with an single asterisk (*) next to the Cite No. are not supplied (under 37 CFR 1.98(a)(2)(iii)) because that application was filed after June 30, 2003 or is available in the IFW. ** CITE NO.: Those document(s) which are marked with an double asterisk (**) next to the Cite No. are not supplied because they were previously cited by or submitted to the Office in a prior application relied upon in this application for an earlier filing date under 35 U.S.C. 120. [1] Applicant's unique citation designation number (optional). [2] See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [6] Applicant is to place a check mark here if English language Translation is attached.

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	CA**	VAN GEEL, R. et al.; Chemoenzymatic Conjugation of Toxic Payloads to the Globally Conserved NGlycan of Native mAbs Provides Homogeneous and Highly Efficacious AntibodyDrug Conjugates", Bioconjugate Chemistry, 2015, 26, pgs. 2233-2242.	
	CB**	International Search Report from corresponding PCT Application No. PCT/KR2020/003282, dated 16 June 2020.	
	CC**	Written Opinion from corresponding PCT Application No. PCT/KR2020/003282, dated 16 June 2020.	
	CD**	Extended European Search Report from corresponding European Patent Application No. 20770235.8, dated 10 October 2022.	
	CE**	KUROSAKI HIROMASA et al: "Irreversable Inhibition of Metallo-[beta]-lactamase (IMP-1) by 3-(30Mercaptopropionylsulfanyl)propionic Acid Pentafluorophenyl Ester", Angewandte Chemie International Edition, vol. 44, no. 25, 10 June 2005, pp. 3861-3864.	
	CF**	Office Action from corresponding Japanese Patent Application No. 2021-553121, dated 21 October 2022.	
	CG**	Yamaguchi, Y., et al.; "Structure-Function Analysis and Development of Inhibitors of Metallo-β-lactamases Conferring Drug Resistance in Bacteria", Yakugaku Zasshi, vol. 135, no. 11, 2015, pp. 1299-1305.	

Examiner Signature		Date Considered	

KL/ei

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application No.:	To Be Assigned
Filing Date:	16 January 2023
First Named Inventor:	Sang Jeon CHUNG
Group Art Unit:	To Be Assigned
Examiner:	To Be Assigned
Confirmation No.:	To Be Assigned
Title:	SITE-SPECIFIC ANTIBODY CONJUGATION AND ANTIBODY-DRUG CONJUGATE AS SPECIFIC EMBODIMENT THEREOF
Attorney Docket:	18896-000072-US-COA

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

PRELIMINARY AMENDMENT

Prior to examination of the present application, please consider the following:

Amendments to the Claims begin on page **2** of this paper.

Remarks begin on page **8** of this paper.

AMENDMENTS TO THE CLAIMS

The following listing of claims will replace all prior versions and listings of claims in the application.

LISTING OF CLAIMS

1.-53. (Cancelled)

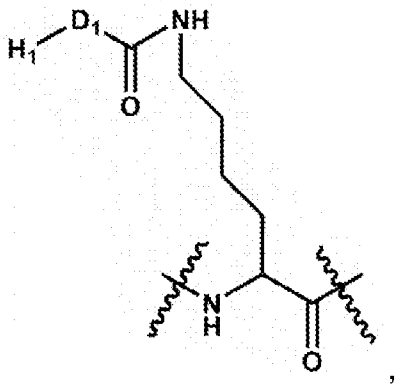
54. (New) A modified antibody or its fragment comprising bio-orthogonal click chemistry functional group,

wherein the modified antibody comprises one or more modified heavy chain,

wherein each of the modified heavy chain comprises a modified lysine residue at 248 position of heavy chain amino acid sequence, wherein the 248 position is determined by an EU numbering system, and

wherein the modified lysine residue comprises a bio-orthogonal click chemistry functional group at terminal end.

55. (New) The modified antibody of claim 54, wherein the modified lysine residue has following structure:



,

wherein H_1 is a group having the bio-orthogonal click chemistry functional group, and

wherein D_1 is bond, or is selected from C_{1-4} alkylene, C_{2-4} alkenylene, C_{2-4} alkynylene and C_{3-8} cycloalkylene.

56. (New) The modified antibody of claim 55, wherein D_1 is bond, or is selected from unsubstituted C_{1-4} alkylene, unsubstituted C_{2-4} alkenylene, unsubstituted C_{2-4} alkynylene and unsubstituted C_{3-8} cycloalkylene.

57. (New) The modified antibody of claim 54, wherein the modified lysine residue does not comprise a terminal bio-reactive functional group.

58. (New) The modified antibody of claim 54, wherein the modified lysine residue does not comprise a terminal bio-reactive functional group selected from terminal hydroxy group, terminal carboxyl group, terminal thiol group, terminal amino group, and terminal aldehyde group.

59. (New) The modified antibody of claim 54, wherein the modified heavy chain comprises an amino acid sequence having a structure of formula 8-2:

[formula 8-2]

G—P—S—V—F—L—F—P—P—K—P-(K)'-D—T—L—M—I,

wherein K' is the modified lysine residue.

60. (New) The modified antibody of claim 55, wherein D_1 comprises one or more heteroatoms.

61. (New) The modified antibody of claim 55,wherein D_1 is $-[CH_2]_a-O-[CH_2]_b-$,

wherein each of a and b is independently selected from 0 to 4, and each of a and b is integer, and

wherein sum of a and b is 0 or more and 4 or less.

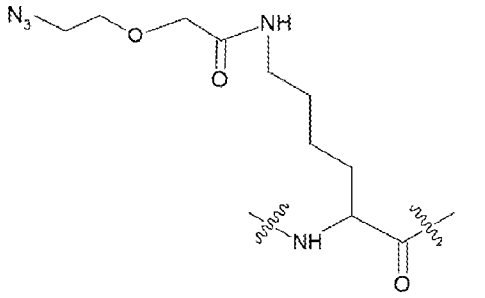
62. (New) The modified antibody of claim 55, wherein D_1 is $-CH_2CH_2OCH_2-$ or $-CH_2OCH_2-$.

63. (New) The modified antibody of claim 54, wherein the modified antibody comprises two modified heavy chains.

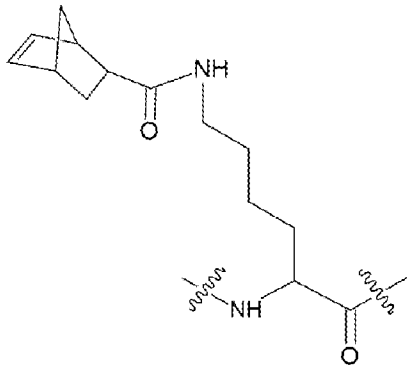
64. (New) The modified antibody of claim 54, wherein the bio-orthogonal click chemistry functional group is a group capable of participating in a click chemistry reaction selected from Huisgen 1,3-dipolar cycloaddition and Diels-Alder reaction.

65. (New) The modified antibody of claim 54, wherein the bio-orthogonal click chemistry functional group is selected from azide group and norbornene group.

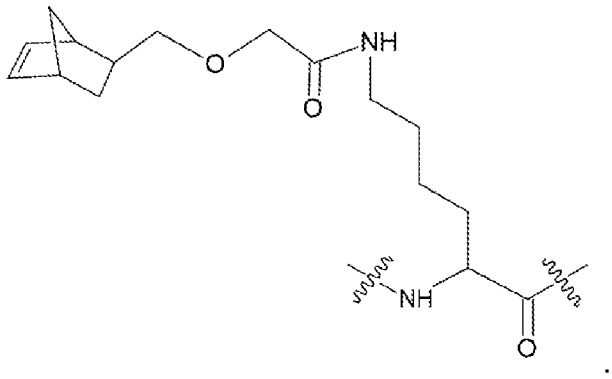
66. (New) The modified antibody of claim 54, wherein the modified lysine residue has following structure:



,



, or



67. (New) A method for preparing antibody-payload conjugate, comprising:

contacting a modified antibody or its fragment comprising a first click chemistry functional group with a payload comprising a cargo unit and a second click chemistry functional group,

wherein the antibody-payload conjugate is formed by the click chemistry reaction between the first click chemistry functional group of the modified antibody and the second click chemistry functional group of the payload,

wherein the modified antibody comprises:

one or more modified heavy chain,

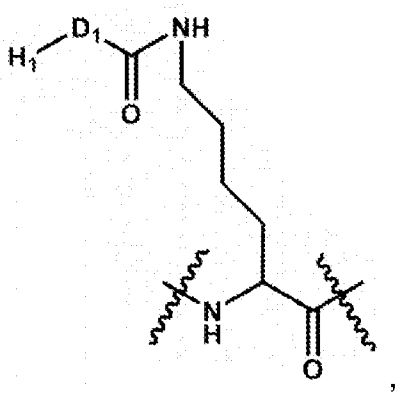
wherein each of the modified heavy chain comprises a modified lysine residue at 248 position of heavy chain amino acid sequence, wherein the 248 position is determined based on EU numbering system,

wherein the modified lysine residue comprises the first click chemistry functional group which is a bio-orthogonal click chemistry functional group at terminal end,

wherein the antibody-payload conjugate comprises an antibody unit derived from the modified antibody and a payload unit derived from the payload,

wherein the payload unit of the antibody-payload conjugate is linked to the antibody unit through K248 of the antibody unit.

68. (New) The method of claim 67, wherein the modified lysine residue has the following structure:



,

wherein H_1 is a group having the bio-orthogonal click chemistry functional group,

wherein D_1 is bond, or is selected from C_{1-4} alkylene, C_{2-4} alkenylene, C_{2-4} alkynylene and C_{3-8} cycloalkylene.

69. (New) The method of claim 68, wherein D_1 is bond, or is selected from unsubstituted C_{1-4} alkylene, unsubstituted C_{2-4} alkenylene, unsubstituted C_{2-4} alkynylene and unsubstituted C_{3-8} cycloalkylene.

70. (New) The method of claim 68, wherein the modified lysine residue does not comprise terminal bio-reactive group selected from terminal hydroxy group, terminal carboxyl group, terminal thiol group, terminal amino group, and terminal aldehyde group.

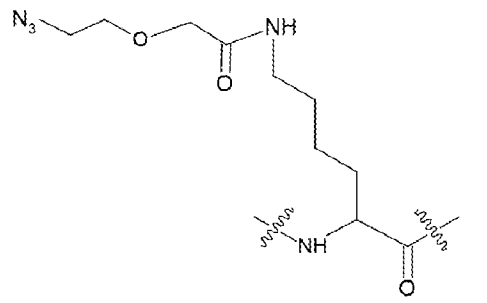
71. (New) The method of claim 68, wherein D_1 comprises one or more heteroatoms.

72. (New) The method of claim 69, wherein D_1 is $-CH_2CH_2OCH_2-$ or $-CH_2OCH_2-$.

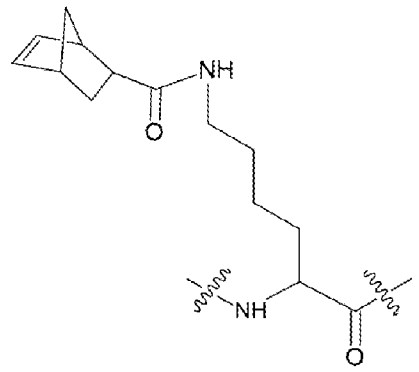
73. (New) The method of claim 68, wherein the bio-orthogonal click chemistry functional group is a group capable of participating in a click chemistry reaction selected from Huisgen 1,3-dipolar cycloaddition and Diels-Alder reaction.

74. (New) The method of claim 68, wherein the bio-orthogonal click chemistry functional group is selected from azide group and norbornene group.

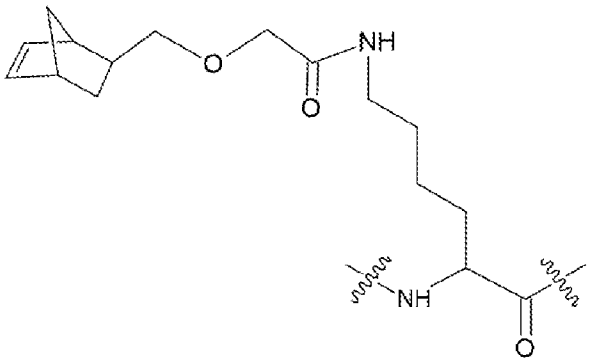
75. (New) The method of claim 68, wherein the modified lysine residue has following structure:



,



, or



.

76. (New) The method of claim 68, wherein the cargo unit comprises a carrier moiety, a fluorescent moiety, a drug moiety, or a radioactive moiety.

REMARKS

Please enter this Preliminary Amendment into the file of the above application prior to examination. Applicant respectfully requests entry of this amendment. The Commissioner is authorized to charge any required fees or credit any overpayment of fees to Deposit Account No. **08-0750**.

If the Examiner believes that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at (314) 446-7670.

Respectfully submitted,

Dated: 16 January 2023

By: /Kisuk Lee/
Kisuk Lee, Reg. No. 66,861

HARNESS, DICKEY & PIERCE, P.L.C.
7700 Bonhomme, Ste. 400
St. Louis, MO 63105
(314) 446-7670 (direct)
(314) 726-7501 (facsimile)

DECLARATION AND ASSIGNMENT[1]

The undersigned acknowledges that this document is being used both as an assignment of the invention and as the declaration (37 CFR 1.63) for a Utility or Design Application entitled:

SITE-SPECIFIC ANTIBODY CONJUGATION AND ANTIBODY-DRUG CONJUGATE AS SPECIFIC EMBODIMENT THEREOF

As a below named inventor, I hereby declare that:

This declaration is directed to (check one):

- ☒ The attached application, or
- ☐ United States application or PCT international application number filed on .

The above-identified application was made or authorized to be made by me.

I believe that I am the original inventor or an original joint inventor of a claimed invention in the above-identified application.

I hereby acknowledge that any willful false statement made in this Declaration and Assignment is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

I hereby state that I have reviewed and understand the contents of the above identified application, including the claims.

I acknowledge that I am aware of the duty to disclose information which is material to patentability as defined in 37 CFR § 1.56.

For good and valuable consideration, the receipt and adequacy whereof I hereby acknowledge, I hereby confirm any prior assignment to **ABTIS CO., LTD.,** #A 815, 142 10, Saneop-ro 156beon-gil, Gwonseon-gu, Suwon-si, Gyeonggi-do, 16648, Republic of Korea and **RESEARCH & BUSINESS FOUNDATION SUNGKYUNKWAN UNIVERSITY,** 2066, Seobu-ro, Jangan-gu, Suwon-si, Gyeonggi-do, 16419, Republic of Korea (hereinafter "Assignees"), and to the extent that I have not already done so, agree to assign, and hereby do, sell, assign and transfer unto Assignees and their successors in interest, the full and exclusive right, title and

[1] This form requires an ADS if one has not yet been filed.

DECLARATION AND ASSIGNMENT

interest in the United States of America and throughout the world, including the right to claim priority under the laws of the United States, the Paris Convention, and any foreign countries, to the inventions as described in the above-identified application, to the above-identified application itself, and all divisions, continuations, continuations-in-part, or other applications claiming priority directly or indirectly from the above-identified application, and any United States or foreign Letters Patent, utility model, or other similar rights which may be granted thereon, including reissues, reexaminations and extensions thereof, and all copyright rights throughout the world in the aforesaid application and the subject matter disclosed therein, these rights, title and interest to be held and enjoyed by Assignees to the full end of the term for which the Letters Patent, utility model, or other similar rights, are granted and any extensions thereof as fully and entirely as the same would have been held by me had this assignment and sale not been made, and the right to sue for, and recover for past infringements of, or liabilities for, any of the rights relating to any of the applications, patents, utility models, or other similar rights, resulting therefrom, and the copyright rights.

I hereby covenant and agree to execute all instruments or documents required or requested for the making and prosecution of any applications of any type for patent, utility model, or other similar rights, and for copyright, in the United States and in all foreign countries including, but not limited to, any provisional, continuation, continuation-in-part, divisional, renewal or substitute thereof, any derivation proceedings relating thereto, and as to Letters Patent any supplemental examination, derivation proceeding, opposition, post grant review, reissue, re-examination, inter partes review, or extension thereof, and for litigation regarding, or for the purpose of protecting title and to the said invention, the United States application for patent, or Letters Patent therefor, and to testify in support thereof, for the benefit of Assignees without further or other compensation than that above set forth.

I hereby covenant that I have not made and will not make any assignment, sale, license, agreement or encumbrance which would conflict with this Declaration and Assignment.

I hereby request the United States Patent and Trademark Office to issue the Letters Patent of the United States of America to Assignees, and request that any official of any country or countries foreign to the United States, whose duty it is to issue or grant patents and applications as aforesaid, to issue the Letters Patent, Utility Model Registration or other similar right to Assignees.

DECLARATION AND ASSIGNMENT

I hereby grant the law firm of Harness, Dickey & Pierce, P.L.C. the power to insert on this Declaration and Assignment any further identification which may be necessary or desirable in order to comply with the rules of the U.S. Patent and Trademark Office for recordation of this document.

Sang Jeon CHUNG

Sang Jeon CHUNG

09/04/20

Dated

Witness

Witness

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Ju Hwan KIM

Ju Hwan KIM

Witness

09/09/20

Dated

Witness

DECLARATION AND ASSIGNMENT

Young Geun Lee
Young Geun LEE

Witness

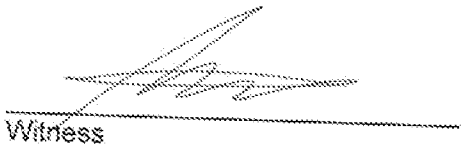
09/04/20
Dated

Witness

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Tae Jin LEE

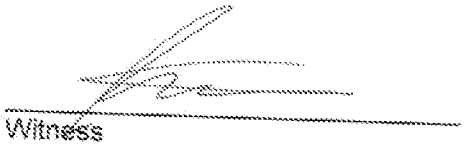
Tae Jin LEE



Witness

09/04/2008

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Jin Woo SEO
Jin Woo SEO

Witness

09/04/20
Dated

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P.O. Box 1450
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ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
18/154,998	**01/16/2023 05:06:02 PM ET**	**18896-000072-US-COA**

Title of Invention

SITE-SPECIFIC ANTIBODY CONJUGATION AND ANTIBODY-DRUG CONJUGATE AS SPECIFIC EMBODIMENT THEREOF

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	5143	FILED BY	Erica Imergoot
PATENT CENTER #	61441378	FILING DATE	-
CUSTOMER #	28997	FIRST NAMED INVENTOR	Sang Jeon CHUNG
CORRESPONDENCE ADDRESS	-	AUTHORIZED BY	Kisuk Lee

Documents

TOTAL DOCUMENTS: 28

DOCUMENT	PAGES	DESCRIPTION	SIZE (KB)
000072uscoa_TrackOneRequest.PDF	1	Track One Request	34 KB
000072uscoa_UtilityPatentApplicationTransmittal.PDF	1	Transmittal of New Application	32 KB
2023-01-16 Application Data Sheet Fillable PDF.pdf	10	Application Data Sheet	2174 KB
000072uscoa_Drawings.PDF	73	Drawings-only black and white line drawings	12942 KB
000072uscoa_POA.PDF	2	Power of Attorney	700 KB

000072uscoa_IDSTransmittal.PDF	2	Transmittal Letter	17 KB
000072uscoa_Form1449.PDF	1	Transmittal Letter	31 KB
2020-09-03 CN107496978A.pdf	11	Foreign Reference	574 KB
EP 3 299 383 A1.PDF	62	Foreign Reference	4235 KB
EP 3617235 A1.PDF	356	Foreign Reference	16738 KB
2020-09-03 KR20180002734A.pdf	65	Foreign Reference	3025 KB
2020-09-03 WO2018199337A1.pdf	532	Foreign Reference	17866 KB
vanGeel.PDF	10	Non Patent Literature	133 KB
ISR.PDF	5	Non Patent Literature	516 KB
WrittenOpinion.PDF	6	Non Patent Literature	731 KB
EP 20770235.PDF	9	Non Patent Literature	341 KB
Kurosaki et al.PDF	4	Non Patent Literature	308 KB
JP 2021-553121_Office Action.PDF	10	Non Patent Literature	216 KB
YAMAGUCHI Y.PDF	7	Non Patent Literature	749 KB
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000072uscoa_PreliminaryAmendment-A.PE.pdf	(1-1)	1	Preliminary Amendment	12 KB
000072uscoa_PreliminaryAmendment-CLM.pdf	(2-7)	6	Claims	83 KB
000072uscoa_PreliminaryAmendment-REM.pdf	(8-8)	1	Applicant Arguments/Remarks Made in an Amendment	12 KB
000072uscoa_Declaration_Assignment.PDF		7	Oath or Declaration filed	573 KB
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2023-01-16 000072uscoa_EnglishSpecification-ABST.pdf	(190-190)	1	Abstract	9 KB
2023-01-16 000072uscoa_Statement3_73c.PDF		2	Miscellaneous Incoming Letter	26 KB

Digest

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New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for 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.

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ELECTRONIC PAYMENT RECEIPT

APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
18/154,998	01/16/2023 05:06:02 PM ET	18896-000072-US-COA

Title of Invention

SITE-SPECIFIC ANTIBODY CONJUGATION AND ANTIBODY-DRUG CONJUGATE AS SPECIFIC EMBODIMENT THEREOF

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	5143	FILED BY	Erica Imergoot
PATENT CENTER #	61441378	AUTHORIZED BY	Kisuk Lee
CUSTOMER #	28997	FILING DATE	-
CORRESPONDENCE ADDRESS	-	FIRST NAMED INVENTOR	Sang Jeon CHUNG

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SITE-SPECIFIC ANTIBODY CONJUGATION AND ANTIBODY-DRUG CONJUGATE AS SPECIFIC EMBODIMENT THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation application of U.S. Patent Application No. 16/980,503, filed on 14 September 2020, which is a national phase application of PCT Application No. PCT/KR2020/003282, filed on March 9, 2020, which claims priority to U.S. Provisional Patent Application No. 62/815,557, filed on March 8, 2019. The entire disclosure of the applications identified in this paragraph is incorporated herein by reference.

FIELD

[0002] The present invention relates to technology for selectively labeling a certain amino acid residue of an antibody with substances (low-molecular-weight compounds, synthetic polymers, biopolymers (i.e., peptides, carbohydrates, proteins, and the like)) or linking a molecule (hereinafter referred to as a "cargo") to be delivered to a certain target cell or tissue to the antibody. Also, the present invention relates to technology for labeling a certain site of an antibody with a desired number of substances or linking a desired number of cargos to certain amino acid residues of the antibody. In addition, the present invention encompasses an antibody complex prepared by the method or a method using a fragment complex of the antibody.

BACKGROUND

[0003] Antibodies are biomolecules that have a function of recognizing certain molecules, and have been used for various industrial applications. For example, an antibody may be used to detect or screen for a certain substance and check a route through which the certain substance moves in the body or cells. Also, the antibody may be used for therapeutic purposes by inducing an immune response against the certain substance.

[0004] To expand the functions of such an antibody, there has been attempts to improve the antibody's ability. Typically, there has been an attempt to label an antibody with foreign substances in order to supplement or expand the antibody's functions. Typically, when an antibody is labeled with a fluorescent substance, the antibody may be used for a fluorescence assay, or when an antibody is labeled with an agent for treating a certain disease, the antibody may be used to maximize a therapeutic effect of the antibody. These attempts and technology are generally referred to as "antibody labeling." The present invention relates to a novel method of labeling an antibody.

[0005] In the beginning, the antibody labeling was achieved by randomly attaching a foreign substance to an antibody. However, this method has a lot of problems. The antibodies thus prepared have a problem in that they have poor homogeneity. These antibodies have a problem in that their effects remain uneven because there are a difference in the number of substances attached to each of the antibodies and a difference in binding sites for the antibodies. This problem is a great barrier to the development of antibody-drug conjugate (ADC) technology that requires high safety and reproducibility.

[0006] Also, the method has a problem in that an antibody's ability to recognize may be significantly degraded. An antibody consists of an Fab domain including an antigen-binding domain that recognizes an antigen, and an Fc domain involved in the crystallization of the antibody. Random labeling resulted in a highly degraded antibody's ability to recognize by allowing a foreign substance to bind to an antigen-binding domain of an antibody or a site adjacent to the antigen-binding domain.

[0007] Therefore, there has been a demand for technology for uniformly labeling an antibody in a site-specific manner in the related art. Although some techniques were developed, e.g., genetically modulating or modifying an antibody and the like, most of them are ineffective in technical and economic aspects. Accordingly, the present invention is designed to solve the problems, and thus is directed to technology capable of specifically linking a certain substance (or a “moiety”) to a certain site of an antibody without any additional modulation of the antibody and delivering a certain substance (a drug or a labeled substance) into cells or tissues using the antibody as well.

SUMMARY

Technical Problem

[0008] The present invention provides technology for specifically transferring a chemical functional group to a certain site of an antibody. In one specific embodiment, the present invention provides technology for specifically transferring a chemical functional group to lysine 246 in the antibody. In another specific embodiment, the present invention provides technology for specifically transferring a chemical functional group to lysine 248 in the antibody. In still another specific embodiment, the present invention provides technology for specifically transferring a chemical functional group to lysine 246 and 248 in the antibody.

[0009] The present invention provides technology capable of linking a desired number of chemical functional groups to an antibody. In one specific embodiment, the present invention provides an antibody or a fragment thereof, which has two certain moieties bound thereto. In

another specific embodiment, the present invention provides an antibody or a fragment thereof, which has four certain moieties bound thereto.

[0010] The present invention provides technology for specifically linking a cargo moiety to a certain site of an antibody. In one specific embodiment, the present invention provides technology for specifically linking a cargo moiety to lysine 246 in the antibody. In another specific embodiment, the present invention provides technology for specifically linking a cargo moiety to lysine 248 in the antibody. In still another specific embodiment, the present invention provides technology for specifically linking a cargo moiety to lysine 246 and 248 in the antibody.

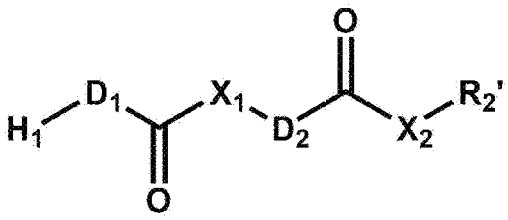
[0011] The present invention provides technology for linking a desired number of cargo moieties to an antibody. In one specific embodiment, the present invention provides an antibody or a fragment thereof, which has two cargo moieties bound thereto. In another specific embodiment, the present invention provides an antibody or a fragment thereof, which has four cargo moieties bound thereto.

[0012] The present invention provides a method of using the aforementioned antibody or antibody fragment. In one specific embodiment, the present invention provides a method of treating a certain disease using an antibody-drug complex.

Technical Solution

[0013] The present application provides A compound of Formula 2:

[0014] [formula 2]



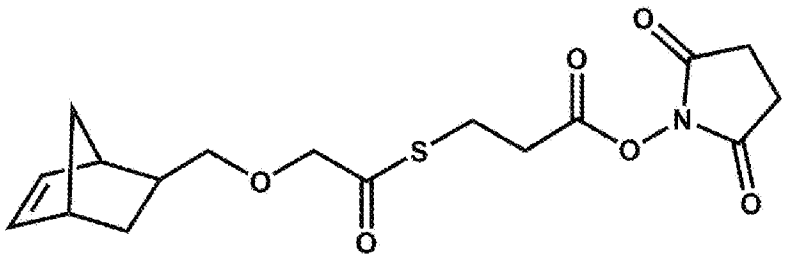
[0015] wherein, H_1 is a first click chemistry functional group, D_1 is selected from a covalent bond, $C_{1\sim4}$alkylene, $C_{2\sim4}$alkenylene, $C_{2\sim4}$alkynlene, and $C_{3\sim8}$cycloalkylene, X_1 is S, D_2 is selected from $C_{1\sim7}$alkylene, $C_{2\sim7}$alkenylene, $C_{2\sim7}$alkynylene, and $C_{3\sim8}$cycloalkynlene, X_2 is O, R_2' is N-succinimide, p-nitrophenyl, or pentafluorophenyl.

[0016] Also, the present application provides the compound wherein H_1 is selected from terminal alkyne, azide, strained alkyne, diene, dienophile, alkene, thiol, and tetrazine. Furthermore, the present application provides the compound wherein H_1 is selected from norbornene, tetrazine, azide and dibenzocyclooctyne-amine.

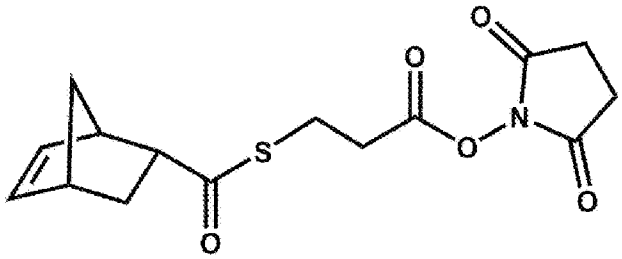
[0017] Additionally, the present application provides the compound wherein R_2' is N-succinimide.

[0018] Additionally, the present application provides the compound wherein the formula 2 is formula 2-1, 2-2, or 2-3:

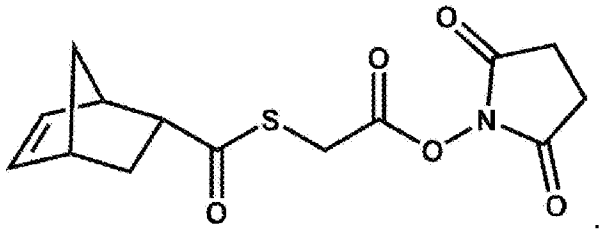
[0019] [formula 2-1]



[0020] [formula 2-2]



[0021] [formula 2-3]

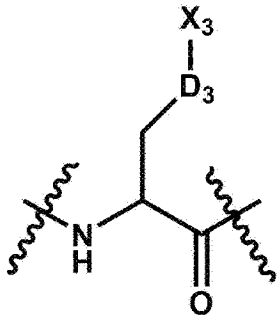


[0022] the present application provides a peptide of formula 4-2:

[0023] [formula 4-2]

$$(Xaa)_{1-3}\text{-}C\text{-}(Xaa)_2\text{-}H\text{-}Xa_1\text{-}G\text{-}Xa_2\text{-}L\text{-}V\text{-}Xa_3\text{-}C\text{-}(Xaa)_{1-3}$$

[0024] wherein, each Xaa is independently any amino acid residue that is not a cysteine residue, C is a cysteine residue, H is a histidine residue, G is a glycine residue, Xa_2 is a glutamic acid residue or an asparagine residue, L is a leucine residue, V is a valine residue, Xa_3 is selected from a tryptophan residue, a naphthylalanine residue, and a phenylalanine residue, and Xa_1 is



, wherein D_3 is a covalent bond or $C_{1\sim3}$alkylene and X_3 is NH_2, wherein the peptide consists of 13 to 17 amino acid residues, wherein the peptide exhibits the activity of binding to human immunoglobulin G (IgG), and wherein the cysteine residue that is between two to four amino acids from the N-terminus of formula 4-2 and the cysteine residue that is between two to four amino acids from the C-terminus of formula 4-2 are optionally linked.

[0025] Also, the present application provides the peptide wherein D_3 is a covalent bond, methylene, or ethylene

[0026] Additionally, the present application provides the peptide wherein the formula 4-2 is formula 4-6:

[0027] [formula 4-6]

$$\mathbf{D\text{-}C\text{-}A\text{-}W\text{-}H\text{-}Xa_1\text{-}G\text{-}E\text{-}L\text{-}V\text{-}W\text{-}C\text{-}T}$$

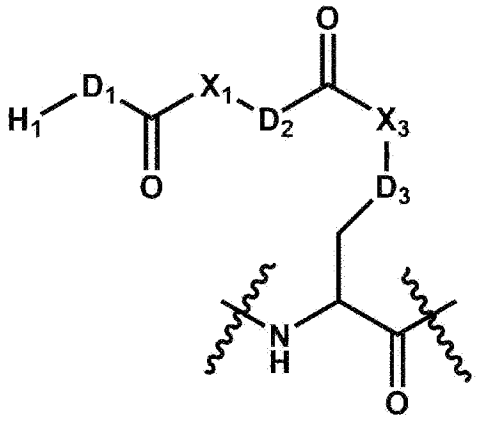
[0028] wherein, D is an aspartic acid residue, A is an alanine residue, E is a glutamic acid residue, W is a tryptophan residue, and T is a threonine residue.

[0029] The present application provides a peptide-compound conjugate of formula 6-2:

[0030] [formula 6-2]

$$\mathbf{(Xaa)_{1\text{-}3}\text{-}C\text{-}(Xaa)_2\text{-}H\text{-}(Xa_1)'\text{-}G\text{-}Xa_2\text{-}L\text{-}V\text{-}Xa_3\text{-}C\text{-}(Xaa)_{1\text{-}3}}$$

[0031] wherein, each Xaa is independently any amino acid residue that is not a cysteine residue, C is a cysteine residue, H is a histidine residue, G is a glycine residue, Xa_2 is a glutamic acid residue or an asparagine residue, L is a leucine residue, V is a valine residue, Xa_3 is selected from a tryptophan residue, a naphthylalanine residue, and a phenylalanine residue, and



[0032] $(Xa_1)'$ is , wherein H_1 is a first click chemistry functional group, D_1 is selected from a covalent bond, $C_{1\sim4}$alkylene, $C_{2\sim4}$alkenylene, $C_{2\sim4}$alkynylene, and $C_{3\sim8}$cycloalkynlene, X_1 is S, D_2 is selected from $C_{1\sim7}$alkylene, $C_{2\sim7}$alkenylene, $C_{2\sim7}$alkynylene, and $C_{3\sim8}$cycloalkynlene, D_3 is a covalent bond, or $C_{1\sim3}$alkylene, and X_3 is NH, wherein the peptide consists of 13 to 17 amino acid residues, wherein the peptide exhibits the activity of binding to human immunoglobulin G (IgG), and wherein the cysteine residue that is between two to four amino acids from the N-terminus of formula 6-2 and the cysteine residue that is between two to four amino acids from the C-terminus of formula 6-2 are optionally linked.

[0033] Also, the present application provides the peptide-compound conjugate wherein a distance from a beta carbon of the $(Xa_1)'$ to a first carbonyl carbon of the $(Xa_1)'$ is less than approx. 11.668Å.

[0034] Additionally, the present application provides the peptide-compound conjugate wherein D_2 is $C_y$alkylene, $C_y$alkenylene, or $C_y$alkynylene, D_3 is $C_x$alkylene, wherein y is an integer greater than or equal to 1, and $1 \leq x+y \leq 5$.

[0035] Additionally, the present application provides the peptide-compound conjugate wherein a distance from a beta carbon of the $(Xa_1)'$ to a first carbonyl carbon of the $(Xa_1)'$ is greater than approx. 16.208Å.

[0036] Additionally, the present application provides the peptide-compound conjugate wherein D_2 is $C_y$alkylene, $C_y$alkenylene, or $C_y$alkynylene, and D_3 is $C_x$alkylene, wherein y is an integer greater than or equal to 1, and $9 \leq x+y \leq 12$. Furthermore, the present application provides the peptide-compound conjugate wherein D_2 is $C_y$alkenylene, or $C_y$alkynylene.

[0037] Additionally, the present application provides the peptide-compound conjugate wherein a distance from a beta carbon of the $(Xa_1)'$ to a first carbonyl carbon of the $(Xa_1)'$ is approx. 11.668Å to approx. 16.208Å.

[0038] Additionally, the present application provides the peptide-compound conjugate wherein D_2 is $C_y$alkylene, $C_y$alkenylene, or $C_y$alkynylene, and D_3 is $C_x$alkylene, wherein y is an

integer greater than or equal to 1, and $6 \leq x+y \leq 8$.

[0039] Additionally, the present application provides the peptide-compound conjugate wherein the formula 6-2 is formula 6-3:

[0040] [formula 6-3]

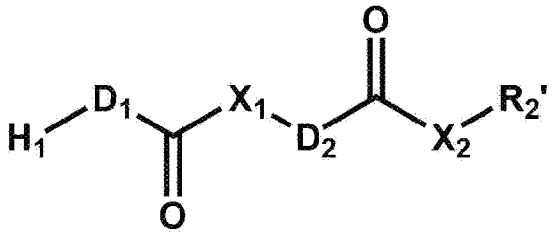
$$D\text{-}C\text{-}A\text{-}W\text{-}H\text{-}(Xa_1)'\text{-}G\text{-}E\text{-}L\text{-}V\text{-}W\text{-}C\text{-}T$$

[0041] wherein, D is an aspartic acid residue, A is an alanine residue, E is a glutamic acid residue, W is a tryptophan residue, T is a threonine residue.

[0042] Additionally, the present application provides the peptide-compound conjugate wherein D_1 is a covalent bond and D_2 is methylene.

[0043] The present application provides a method for preparing an agent for transferring a first click chemical functional group to an antibodycomprising reacting a compound of formula 2:

[0044] [formula 2]

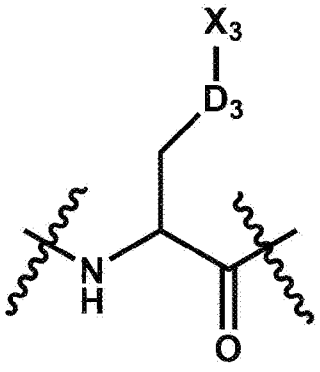


[0045] wherein, H_1 is a first click chemistry functional group, D_1 is selected from a covalent bond, $C_{1\sim4}$alkylene, $C_{2\sim4}$alkenylene, $C_{2\sim4}$alkynylene, and $C_{3\sim8}$cycloalkynlene, X_1 is S, D_2 is selected from $C_{1\sim7}$alkylene, $C_{2\sim7}$alkenylene, $C_{2\sim7}$alkynylene, and $C_{3\sim8}$cycloalkynlene, X_2 is O, R_2' is N-succinimide, p-nitrophenyl, or pentafluorophenyl, with a peptide of formula 4-2:

[0046] [formula 4-2]

$$(Xaa)_{1\text{-}3}\text{-}C\text{-}(Xaa)_2\text{-}H\text{-}Xa_1\text{-}G\text{-}Xa_2\text{-}L\text{-}V\text{-}Xa_3\text{-}C\text{-}(Xaa)_{1\text{-}3}$$

[0047] wherein, each Xaa is independently any amino acid residue that is not a cysteine residue, C is a cysteine residue, H is a histidine residue, G is a glycine residue, Xa_2 is a glutamic acid residue or an asparagine residue, L is a leucine residue, V is a valine residue, Xa_3 is selected from a tryptophan residue, a naphthylalanine residue, and a phenylalanine residue, and



[0048] Xa_1 is , wherein D_3 is a covalent bond or $C_{1\sim3}$alkylene and X_3 is NH_2, wherein the peptide consists of 13 to 17 amino acid residues, wherein the peptide exhibits the activity of binding to human immunoglobulin G (IgG), and wherein the cysteine residue that is between two to four amino acids from the N-terminus of formula 4-2 and the cysteine residue that is between two to four amino acids from the C-terminus of formula 4-2 are optionally linked.

[0049] Also, the present application provides the method for preparing an agent for transferring a first click chemical functional group to an antibodywherein D_2 is $C_y$alkylene, $C_y$alkenylene, or $C_y$alkynylene, and D_3 is $C_x$alkylene, wherein y is an integer greater than or equal to 1, and $1 \leq x+y \leq 5$, characterized in that the agent for transferring a first click chemical functional group to an antibodyprepared by the method can react with an antibody to deliver the first click chemistry functional group specifically to a 248 a lysine residue of an Fc domain of the antibody.

[0050] Additionally, he present application provides the method for preparing an agent for transferring a first click chemical functional group to an antibodywherein D_2 is $C_y$alkylene, $C_y$alkenylene, or $C_y$alkynylene, and D_3 is $C_x$alkylene, wherein y is an integer greater than or equal to 1, and $1 \leq x+y \leq 5$, characterized in that the agent for transferring a first click chemical functional group to an antibodyprepared by the method can react with an antibody to deliver the first click chemistry functional group specifically to a 248 a lysine residue of an Fc domain of the antibody.

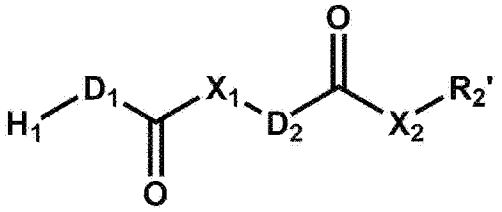
[0051] Additionally, the present application provides the method for preparing an agent for transferring a first click chemical functional group to an antibodywherein D_2 is $C_y$alkylene, $C_y$alkenylene, or $C_y$alkynylene, and D_3 is $C_x$alkylene, wherein y is an integer greater than or equal to 1, and $9 \leq x+y \leq 12$, characterized in that the agent for transferring a first click chemical functional group to an antibodyprepared by the method can react with an antibody to deliver the first click chemistry functional group specifically to a 246 a lysine residue of an Fc domain of the antibody.

[0052] Additionally, the present application provides the method for preparing an agent for transferring a first click chemical functional group to an antibodywherein D_2 is $C_y$alkylene, $C_y$alkenylene, or $C_y$alkynylene, and D_3 is $C_x$alkylene, wherein y is an integer greater than or equal

to 1, and 6≤x+y≤8, characterized in that the agent for transferring a first click chemical functional group to an antibodyprepared by the method can react with an antibody to deliver the first click chemistry functional group selectively to a 246 a lysine residue or a 248 a lysine residue of an Fc domain of the antibody

[0053] The present application provides a kit for preparing a first click chemistry functional group transferring to an antibody comprising a compound of formula 2:

[0054] [formula 2]

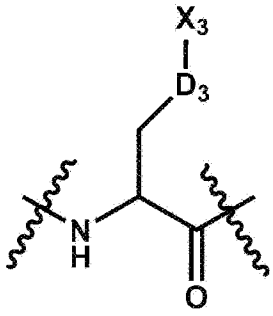


[0055] wherein, H_1 is a first click chemistry functional group, D_1 is selected from a covalent bond, $C_{1\sim4}$alkylene, $C_{2\sim4}$alkenylene, $C_{2\sim4}$alkynylene, and $C_{3\sim8}$cycloalkynlene, X_1 is S, D_2 is selected from $C_{1\sim7}$alkylene, $C_{2\sim7}$alkenylene, $C_{2\sim7}$alkynylene, and $C_{3\sim8}$cycloalkynlene, X_2 is O, R_2' is N-succinimide, p-nitrophenyl, or pentafluorophenyl; and a peptide of formula 4-2:

[0056] [formula 4-2]

$$\mathbf{(Xaa)_{1\text{-}3}\text{-}C\text{-}(Xaa)_2\text{-}H\text{-}Xa_1\text{-}G\text{-}Xa_2\text{-}L\text{-}V\text{-}Xa_3\text{-}C\text{-}(Xaa)_{1\text{-}3}}$$

[0057] wherein, each Xaa is independently any amino acid residue that is not a cysteine residue, C is a cysteine residue, H is a histidine residue, G is a glycine residue, Xa_2 is a glutamic acid residue or an asparagine residue, L is a leucine residue, V is a valine residue, Xa_3 is selected from a tryptophan residue, a naphthylalanine residue, and a phenylalanine residue, and

[0058] Xa_1 is 

, wherein D_3 is a covalent bond or $C_{1\sim3}$alkylene and X_3 is NH_2, wherein the peptide consists of 13 to 17 amino acid residues, wherein the peptide exhibits the activity of binding to human immunoglobulin G (IgG), and wherein the cysteine residue that is between two to four amino acids from the N-terminus of formula 4-2 and the cysteine residue that is between two to four amino acids from the C-terminus of formula 4-2 are optionally linked..

[0059] The present application provides an antibody or a fragment thereof comprising one or more amino acid sequence selected from formula 8-1, formula 8-2, and formula 8-3:

[0060] [formula 8-1]

G—P—S—V—F—L—F—P—P-(K)'-P—K—D—T—L—M—I

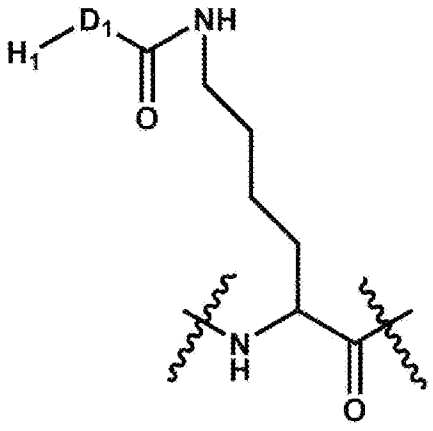
[0061] [formula 8-2]

G—P—S—V—F—L—F—P—P—K—P-(K)'-D—T—L—M—I

[0062] [formula 8-3]

G—P—S—V—F—L—F—P—P-(K)'-P-(K)'-D—T—L—M—I

[0063] wherein, G is a glycine residue, P is a proline residue, S is a serine residue, V is a valine residue, F is a phenylalanine residue, L is a leucine residue, K is a lysine residue, D is an aspartic acid residue, T is a threonine residue, M is a methionine residue, I is an isoleucine residue, and

[0064] (K)' is 

, wherein H_1 is a first click chemistry functional group, D_1 is selected from a covalent bond, $C_{1\sim4}$alkylene, $C_{2\sim4}$alkenylene, $C_{2\sim4}$alkynylene, and $C_{3\sim8}$cycloalkynlene.

[0065] Also, the present application provides the antibody or a fragment thereof wherein D_1 is a covalent bond.

[0066] Additionally, the present application provides the antibody or a fragment thereof comprising the amino acid sequence of formula 8-1 and not comprising the amino acid sequence of formula 8-2 and 8-3. Furthermore, the present application provides the antibody or a fragment thereof comprising the amino acid sequence of formula 8-1 in both two Fc domains.

[0067] Additionally, the present application provides the antibody or a fragment thereof comprising the amino acid sequence of formula 8-2 and not comprising the amino acid sequence of formula 8-1 and 8-3. Furthermore, the present application provides the antibody or a fragment thereof comprising the amino acid sequence of formula 8-2 in both two Fc domains

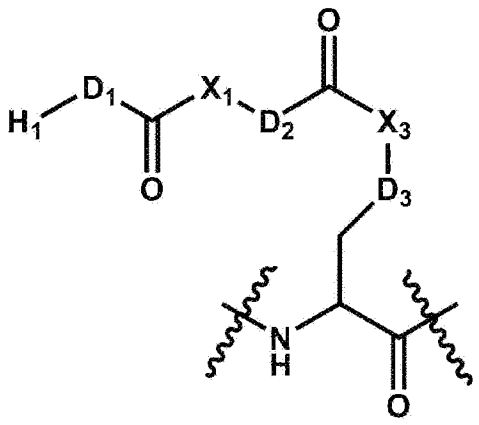
[0068] Additionally, the present application provides the antibody or a fragment thereof comprising the amino acid sequence of formula 8-3 and not comprising the amino acid sequence of formula 8-1 and 8-2. Furthermore, the present application provides the antibody or a fragment thereof comprising the amino acid sequence of formula 8-3 in both two Fc domains.

[0069] The present application provides a method for preparing an antibody or a fragment thereof comprising first click chemistry functional group comprising reacting a peptide-compound conjugate of formula 6-2:

[0070] [formula 6-2]

$$(Xaa)_{1-3}\text{-}C\text{-}(Xaa)_2\text{-}H\text{-}(Xa_1)'\text{-}G\text{-}Xa_2\text{-}L\text{-}V\text{-}Xa_3\text{-}C\text{-}(Xaa)_{1-3}$$

[0071] wherein, each Xaa is independently any amino acid residue that is not a cysteine residue, C is a cysteine residue, H is a histidine residue, G is a glycine residue, Xa_2 is a glutamic acid residue or an asparagine residue, L is a leucine residue, V is a valine residue, Xa_3 is selected from a tryptophan residue, a naphthylalanine residue, and a phenylalanine residue, and

[0072] $(Xa_1)'$ is  , wherein H_1 is a first click chemistry functional group, D_1 is selected from a covalent bond, $C_{1\sim4}$alkylene, $C_{2\sim4}$alkenylene, $C_{2\sim4}$alkynylene, and $C_{3\sim8}$cycloalkynlene, X_1 is S, D_2 is selected from $C_{1\sim7}$alkylene, $C_{2\sim7}$alkenylene, $C_{2\sim7}$alkynylene, and $C_{3\sim8}$cycloalkynlene, D_3 is a covalent bond or $C_{1\sim3}$alkylene and X_3 is NH, wherein the peptide consists of 13 to 17 amino acid residues, wherein the peptide exhibits the activity of binding to human immunoglobulin G (IgG), and wherein the cysteine residue that is between two to four amino acids from the N-terminus of formula 6-2 and the cysteine residue that is between two to four amino acids from the C-terminus of formula 6-2 are optionally linked, with an antibody or a fragment thereof.

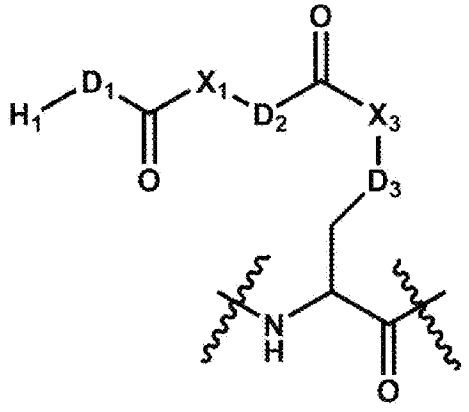
[0073] The present application provides a kit for preparing an antibody or a fragment thereof comprising first click chemistry functional group comprising

[0074] a peptide-compound conjugate of formula 6-2:

[0075] [formula 6-2]

$$(Xaa)_{1-3}\text{-}C\text{-}(Xaa)_2\text{-}H\text{-}(Xa_1)'\text{-}G\text{-}Xa_2\text{-}L\text{-}V\text{-}Xa_3\text{-}C\text{-}(Xaa)_{1-3}$$

[0076] wherein, each Xaa is independently any amino acid residue that is not a cysteine residue, C is a cysteine residue, H is a histidine residue, G is a glycine residue, Xa_2 is a glutamic acid residue or an asparagine residue, L is a leucine residue, V is a valine residue, W is a tryptophan residue, and



[0077] $(Xa_1)'$ is , wherein H_1 is a first click chemistry functional group, D_1 is selected from a covalent bond, $C_{1\sim4}$alkylene, $C_{2\sim4}$alkenylene, $C_{2\sim4}$alkynylene, and $C_{3\sim8}$cycloalkynlene, X_1 is S, D_2 is selected from $C_{1\sim7}$alkylene, $C_{2\sim7}$alkenylene, $C_{2\sim7}$alkynylene, and $C_{3\sim8}$cycloalkynlene, D_3 is a covalent bond or $C_{1\sim3}$alkylene and X_3 is NH, wherein the peptide consists of 13 to 17 amino acid residues, wherein the peptide exhibits the activity of binding to human immunoglobulin G (IgG), and wherein the cysteine residue that is between two to four amino acids from the N-terminus of formula 6-2 and the cysteine residue that is between two to four amino acids from the C-terminus of formula 6-2 are optionally linked; and an antibody or a fragment thereof.

[0078] The present application provides a compound of formula 9:

[0079] [formula 9]

$$C_m\text{———}H_2$$

[0080] wherein, C_m is a cargo moiety, H_2 is a second click chemistry functional group.

[0081] The present application provides a method for preparing an antibody-drug conjugate comprising reacting an antibody or a fragment thereof comprising one or more amino acid sequence selected from formula 8-1, formula 8-2, and formula 8-3:

[0082] [formula 8-1]

$$G\text{—}P\text{—}S\text{—}V\text{—}F\text{—}L\text{—}F\text{—}P\text{—}P\text{-}(K)'\text{-}P\text{—}K\text{—}D\text{—}T\text{—}L\text{—}M\text{—}I$$

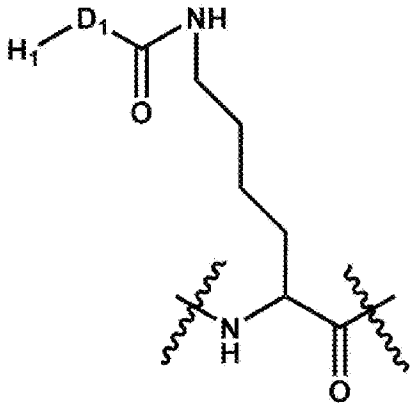
[0083] [formula 8-2]

G—P—S—V—F—L—F—P—P—K—P-(K)'-D—T—L—M—I

[0084] [formula 8-3]

G—P—S—V—F—L—F—P—P-(K)'-P-(K)'-D—T—L—M—I

[0085] wherein, G is a glycine residue, P is a proline residue, S is a serine residue, V is a valine residue, F is a phenylalanine residue, L is a leucine residue, K is a lysine residue, D is an aspartic acid residue, T is a threonine residue, M is a methionine residue, I is an isoleucine residue, and

[0086] (K)' is 

, wherein H_1 is a first click chemistry functional group, D_1 is selected from a covalent bond, $C_{1\sim4}$alkylene, $C_{2\sim4}$alkenylene, $C_{2\sim4}$alkynylene, and $C_{3\sim8}$cycloalkynlene, with a compound of formula 9:

[0087] [formula 9]

C_m———H_2

[0088] wherein, C_m is a cargo moiety, H_2 is a second click chemistry functional group which is complementary with the first click chemistry functional group.

[0089] The present application provides a kit for preparing an antibody-drug conjugate comprising an antibody or a fragment thereof comprising one or more amino acid sequence selected from formula 8-1, formula 8-2, and formula 8-3:

[0090] [formula 8-1]

G—P—S—V—F—L—F—P—P-(K)'-P—K—D—T—L—M—I

[0091] [formula 8-2]

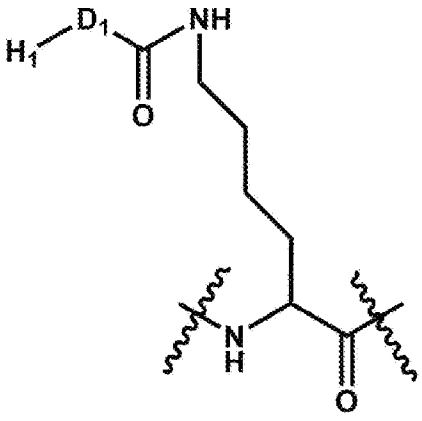
G—P—S—V—F—L—F—P—P—K—P-(K)'-D—T—L—M—I

[0092] [formula 8-3]

G—P—S—V—F—L—F—P—P-(K)'-P-(K)'-D—T—L—M—I

[0093] wherein, G is a glycine residue, P is a proline residue, S is a serine residue, V is a

valine residue, F is a phenylalanine residue, L is a leucine residue, K is a lysine residue, D is an aspartic acid residue, T is a threonine residue, M is a methionine residue, I is an isoleucine residue, and

[0094] (K)' is  , wherein H_1 is a first click chemistry functional group, D_1 is selected from a covalent bond, $C_{1\sim4}$alkylene, $C_{2\sim4}$alkenylene, $C_{2\sim4}$alkynylene, and $C_{3\sim8}$cycloalkynlene; and a compound of formula 9:

[0095] [formula 9]



[0096] wherein, C_m is a cargo moiety, H_2 is a second click chemistry functional group which is complementary with the first click chemistry functional group.

[0097] The present application provides an antibody or a fragment thereof comprising one or more amino acid sequence selected from formula 10-1, formula 10-2, and formula 10-3:

[0098] [formula 10-1]

G—P—S—V—F—L—F—P—P-(K)"-P—K—D—T—L—M—I

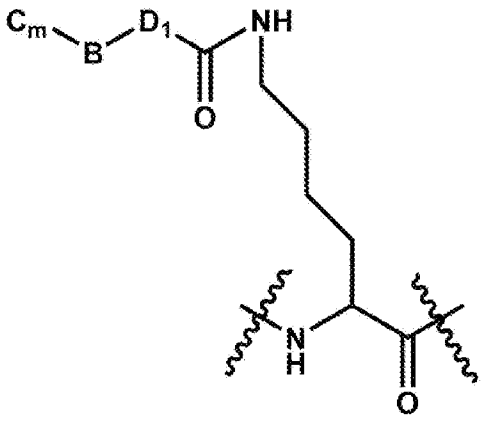
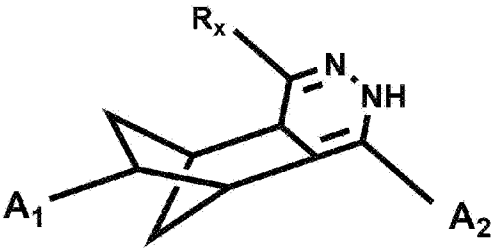
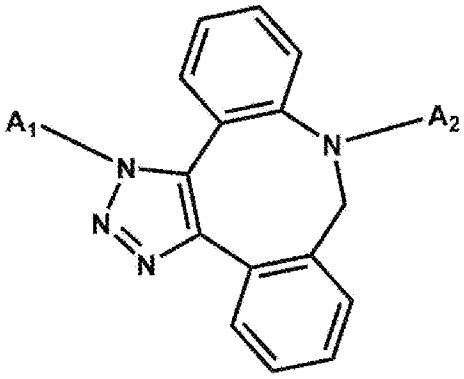
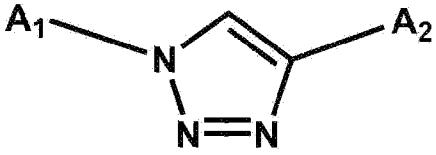
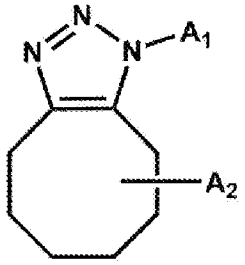
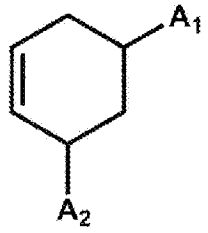
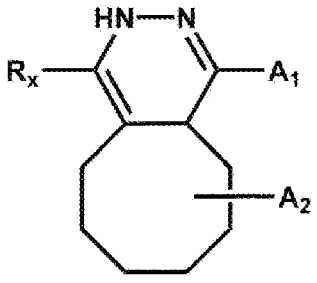
[0099] [formula 10-2]

G—P—S—V—F—L—F—P—P—K—P-(K)"-D—T—L—M—I

[0100] [formula 10-3]

G—P—S—V—F—L—F—P—P-(K)"-P-(K)"-D—T—L—M—I

[0101] wherein, G is a glycine residue, P is a proline residue, S is a serine residue, V is a valine residue, F is a phenylalanine residue, L is a leucine residue, K is a lysine residue, D is an aspartic acid residue, T is a threonine residue, M is a methionine residue, I is an isoleucine residue, and

[0102] (K)" is  , wherein C_m is a cargo moiety, B is selected from  ,  ,  ,  ,  ,  , and  , wherein A_1 and A_2 is connected to the cargo moiety or D_1 and they are not both connected to same, R_x is selected from H, halogen, and $C_{1\sim3}$alkyl, D_1 is selected from a covalent bond, $C_{1\sim4}$alkylene, $C_{2\sim4}$alkenylene, $C_{2\sim4}$alkynylene, and $C_{3\sim8}$cycloalkynlene.

[0103] Also, the present application provides the antibody or a fragment thereof comprising the amino acid sequence of formula 10-1 and not comprising the amino acid sequence of formula 10-2 and 10-3. Furthermore, the present application provides the antibody or a

fragment thereof comprising the amino acid sequence of formula 10-1 in both two Fc domains.

[0104] Additionally, the present application provides the antibody or a fragment thereof comprising the amino acid sequence of formula 10-2 and not comprising the amino acid sequence of formula 10-1 and 10-3. Furthermore, the present application provides the antibody or a fragment thereof comprising the amino acid sequence of formula 10-2 in both two Fc domains.

[0105] Additionally, the present application provides the antibody or a fragment thereof comprising the amino acid sequence of formula 10-3 and not comprising the amino acid sequence of formula 10-1 and 10-2. Furthermore, the present application provides the antibody or a fragment thereof comprising the amino acid sequence of formula 10-3 in both two Fc domains.

[0106] Additionally, the present application provides the antibody or a fragment thereof wherein the cargo moiety comprises a drug moiety. Further, the present application provides the antibody or a fragment thereof wherein the cargo moiety comprises more than one drug moiety. Or further, the present application provides the antibody or a fragment thereof wherein the drug moiety is an anticancer agent. Furthermore, the present application provides the antibody or a fragment thereof wherein the anticancer agent is at least one selected from DM1, DM3, DM4, abrin, ricin A, pseudomonas exotoxin, cholera toxin, diphtheria toxin, tumor necrosis factor, α-interferon, β-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, a cytokine, an apoptotic agen, an anti-angiogenic agent, a lymphokine, taxane, a DNA-alkylating agent, anthracycline, tubulysin analogs, duocarmycin analogs, auristatin E, auristatin F, maytansinoid, a cytotoxic agent comprising a reactive polyethylene glycol moiety, taxon, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, t. colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoid, procaine, tetracaine, lidocaine, propranolol, puromycin, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine, mechlorethamine, thiotepachlorambucil, meiphalan, carmustine, lomustine, cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, cisplatin, dactinomycin, bleomycin, anthramycin, calicheamicin, Gemcitabine, bendamustine, bortezomib, carboplatin, cabazitaxel, dasatinib, docetaxel, epirubicin, erlotinib, everolimus, gemcitabine, gefitinib, idarubicin, imatinib, hydroxyurea, lapatinib, leuprorelin, melphalan, nedaplatin, nilotinib, oxaliplatin, pazopanib, pemetrexed, picoplatin, romidepsin, satraplatin, sorafenib, vemurafenib, sunitinib, teniposide, triplatin, and vinorelbine.

[0107] The present application provides a pharmaceutical composition for treating cancer comprising aforementioned antibody-drug conjugate

[0108] Also, the present application provides the pharmaceutical composition for treating

cancer wherein the cancer is selected from bladder cancer, bone cancer, brain tumor, breast cancer, heart cancer, cervical cancer, colorectal cancer, rectal cancer, esophageal cancer, fibrosarcoma, gastric cancer, gastrointestinal cancer, head and neck cancer, Kaposi sarcoma, renal cancer, leukemia, liver cancer, lung cancer, lymphoma, melanoma, myeloma, ovarian cancer, pancreatic cancer, penile cancer, prostate cancer, testicular germ cell cancer, thymoma and thymic carcinoma.

[0109] Additionally, the present application provides the pharmaceutical composition for treating cancer wherein the cancer is breast cancer.

[0110] The present application provides a method for treating cancer comprising administering a pharmaceutical composition comprising aforementioned antibody-drug conjugate to a subject.

[0111] Also, the present application provides the method for treating cancer wherein the cancer is selected from bladder cancer, bone cancer, brain tumor, breast cancer, heart cancer, cervical cancer, colorectal cancer, rectal cancer, esophageal cancer, fibrosarcoma, gastric cancer, gastrointestinal cancer, head and neck cancer, Kaposi sarcoma, renal cancer, leukemia, liver cancer, lung cancer, lymphoma, melanoma, myeloma, ovarian cancer, pancreatic cancer, penile cancer, prostate cancer, testicular germ cell cancer, thymoma and thymic carcinoma.

[0112] Furthermore, the present application provides the method for treating cancer wherein the cancer is breast cancer.

[Advantageous Effects]

[0113] An antibody product according to the present invention can have a certain number of chemical functional groups or cargo moieties labeled at a certain site thereof. Therefore, the present invention can provide an antibody product having high uniformity. Also, the present invention can provide an antibody product whose antibody functions are not degraded. That is, the present invention can provide an antibody product whose antibody binding affinity and half-life are not degraded. The present invention is of great significance as being the first technology allowing site-specific labeling of an antibody without any complicated processes.

BRIEF DESCRIPTION OF THE DRAWINGS

[0114] FIG. 1 shows a partial sequence of an Fc domain and numbers in the sequence numbered according to the EU numbering system.

[0115] FIG. 2 shows positions of lysine residues in an Fc domain including lysine 246 and 248.

[0116] FIG. 3 and FIG. 4 show the topology between SSFI and the Fc domain.

[0117] FIG. 5 shows Xa_1 of the SSFI and lysine 246 and 248 in the Fc domain.

[0118] FIG. 6 shows a distance between an amine group of the lysine 246 and a beta carbon of the Xa_1 of the SSFI.

[0119] FIG. 7 shows a distance between an amine group of the lysine 248 and a beta carbon of the Xa_1 of the SSFI.

[0120] FIG. 8 shows a structure of R_1'-L_2-SSFI and a distance (L_c) between a beta carbon and a first carbonyl carbon of (Xa_1)'.

[0121] FIG. 9 shows the topology between the R1'-L2-SSFI and the Fc domain so that a side chain of (Xa_1)' has a direction (a dotted arrow) parallel with the x axis on the drawing.

[0122] FIG. 10 shows the conditions used to allow the first carbonyl carbon to react well with lysine 248.

[0123] FIG. 11 shows the conditions used to allow the first carbonyl carbon to react well with lysine 246.

[0124] FIG. 12 shows the conditions used to allow the first carbonyl carbon to selectively react with lysine 246 or 248.

[0125] FIG. 13 shows an FcRn binding site and lysine 246 and 248 in the Fc domain.

[0126] FIG. 14 shows the analysis of a binding structure between SSFI and the Fc domain in comparison with a binding site between Fc and of FcRn.

[0127] FIG. 15 shows a method of synthesizing Compound I.

[0128] FIG. 16 shows the results of confirming a structure of Compound I by means of mass spectrometry.

[0129] FIG. 17 shows a method of synthesizing Compound II.

[0130] FIG. 18 shows the results of confirming a structure of Compound II by means of mass spectrometry.

[0131] FIG. 19 shows a method of synthesizing Compound III.

[0132] FIG. 20 shows the results of confirming a structure of Compound III by means of mass spectrometry.

[0133] FIG. 21 and FIG. 22 show a method of synthesizing Compound IV.

[0134] FIG. 23 shows the results of confirming a structure of Compound IV by means of mass spectrometry.

[0135] FIG. 24 shows the results of confirming a structure of SSFI (6Lys) by means of mass spectrometry.

[0136] FIG. 25 shows the results of confirming a structure of SSFI (6Orn) by means of

mass spectrometry.

[0137] FIG. 26 shows the results of confirming a structure of SSFI (6Dab) by means of mass spectrometry.

[0138] FIG. 27 shows the results of confirming a structure of SSFI (6Dap) by means of mass spectrometry.

[0139] FIG. 28 shows the results of confirming a structure of DD2 by means of mass spectrometry.

[0140] FIG. 29 shows the results of confirming a structure of DD3 by means of mass spectrometry.

[0141] FIG. 30 shows the results of confirming a structure of DD4 by means of mass spectrometry.

[0142] FIG. 31 shows the results of confirming a structure of DD5 by means of mass spectrometry.

[0143] FIG. 32 shows the results of confirming a structure of DD6 by means of mass spectrometry.

[0144] FIG. 33 shows the results of confirming a structure of Compound I-SSFI (6Lys) by means of mass spectrometry.

[0145] FIG. 34 shows the results of confirming a structure of Compound II-SSFI (6Lys) by means of mass spectrometry.

[0146] FIG. 35 shows the results of confirming a structure of Compound III-SSFI (6Lys) by means of mass spectrometry.

[0147] FIG. 36 shows the results of confirming a structure of Compound III-SSFI (6Orn) by means of mass spectrometry.

[0148] FIG. 37 shows the results of confirming a structure of Compound III-SSFI (6Dab) by means of mass spectrometry.

[0149] FIG. 38 shows the results of confirming a structure of Compound III-SSFI (6Dap) by means of mass spectrometry.

[0150] FIG. 39 shows the results of confirming a structure of Compound IV-SSFI (6Dap) by means of mass spectrometry.

[0151] FIG. 40 shows the results of observing a binding reaction using trastuzumab and Compound I-SSFI (6Lys) by means of HIC-HPLC.

[0152] FIG. 41 shows the results of observing a binding reaction using trastuzumab and Compound II-SSFI (6Lys) by means of HIC-HPLC.

[0153] FIG. 42 shows a reaction of an antibody with Compound III-SSFI (6Dap, Dab, Orn, or Lys).

[0154] FIG. 43 shows a structure of Ab (246/248)-Norbornene as the final product.

[0155] FIG. 44 shows the results of observing a binding reaction using trastuzumab and Compound III-SSFI (6Dap) by means of HIC-HPLC.

[0156] FIG. 45 shows the results of observing a binding reaction using trastuzumab and Compound III-SSFI (6Dab) by means of HIC-HPLC.

[0157] FIG. 46 shows the results of observing a binding reaction using trastuzumab and Compound III-SSFI (6Orn) by means of HIC-HPLC.

[0158] FIG. 47 shows the results of observing a binding reaction using trastuzumab and Compound III-SSFI (6Lys) by means of HIC-HPLC.

[0159] FIG. 48 shows the results of observing a binding reaction using trastuzumab and Compound IV-SSFI (6Dap) by means of HIC-HPLC.

[0160] FIG. 49 and FIG. 50 show an increase of molecular weight spectra by antibody-norbornene binding.

[0161] FIG. 51, FIG. 52, FIG. 53 and FIG. 54 show the MS/MS chromatogram results of trastuzumab and antibody-norbornene complexes.

[0162] FIG. 55 shows the sequence matching results by means of the MS/MS spectrum.

[0163] FIG. 56 shows the mass spectrum of trastuzumab measured to confirm a trastuzumab-azide structure.

[0164] FIG. 57 shows the mass spectrum of a trastuzumab-azide complex measured to confirm the trastuzumab-azide structure.

[0165] FIG. 58 shows the results of confirming a structure of tetrazine-DM1 by means of mass spectrometry.

[0166] FIG. 59 shows a structure of a Compound III-SSFI (6Dap)-based trastuzumab-DM1 conjugate.

[0167] FIG. 60 shows the results of observing a formation reaction of a trastuzumab-DM1 conjugate by means of HIC-HPLC.

[0168] FIG. 61 and FIG. 62 show an increase of molecular weight spectra by norbornene-tetrazine-DM1 binding.

[0169] FIG. 63 shows structures of three designs of payloads bound to an antibody labeled with norbornene.

[0170] FIG. 64 shows the results of analyzing antigen binding affinity of an antibody-drug

conjugate.

[0171] FIG. 65, FIG. 66 and FIG. 67 show the results of analyzing serum stability of the antibody-drug conjugate.

[0172] FIG. 68, FIG. 69 and FIG. 70 show the results of evaluating a medicinal effect of the antibody-drug conjugate at a cellular level.

[0173] FIG. 71 and FIG. 72 show the results of evaluating a medicinal effect of the antibody-drug conjugate at an animal level.

[0174] FIG. 73 shows the results of a pharmacokinetics test on the antibody-drug conjugate.

DETAILED DESCRIPTION

[0175] Definitions

[0176] Unless otherwise defined, all technical and scientific terms used in the present invention have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. The following references are provided to give a number of common definitions of the terms used in the present invention to those skilled in the related art: Singleton *et al.*, Dictionary of Microbiology and Molecular Biology (2nd ed. 1994); The Cambridge Dictionary of Science and Technology (Walker ed., 1988); The Glossary of Genetics, 5th Ed., R. Rieger *et al.* (eds.), Springer Verlag (1991); and Hale & Marham, The Harper Collins Dictionary of Biology (1991). Unless otherwise clearly specified, the following terms used in the present invention shall have the meanings ascribed to them as follows.

[0177] In some embodiments, a chemical structure is disclosed together with the corresponding chemical name. When the terms contradict or conflict with each other, the chemical structure takes precedence over the chemical name to understand the meaning of a compound through the chemical structure.

[0178] The term “hetero” used in the present invention refers to a compound or a group of compounds including at least one heteroatom. The term “heteroatom” refers to an atom other than a carbon or hydrogen atom, and, for example, includes B, Si, N, P, O, S, and Se. Preferably, the heteroatom includes, among others, polyvalent elements such as N, O, and S, or monovalent elements such as F, Cl, Br, and I, but the present invention is not limited thereto.

[0179] The term “lower” used in the present invention is used to modify hydrocarbons, for example, alkylenes, and the like, and thus means that the corresponding hydrocarbon has 6 or less carbon atoms. For example, a C_{1-6} linear or branched alkyl group are referred to by another

name such as a "lower alkyl" group.

[0180] The term "oxy" used in the present invention refers to a secondary radical (-O-) of an oxygen atom.

[0181] The term "alkyl" or "alkane" refers to a linear or branched non-aromatic hydrocarbon that is completely saturated. Unless otherwise defined, a linear or branched alkyl group typically has a 1 to approximately 20 carbon atoms, preferably 1 to approximately 10 carbon atoms. The linear and branched alkyl group includes methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, tert-butyl, pentyl, hexyl, pentyl, and octyl.

[0182] The term "alkenyl" or "alkene" refers to a linear or branched non-aromatic hydrocarbon that contains at least one double bond. Unless otherwise defined, a linear or branched alkenyl group typically has 1 to approximately 20 carbon atoms, preferably 1 to approximately 10 carbon atoms.

[0183] The term "alkynyl" or "alkyne" refers to a linear or branched non-aromatic hydrocarbon that contains at least one triple bond. Unless otherwise defined, a linear or branched alkynyl group typically has 1 to approximately 20 carbon atoms, preferably 1 to approximately 10 carbon atoms.

[0184] The term "cycloalkane" or "cycloalkyl" group refers to a completed saturated cyclic hydrocarbon. The "cycloalkyl" includes monocyclic and polycyclic rings. Unless otherwise defined, a monocyclic cycloalkyl group generally has 3 to approximately 10 carbon atoms, more generally 3 to 8 carbon atoms. Rings other than the first ring of the polycyclic cycloalkyl may be selected from saturated, unsaturated, and aromatic rings. The cycloalkyl includes a bicyclic molecule that contains 1, 2, or 3 or more atoms shared between two rings. The term "fused cycloalkyl" refers to a polycyclic cycloalkyl in which one ring shares two adjacent atoms with another ring. Rings other than the first ring of the fused polycyclic cycloalkyl may be selected from saturated, unsaturated, and aromatic rings.

[0185] The term "cycloalkyne" or "cycloalkynyl" refers to a cyclic hydrocarbon that contains at least one triple bond, which is also referred to as a "strained alkyne." The "cycloalkynyl" includes monocyclic and polycyclic rings. Unless otherwise defined, a monocyclic cycloalkynyl generally has 3 to approximately 10 carbon atoms, more generally 3 to 8 carbon atoms. Rings other than the first ring of the polycyclic cycloalkynyl may be selected from saturated, unsaturated and aromatic rings. The cycloalkynyl is a bicyclic molecule that contains 1, 2, or 3 or more atoms shared between two rings. The term "fused cycloalkynyl" refers to a polycyclic cycloalkynyl in which one ring shares two adjacent atoms with another ring. Rings other than the first ring of the fused polycyclic cycloalkynyl may be selected from saturated,

unsaturated, and aromatic rings.

[0186] The term "alkylene" used as a molecule itself or used as part of another molecule refers to a divalent radical derived from an alkane. For example, the group includes -CH_2CH_2-, and - $CH_2CH_2CH_2CH_2$-, all of which contain 10 or less carbon atoms, but the present invention is not limited thereto. The term "lower alkylene" refers to a short alkylene group that generally has 6 or less carbon atoms. Unless stated otherwise, the term "alkylene" is intended to encompass groups represented by the "heteroalkylene" in the present invention.

[0187] The term "heteroalkylene" refers to a divalent radical derived from a heteroalkyl, and, for example, includes -CH_2-CH_2-S-CH_2-CH_2-, and -CH_2-S-CH_2-CH_2-NH-CH_2-, but the present invention is not limited thereto. A heteroalkylene group may contain the same or different heteroatoms at each end or all ends of a chain thereof (including an alkyleneoxy, an alkylenedioxy, an alkyleneamino, an alkylenediamino, an aminooxyalkylene, and the like, but the present invention is not limited thereto). Further, an indication of connection of both ends of the chain is independent of the arrangement of groups of a formula. For example, the formula -$C(O)_2$R'- refers to both -$C(O)_2$R'- and -R'$(O)_2$C-.

[0188] The term "alkenylene" used as a molecule itself or used as part of another molecule refers to a divalent radical derived from an alkene. For example, the group includes -CH=CH-, -CH_2CH=CHCH_2-, and -CH=CH-CH=CH-, all of which have 10 or less carbon atoms, but the present invention is not limited thereto. Unless stated otherwise, the term "alkenylene" is intended to encompass heteroalkenylenes in the present invention.

[0189] The term "alkynylene" used as a molecule itself or used as part of another molecule refers to a divalent radical derived from an alkyne. For example, the group includes -C≡C-, -CH_2C≡CCH_2-, and -C≡C-C≡C-, all of which have 10 or less carbon atoms, but the present invention is not limited thereto. Unless stated otherwise, the term "alkynylene" is intended to encompass heteroalkynylenes in the present invention.

[0190] The term "cycloalkylene" used as a molecule itself or used as part of another molecule refers to a divalent radical derived from a cycloalkene. Unless stated otherwise, the "cycloalkylene" is intended to encompass heterocycloalkylenes in the present invention.

[0191] The term "alkylene" used in this specification, examples, and the claims is intended to encompass both "unsubstituted alkylene" and "substituted alkylene." Among these, the latter refers to an alkylene group that has a substituent replacing a hydrogen atom on one or more carbon atoms of the hydrocarbon. Unless otherwise clearly specified, the substituent may, for example, include a halogen, a hydroxyl group, a carbonyl group (for example, carboxyl, alkoxycarbonyl, formyl, or acyl), a thiocarbonyl group (for example, thioester, thioacetate, or

thioformate), an alkoxy group, a phosphoryl group, a phosphate group, a phosphonate group, a phosphinate group, an amino group, an amido group, an amidine group, an imine group, a cyano group, a nitro group, an azido group, a sulfhydryl group, an alkylthio group, a sulfate group, a sulfonate group, a sulfamoyl group, a sulfonamido group, a sulfonyl group, a heterocyclyl group, an aralkyl group, or an aromatic or heteroaromatic group. When properly substituted, it may be understood by those skilled in the related art that a substituted residue on a hydrocarbon chain may itself be substituted. For example, the substituent of the substituted alkylene may include substituted and unsubstituted amino, azido, imino, amido, phosphoryl (including phosphonates and phosphinates), sulfonyl (including sulfates, sulfonamidos, sulfamoyls, and sulfonates), and silyl groups, and may also include ethers, alkylthios, carbonyls (including ketones, aldehydes, carboxylates, and esters), -CF3, -CN, and equivalents thereof. Exemplary substituted alkyls are as described below. The cycloalkylene may be further substituted with an alkyl, an alkenyl, an alkoxy, an alkylthio, an aminoalkyl, a carbonyl-substituted alkyl, -CF3, -CN, and equivalents thereof. This content is also equally applicable to the alkenylene and the alkynylene.

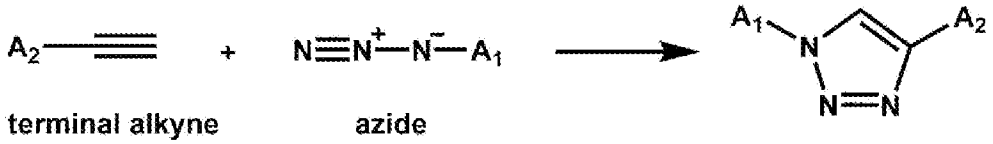
[0192] When used together with a residue such as an alkylene, an alkenylene, or an alkynylene, the term "C_{x-y}" is, for example, intended to encompass residues containing x to y carbon atoms in the chain thereof. For example, the term "C_{x-y} alkylene" refers to a substituted or unsubstituted, linear or branched alkylene group that contains x to y carbon atoms in the chain thereof. For example, it is meant to exemplarily include a haloalkylene group such as difluoromethylene, 2,2,2-trifluoroethylene, and the like. The C_0 alkylene refers to a covalent bond. The terms "C_{2-y} alkenylene" and "C_{2-y} alkynylene" refers to a substituted or unsubstituted unsaturated aliphatic residue to which a definition of length and possible substitution is applied as described in the definition of the alkylene. However, this means that each contains at least one double or triple bond.

[0193] The term "click-chemistry reaction" is used as a chemical concept introduced by K. Barry Sharpless of the Scripps Research Institute to explain complementary chemical functional groups and a chemical reaction designed so that two molecules can rapidly and stably form a covalent bond. The click-chemistry reaction does not refer to a certain reaction, but refers to a concept of such a rapid and stable reaction. In any embodiment, the click-chemistry reaction must be modular, wide in scope, give high yields, generate only insignificant by-products, be stereospecific, physiologically stable, driven by a thermodynamic driving force (for example, greater than 84 kJ/mol), and/or have high atom economy. Some reactions are known to satisfy the requirements:

[0194] (1) Huisgen 1,3-dipolar cycloaddition (for example, including a Cu(I)-catalyzed

cycloaddition reaction, which is often commonly referred to as a "click reaction"; see Tornoe *et al.*, Journal of Organic Chemistry (2002) 67: 3057-3064): Copper or ruthenium is generally used as a catalyst;

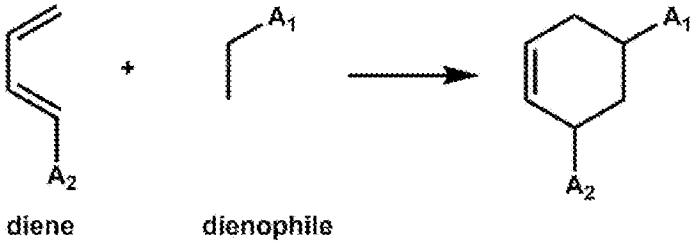
[0195] [Schematic diagram of Huisgen 1,3-dipolar cycloaddition]



1,3-dipolar cycloaddition

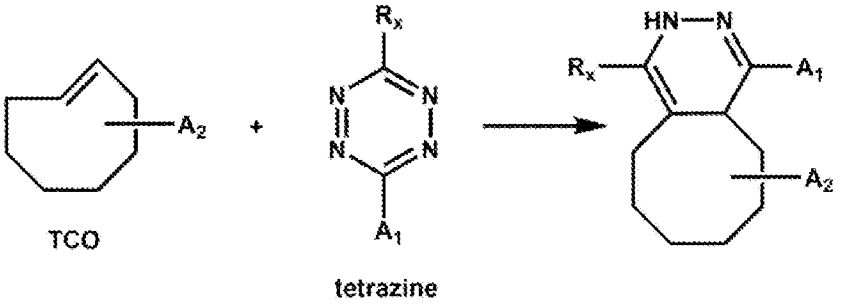
[0196] (2) Diels-Alder reaction, for example cycloaddition (for example, strain-promoted cycloaddition (SPAAC)) including a normal electron-demand Diels-Alder reaction and an inverse electron-demand Diels-Alder reaction, but the present invention is not limited thereto);

[0197] [Schematic diagram of Diels-Alder reaction]

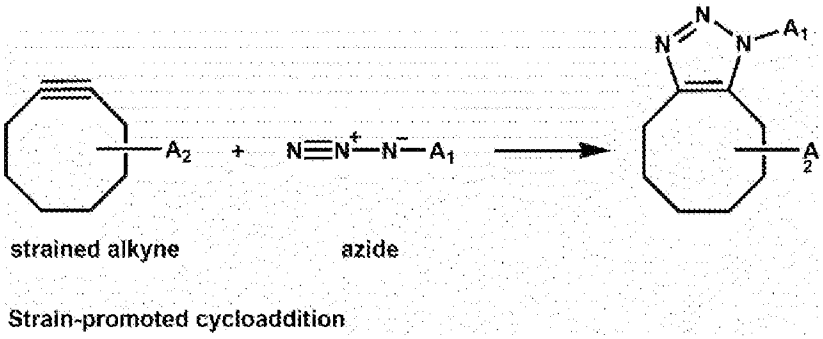


Diels-Alder reaction

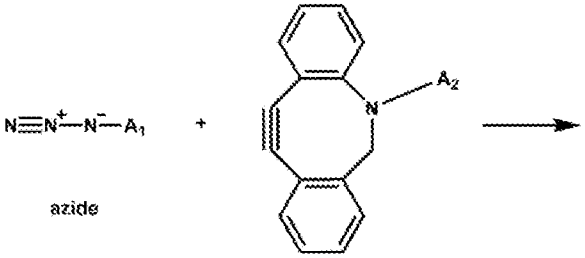
[0198] [Example of Diels-Alder reaction; TCO and tetrazine]

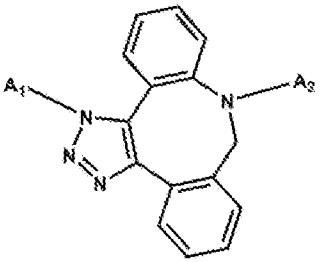


[0199] [Schematic diagram of strain-promoted cycloaddition]



[0200] [Example of strain-promoted cycloaddition; Azide and DBCO]



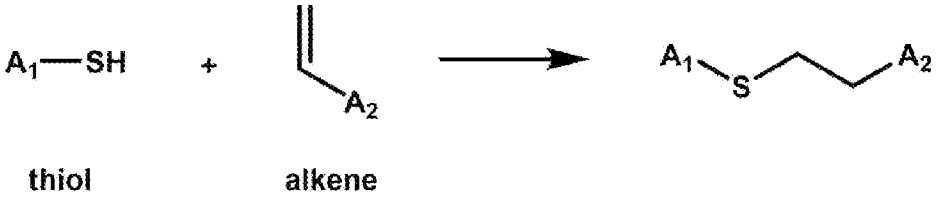


[0201] (3) Nucleophilic addition to small strained rings such as epoxides and aziridines;

[0202] (4) Nucleophilic addition to activated carbonyl groups;

[0203] (5) Addition to carbon-carbon double bonds or triple bonds.

[0204] [Addition of thiol and alkene]



[0205] The term “click-chemistry functional group” used in the present invention refers to a functional group that participates in a click-chemistry reaction. For example, a strained alkyne (for example, a cyclooctyne) corresponds to a click-chemistry functional group. In general, the click-chemistry reaction requires at least two molecules, each of which contains click-chemistry functional groups complementary to each other. In this way, a pair of click-chemistry functional

groups having reactivity with each other is often referred to as "partner click-chemistry functional groups" in the present invention. In strain-promoted cycloaddition of a cyclooctyne with an azide, for example, the azide is a partner click-chemistry functional group for the cyclooctyne and other alkynes. Exemplary click-chemistry functional groups used in the present invention include a terminal alkyne, an azide, a strained alkyne, a diene, a dienophile, a trans-cyclooctene, an alkene, a thiol, and a tetrazine, but the present invention is not limited thereto. Other click-chemistry functional groups are known to those skilled in the related art.

[0206] The term "leaving group" used in the present invention has the same concept as well known to those skilled in the related art (Advanced Organic Chemistry: reactions, mechanisms and structure- Fourth Edition of Jerry March, John Wiley and Sons Ed.; 1992, pages 351-357), and refers to a chemical functional group linked to any reactant, which moves when the reactant is subjected to a substitution reaction (displacement reaction), for example, a nucleophilic substitution reaction. A good leaving group refers to a leaving group that easily moves during the nucleophilic substitution reaction. Exemplary good leaving groups include a halogen (F, Cl, Br, and I), a tosylate, a mesylate, a triflate, an acetate, a trifluoromethylacetate, a camphorsulfonate, 2-thioxobenzo[d]thiazol-3(2H)-yl, N-hydrosuccinimide, N-aryloxide, and an aryloxide substituted with one or more electron-withdrawing groups (EWGs), but the present invention is not limited thereto. For example, the aryloxide substituted with one or more electron-withdrawing groups include 2-nitrophenoxide, 4-nitrophenoxide, 2,4-dinitrophenoxide, pentafluorophenoxide, 2-chloro-4-nitrophenoxide, 2,4-dichlorophenoxide, and 2,4,6-chlorophenoxide, and the electron-withdrawing group, for example, includes a halogen (F, Cl, Br, or I), $-NO_2$, -CN, $-C(O)(C_{1-6}$ alkyl), -C(O)(aryl), $-C(O)O(C_{1-6}$ alkyl), -C(O)O(aryl), and the like.

[0207] The term "interactome" used in the present invention refers to a protein or a peptide that participates in a protein-protein interaction when there is a protein-protein interaction (PPI) between proteins or between peptides. For example, a chaperone protein and its passenger protein are mutual interactomes. The "protein-protein interaction" means that two or more proteins or peptide molecules interact to come into physical contact with each other with high specificity. In this case, the causative interaction includes an electromagnetic force, a hydrogen bond, and a hydrophobic interaction, but the present invention is not limited thereto.

[0208] The term "antibody interactome" used in the present invention refers to an interactome for an antibody including immunoglobulins. Exemplary antibody interactomes are listed in Table 1. Among these, peptides such as Fc-III, and the like are known to have binding activity for an Fc domain of an immunoglobulin. In this case, the peptides are also referred to as "Fc interactomes."

<Table 1> List of SSAIs

Peptide	Binding Constant	Binding Capacity	Elution pH	Remarks
PAM	K_d = 0.3μM	n.a.	3 or 9	Dendrimer comb. library SpA mimic
D-PAM	n.a.	50	3.5	
D-PAM-Φ	n.a.	10	4	
TWKTSRSIF	n.a.	n.a.	n.a.	Phage display library SpA mimic
FGRLYSSAIRY	n.a.	n.a.	n.a.	
Fc-III (DeLano et al.) Fc-III-(Sepharose)	K_d = 16nM	26.6mg/μmol	3.5	Phage display cyclic peptide library SpA mimic
FcBP-2	K_d = 1.6nM	n.a.	n.a.	Bicyclic peptide
Fc-III-4C	K_d = 2.5nM	n.a.	3.5	Bicyclic peptide
EPIHRSTLTALL	n.a.	320μg/g	n.a.	Phage display library SpA mimic
APAR	K_d = 94nM	9.1	n.a.	Comb. tetrapeptide library
FcRM	K_d = 20μM	n.a.	2.7	Synthetic cyclic peptide library, Fcγ-receptor mimic

<Table 1> (Continued)

Peptide	Binding Constant	Binding Capacity	Elution pH	Remarks
FWRGWV	K_d = 10μM	28.4	4	Comb. library (one-bead-one-peptide) SpA mimic
HYFKFD	K_d = 11μM	27.0	n.a.	
HFRRHL	K_d = 29μM	33.6	n.a.	
FWOrGWV	K_d = 106μM	72	n.a.	
DAAG	K_a = 7.9x10^{5}M^{-1}	36.2	3.6	Comb. library SpA mimic
DAAG	Ka = 2.6x10^{5}M^{-1}	49.6	3.6	
cyclo[(Ne-Ac)KA)-FWHYFK-Lact-E]	n.a.	n.a.	3.5	Cyclic peptide
cyclo[(Ne-Ac)-Dap(A)-FWHYFK-Lact-E]	n.a.	n.a.	3.5	
cyclo[Link-M-WPRHYK]	K_d = 7.6μM	19.7	4.0	mRNA display library SpA mimic
NKFRGRYK	K_a = 3.6x10^{6}M^{-1}	DBC-4.9	4.0	Spot peptide array Fcγ mimic
NARKFYKG	K_a = 8.5x10^{6}M^{-1}	DBC-5.0	4.0	

<Table 1> (Continued)

Peptide	Binding Constant	Binding Capacity	Elution pH	Remarks
FYWHCLDE(1)	K_d = 1.5μM	104.3	6.0	Biomimetic design strategy Fc-binder (SpA mimic)
FYCHWALE(2)	K_d = 6.1μM	87.6	6.0	
FYCHTIDE(3)	K_d = 5.7μM	63.7	6.0	Dual affinity ligand
Dual 1/3 (2:1)	K_d = 0.69μM	137.9	6.0	
ERGW	K_d = 0.5mM	n.a.	n.a.	Computer design strategy Fc-binder
KHRFNKD	K_d = 20nM	n.a.	n.a.	Phage-display library SpA mimic

* Maximal binding capacity (q_m) in mg/mL. DBC: Dynamic binding capacity. The depicted values are towards IgG and may not be directly compared to each other because they were obtained at different conditions or by different methods. n.a.: Not available.

[0209] According to the present invention, the term “antibody” refers to an immunoglobulin molecule or a fragment thereof. Immunoglobulins are generally well known, and have an ability

to specifically bind to a certain antigen. However, because the antibody according to the present invention is a concept that also encompasses a fragment thereof, the antibody does not have to show binding ability to a certain antigen as in case of the Fc fragment. In addition to the naturally occurring immunoglobulins, the antibody is also intended to encompass all recombinant proteins, fusion proteins, chimeric proteins, human immunoglobulins, non-human animal immunoglobulins, and the like, which have the same or similar structures.

[0210] According to the present invention, the term "conjugate" refers to a heterogeneous molecule made when conjugate partners are taken together to form a covalent bond. The covalent bond may be preferably formed by means of a click-chemistry reaction. The term "conjugate partner" refers to each of molecules intended to form a conjugate. In this case, when one wishing to perform conjugation intends to link a certain molecule to any other molecule, the certain molecule may be generally referred to as a "target molecule" or "target protein," and the any other molecule may be generally referred to as a "cargo molecule" or "cargo moiety" for the sake of convenience.

[0211] According to the present invention, the term "carrier moiety" refers to a portion of molecule that constitutes a conjugate, that is, a molecule that functions to enhance serum stability of a molecule linked thereto or extend the half-life of the molecule. Molecules that may be used as the carrier moiety are well known in the related art. Representative examples of the carrier moiety include albumin, gelatin, elastin (including tropoelastin), an elastin-derived polypeptide (α-elastin and elastin-like polypeptides (ELPs)), gliadin, legumin, zein, a soy protein (for example, a soy protein isolate (SPI)), a milk protein, a whey protein, bilirubin, and the like, but the present invention is not limited thereto.

[0212] According to the present invention, the term "fluorescent moiety" is intended to encompass dyes or dye reagents used for fluorescence. Molecules that may be used as the dyes and dye reagents are well known in the related art. Representative examples of the fluorescent moiety are as listed in Table 2, but the present invention is not limited thereto (Immunotech-Coulter Corp. catalog, "Cytometry Monoclonal Reagent Guide," 8/95, p.3).

<Table 2> List of fluorescent moieties

Fluorochrome	Maximum Absorbance	Excitation at 488 nm	Maximum Emission	Fluorescence
Fluorescein isothiocyanate (FITC)	495 nm	Yes	525 nm	Green
Phycoerythrin (PE)	488; 565 nm	Yes	575 nm	Orange-red
Energy Coupled Dye [ECD] (PE-Texas-Red)	488; 565 nm	Yes	610-635 nm	Red
Phycoerythrin-Cyanin 5 (PE-Cy5)	488; 565; 652 nm	Yes	670 nm	Deep-red

[0213] According to the present invention, the term "drug moiety" refers to a molecule that has a therapeutic effect on any disease. The drug moiety according to the present invention includes those known to a person having ordinary skill in the art as being effective against any disease. Typically, the drug moiety having an anti-cancer effect includes DM1, DM3, DM4, Abrin, Ricin A, a *Pseudomonas* exotoxin, a *Cholera* toxin, a *Diphtheria* toxin, a tumor necrosis factor, α interferon, β interferon, a nerve growth factor, a platelet-derived growth factor, a tissue plasminogen activator, a cytokine, an apoptosis-inducing agent, an anti-angiogenic agent, a lymphokine, taxane, a DNA-alkylating agent, anthracyclin, a Tubulysin analogue, a duocarmycin analogue, auristatin E, auristatin F, a maytansinoid, a cytotoxic agent including a reactive polyethylene glycol residue, taxon, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, T. Colchicine, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dihydrotestosterone, a glucocorticoid, procaine, tetracaine, lidocaine, propranolol, puromycin, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine, mechlorethamine, thiotepa, chlorambucil, Meiphalan, carmustine, lomustine, cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, cisplatin, dactinomycin, bleomycin, anthramycin, calicheamicin, abiraterone, bendamustine, bortezomib, carboplatin, cabazitaxel, dasatinib, doxetaxel, epirubicin, erlotinib, everolimus, gemcitabine, gefitinib, idarubicin, imatinib, hydroxyurea, lapatinib, leuprorelin, melphalan, nedaplatin, nilotinib, oxaliplatin, pazopanib, pemetrexed, picoplatin, romidepsin, satraplatin, sorafenib, vemurafenib, sunitinib, teniposide, triplatin, and vinorelbine, but the present invention is not limited thereto.

[0214] According to the present invention, the term "radioactive moiety" refers to a moiety including a radioisotope. Radioisotopic labeling is useful for diagnostic imaging and radiotherapy. Representative radioactive moieties include ^{18}F, ^{11}C, ^{125}I, ^{123}I, ^{124}I, ^{131}I, and ^{99m}Tc, but the present invention is not limited thereto.

[0215] The term "pharmaceutically acceptable carrier" may be used in the sense of including an excipient, a diluent, or an adjuvant. The carrier may be, for example, selected from the group consisting of lactose, dextrose, sucrose, sorbitol, mannitol, xylitol, erythritol, maltitol, starch, acacia gum, alginate, gelatin, calcium phosphate, calcium silicate, cellulose, methyl cellulose, polyvinyl pyrrolidone, water, physiological saline, a buffer such as PBS, methylhydroxy benzoate, propylhydroxy benzoate, talc, magnesium stearate, and mineral oil. The carrier may include a filler, an anti-agglomerating agent, a lubricant, a wetting agent, a flavoring agent, an emulsifying agent, a preservative, or a combination thereof.

[0216] The term "pharmaceutically acceptable salt" refers to a salt in which biological effectiveness and properties of a proteome and a compound according to the present invention are preserved and that is not undesirable in biological or other aspects. In many cases, the proteome and compound of the present invention may form an acidic and/or basic salt in the presence of a charged group, for example, a charged amino and/or carboxyl group or the likes. A pharmaceutically acceptable acid addition salt may be prepared from an inorganic and organic acid, and a pharmaceutically acceptable base addition salt may be prepared from an inorganic and organic base.

[0217] The term "treatment" refers to an approach to obtain beneficial or desirable clinical outcomes. For the object of the present invention, non-limiting examples of the beneficial or desirable clinical outcomes include palliation of symptoms, reduction of a disease range, stabilization (i.e., not worsening) of a disease state, delay of progression or reduction of a progression rate of a disease, (partially or totally) improvement or temporal palliation and mitigation of a disease condition, and whether detectable or not. The treatment denotes all therapeutic treatments, and prophylactic or preventative methods. The treatments include treatments required for disorders to be prevented and already-occurring disorders. "Palliating" a disease means that a range of disease conditions and/or undesirable clinical signs are lowered and/or a time course of progression of the disease is delayed or extended, compared to when the disease is not treated.

[0218] The "therapeutically effective amount" (or "effective amount") refers to a sufficient amount of an active ingredient, for example, an agent according to the present invention, to achieve the treatment when administered to a subject or a patient. Therefore, what constitutes a therapeutically effective amount of a composition according to the present invention may be easily determined by those skilled in the related art. In the context of vision therapy, the "therapeutically effective amount" is an amount that causes an objectively measured change in one or more parameters associated with the treatment of eye diseases or conditions, which

include an increase or decrease in expression of one or more genes associated with the eye diseases or conditions, an induction of apoptosis or other cell death pathways, a clinical improvement in symptoms, a decrease in abnormal neovascularization or inflammation, and the like. Of course, the therapeutically effective amount may vary depending on a certain subject and condition to be treated, the weight and age of a subject, the severity of a disease condition, a certain compound to be selected, a subsequent dosing schedule, the administration time adjustment, a mode of administration, and the like, all of which may be easily determined by those skilled in the related art. In the context of combination therapy, it should be understood that what constitutes a therapeutically effective amount of a certain active ingredient may be different from what constitutes a therapeutically effective amount of an active ingredient that is administered for monotherapy (that is, a treatment regimen using one chemical entity as the active ingredient).

[0219] The "subject" or "patient" refers to an animal in need of treatment which may be achieved by the molecule of the present invention. The animal to be treated according to the present invention includes a vertebrate. Particularly preferred examples of the animal include mammals, for example, bovine, canine, equine, feline, ovine, porcine, and primate animals (including human and non-human primates).

[0220] The term "about" or "approximately" refers to an amount, level, value, number, frequency, percentage, dimension, size, quantity, weight, or length that varies by 30, 25, 20, 25, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1% with respect to the reference amount, level, value, number, frequency, percentage, dimension, size, quantity, weight, or length.

[0221] 1. Antibody

[0222] It should be understood that the description of the present invention is intended to provide a description of the structure, academic system, and physiological action of an antibody provided to aid in understanding the present invention, and the antibody falling within the scope of rights of the present invention is not intended to limit the description of the present invention.

[0223] An antibody consists of two heavy chains and two light chains as known in the art. When an antibody is simply divided in a functional aspect, the antibody is divided into a fragment antigen-binding variable region (Fab) including a light chain and a fragment crystallizable region composed as a portion of a heavy chain. The Fab includes a paratope that binds to an antigen, and refers to a region that allows an antibody to have specific binding activity for an antigen as known in the art. As the Fc domain is a ligand for an Fc receptor (FcR) in cells, it plays an important role in inducing an immune response. Also, the Fc domain plays an important role in extending the half-life of an antibody by binding to a neonatal Fc receptor so that the antibody can be repeatedly internalized into the cells.

[0224] From these facts, it is possible to deduce some desirable directivity of labeling an antibody: (1), First, it is desirable to label an antibody at a position spaced apart from a paratope of the antibody. When the labeling is performed on the paratope or at a position adjacent to the paratope, a binding affinity of the antibody for an antigen may be significantly lowered. (2) Second, it is desirable to label an antibody at a position spaced apart from a recognition site of FcR including FcRn. When the labeling is performed on the recognition site of the receptor or at a position adjacent to the recognition site, an immune response-inducing function of the antibody may be reduced, or the half-life of the antibody may be shortened. Information on the binding activity motif of the Fc domain may be found in DeLano, W.L. (2000): Convergent Solutions to Binding at a Protein-Protein Interface; Science, 287(5456), 1279-1283., and W. Lance Martin *et al.* (2001), Molecular Cell, Vol. 7, 867-877, April, 2001.

[0225] In the present invention, when reference to an amino acid sequence of the Fc domain of the antibody is made, numbers in the sequence are numbered according to the EU numbering system unless stated otherwise. The EU numbering system has been widely used as a sequencing system for the Fc domain after research on the sequence of IgG, as described in Edelman GM, *et al.*, The covalent structure of an entire gamma-G immunoglobulin molecule; Proc. Natl. Acad. Sci. USA., 1969 May; 63(1): 78-85.

[0226] 1.1. Search for desirable labeling site

[0227] A labeling site of an antibody may be designed in consideration of the criteria for the labeling site, that is, (1) a site spaced apart from a paratope; and (2) a site spaced apart from a recognition site of FcR including FcRn. Examples of the amino acids used in a bioconjugation reaction typically include lysine, cysteine, and tyrosine. Lysine 246 (Lys_{246}) and lysine 248 (Lys_{248}) present in an Fc domain of the antibody are residues that satisfy all the requirements, and thus are both desirable labeling sites. A sequence of the Fc domain including the Lys_{246} and Lys_{248} residues is GPSVFLFPPKPKDTLMI, and the sequence and numbers in the sequence numbered according to the EU numbering system are shown in FIG. 1 (FIG. 1, SEQ ID NO: 1).

[0228] In specific embodiments, the antibody according to the present invention may include a sequence of SEQ ID NO: 1 or derivatives thereof. Also, the antibody according to the present invention may include a derivative of SEQ ID NO: 1 in which lysine 246 is substituted. In addition, the antibody according to the present invention may include a derivative of SEQ ID NO: 1 in which lysine 248 is substituted. Furthermore, the antibody according to the present invention may include a derivative of SEQ ID NO: 1 in which lysine 246 and 248 are substituted.

[0229] The sequence of SEQ ID NO: 1 or derivatives thereof include sequences mutated in an acceptable range. In specific embodiments, the mutated sequences may have a homology

that is greater than or equal to approximately 90%, approximately 85%, approximately 80%, approximately 75%, or approximately 70% with respect to the sequence of SEQ ID NO: 1 or derivatives thereof. In specific embodiment specified below, it should be understood that the derivatives of SEQ ID NO: 1 represented by Formulas 7-1 to 7-3, 8-1 to 8-3, and 10-1 to 10-3 also include sequences mutated in an acceptable range.

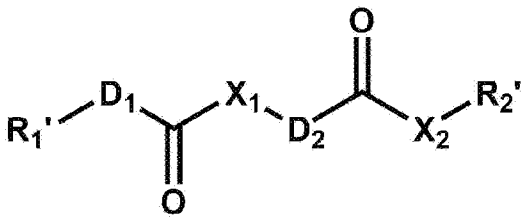
[0230] FIG. 2 shows positions of lysine residues in an Fc domain including lysine 246 and 248.

[0231] 2. Linker (R_1'-L_1)

[0232] The present invention discloses a novel compound capable of being used to label an antibody. For the sake of convenience, such a compound is herein referred to as a linker, which is indicated by the symbol "R_1'-L_1."

[0233] The present invention provides a compound having a structure of the following Formula 1:

[0234] [Formula 1]



[0235] wherein R_1' is a first chemical functional group,

[0236] D_1 is any alkylene, alkenylene, or alkynylene,

[0237] X_1 is an element that is more electronegative than carbon,

[0238] D_2 is any alkylene, alkenylene, or alkynylene,

[0239] X_2 is an element that is more electronegative than carbon, and

[0240] R_2' is a second chemical functional group.

[0241] In Formula 1, a carbonyl group connected to D_1 is referred to as a first carbonyl group. Also, a carbonyl group connected to D_2 is referred to as a second carbonyl group.

[0242] In specific embodiments, R_1' may include a click-chemistry functional group. Also, the click-chemistry functional group may include one or more selected from an alkyne, an azide, a strained alkyne, a diene, a dienophile, an alkene, a thiol, and a tetrazine. Furthermore, the click-chemistry functional group may be selected from an azide, or a strained alkyne. Further, the click-chemistry functional group may be selected from an azide, or dibenzocyclooctyne-amine. Additionally, the click-chemistry functional group may be selected from a diene, or a dienophile. Further, the click-chemistry functional group may be selected from a tetrazine, or a norbornene.

Alternatively, the click-chemistry functional group may be selected from a tetrazine, or a trans-cyclooctene. In addition, R_1' may include two or more click-chemistry functional groups.

[0243] In other specific embodiments, R_1' may include a carrier moiety, a fluorescent moiety, a drug moiety, or a radioactive moiety. Also, R_1' may include a drug moiety. In addition, R_1' may include a VC linker. In other specific embodiments, R_1' may include an antibody or an analogue thereof, which includes a paratope.

[0244] In specific embodiments, D_1 may include any one selected from a covalent bond, a C_{1-4} alkylene, a C_{2-4} alkenylene, a C_{2-4} alkynylene, and a C_{3-8} cycloalkylene. Also, D_1 may be $-CH_2OCH_2-$. In addition, D_1 may be a covalent bond. When D_1 is a covalent bond, R_1' and the carbon of the first carbonyl group are directly connected to each other. Hereinafter, when describing a structure of a compound, all the alkylene, the alkenylene, the alkynylene, and the cycloalkylene are intended to include a heteroalkylene, a heteroalkenylene, a heteroalkynylene, and heterocycloalkylene, respectively, the contents of which are also specified in the section "Definitions."

[0245] In specific embodiments, X_1 may be NR_1, S, or O, wherein R_1 may be H, a halogen, or a substituted or unsubstituted C_{1-3} alkylene. Also, X_1 may be S. X_1 may attract electrons from the carbon of the first carbonyl group to activate the first carbonyl group.

[0246] In specific embodiments, D_2 may include any one selected from a C_{1-7} alkylene, a C_{2-7} alkenylene, a C_{2-7} alkynylene, and a C_{3-8} cycloalkylene. Also, D_2 may be a C_{1-2} alkylene. Furthermore, D_2 may be methylene.

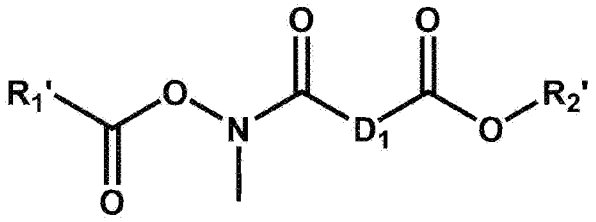
[0247] In specific embodiments, X_2 may be NR_1, S, or O, wherein R_1 may be H, a halogen, or a substituted or unsubstituted C_{1-3} alkylene. Also, X_2 may be O. X_2 may attract electrons from the carbon of the second carbonyl group to activate the second carbonyl group.

[0248] In specific embodiments, R_2' may be a halogen, N-succinimide, p-nitrophenyl, or pentafluorophenyl.

[0249] In specific embodiments, R_2' and X_2 taken together may form a leaving group. For example, X_2 may be O, and R_2' may be N-succinimide, p-nitrophenyl, or pentafluorophenyl. Also, R_2' may be N-succinimide. When a good leaving group is connected to the second carbonyl group, reactivity of the second carbonyl group may be enhanced. For example, an N-hydroxysuccinimide ester (an NHS ester) is known to show very high reactivity.

[0250] The present invention provides a compound having a structure of the following Formula 1-2:

[0251] [Formula 1-2]



[0252] wherein R_1' is a first chemical functional group,

[0253] D_1 is any alkylene, alkenylene, or alkynylene, and

[0254] R_2' is a second chemical functional group.

[0255] In specific embodiments, R_1' may include a click-chemistry functional group. Also, the click-chemistry functional group may include one or more selected from an alkyne, an azide, a strained alkyne, a diene, a dienophile, an alkene, a thiol, and a tetrazine. Furthermore, the click-chemistry functional group may be selected from an azide, or a strained alkyne. Further, the click-chemistry functional group may be selected from an azide, or dibenzocyclooctyne-amine. Additionally, the click-chemistry functional group may be selected from a diene, or a dienophile. Further, the click-chemistry functional group may be selected from a tetrazine, or a norbornene. Alternatively, the click-chemistry functional group may be selected from a tetrazine, or a trans-cyclooctene. In addition, R_1' may include two or more click-chemistry functional groups.

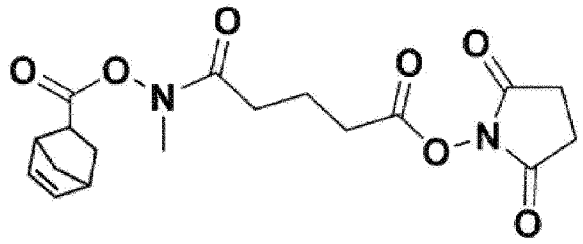
[0256] In other specific embodiments, R_1' may include a carrier moiety, a fluorescent moiety, a drug moiety, or a radioactive moiety. Also, R_1' may include a drug moiety. In addition, R_1' may include a VC linker. In other specific embodiments, R_1' may include an antibody or an analogue thereof, which includes a paratope.

[0257] In specific embodiments, D_1 may include any one selected from a C_{1-7} alkylene, a C_{2-7} alkenylene, a C_{2-7} alkynylene, and a C_{3-8} cycloalkylene. Also, D_1 may be a C_{1-2} alkylene. Furthermore, D_1 may be methylene.

[0258] In specific embodiments, R_2' and O taken together may form a leaving group. In this case, R_2' may be N-succinimide, p-nitrophenyl, or pentafluorophenyl. Also, R_2' may be N-succinimide.

[0259] In specific embodiments, the compound represented by Formula 1-2 may have a structure of the following Formula 1-3:

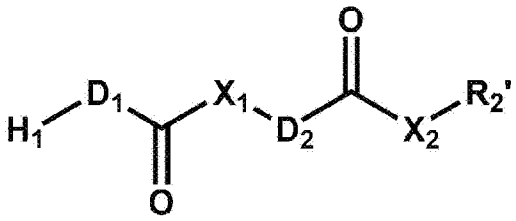
[0260] [Formula 1-3]



[0261] 2.1. Linker including first click-chemistry functional group (H_1-L_1)

[0262] The present invention provides a compound having a structure of the following Formula 2:

[0263] [Formula 2]



[0264] wherein H_1 is a first click-chemistry functional group,

[0265] D_1 is any alkylene, alkenylene, or alkynylene,

[0266] X_1 is an element that is more electronegative than carbon,

[0267] D_2 is any alkylene, alkenylene, or alkynylene,

[0268] X_2 is an element that is more electronegative than carbon, and

[0269] R_2' is a second chemical functional group. The linker having the structure of Formula 2 is herein referred to as a "linker including a first click-chemistry functional group," which is indicated by the symbol "H_1-L_1."

[0270] In Formula 2, a carbonyl group connected to D_1 is referred to as a first carbonyl group. Also, a carbonyl group connected to D_2 is referred to as a second carbonyl group.

[0271] In specific embodiments, H_1 may include any one selected from an alkyne, an azide, a strained alkyne, a diene, a dienophile, an alkene, a thiol, and a tetrazine. Furthermore, H_1 may be an azide, or a strained alkyne. Further, H_1 may be an azide, or dibenzocyclooctyne-amine. Additionally, H_1 may be a diene, or a dienophile. Further, H_1 may be a tetrazine, or a norbornene. Alternatively, H_1 may be a tetrazine, or a trans-cyclooctene.

[0272] In specific embodiments, D_1 may include any one selected from a covalent bond, a C_{1-4} alkylene, a C_{2-4} alkenylene, a C_{2-4} alkynylene, and a C_{3-8} cycloalkylene. Also, D_1 may be $-CH_2OCH_2-$. In addition, D_1 may be a covalent bond.

[0273] In specific embodiments, X_1 may be NR_1, S, or O, wherein R_1 may be H, a halogen,

or a substituted or unsubstituted C_{1-3} alkylene. Also, X_1 may be S.

[0274] In specific embodiments, D_2 may include any one selected from a C_{1-7} alkylene, a C_{2-7} alkenylene, a C_{2-7} alkynylene, and a C_{3-8} cycloalkylene. Also, D_2 may be a C_{1-2} alkylene. Furthermore, D_2 may be methylene.

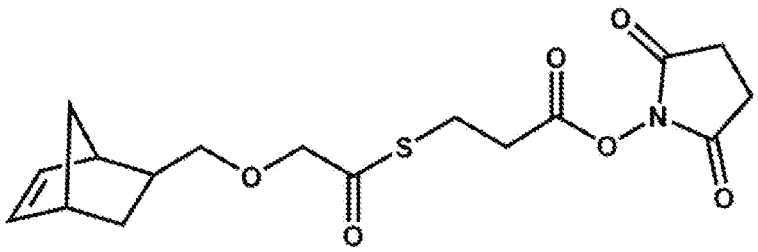
[0275] In specific embodiments, X_2 may be NR_1, S, or O, wherein R_1 may be H, a halogen, or a substituted or unsubstituted C_{1-3} alkylene. Also, X_2 may be O.

[0276] In specific embodiments, R_2' may be a halogen, N-succinimide, p-nitrophenyl, or pentafluorophenyl.

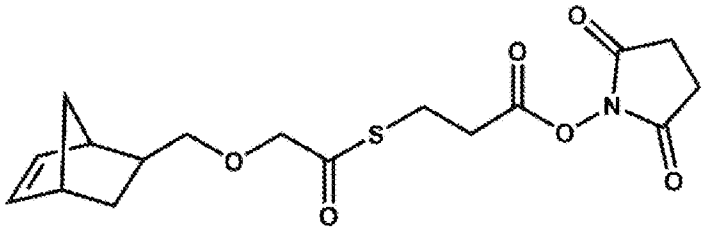
[0277] In specific embodiments, R_2' and X_2 taken together may form a leaving group. For example, X_2 may be O, and R_2' may be N-succinimide, p-nitrophenyl, or pentafluorophenyl. Also, R_2' may be N-succinimide.

[0278] In specific embodiments, the compound represented by Formula 2 may have any one structure selected from the following Formulas 2-1 to 2-3:

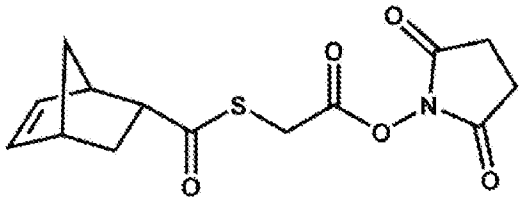
[0279] [Formula 2-1]



[0280] [Formula 2-2]



[0281] [Formula 2-3]

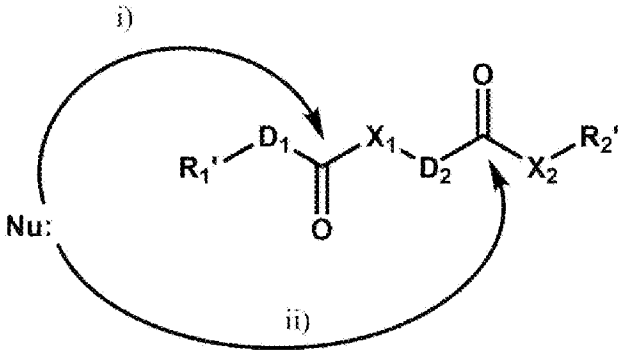


[0282] 2.2. The position at which a substitution reaction occurs may be specifically determined due to the difference in reactivity between the first carbonyl group and the second carbonyl group

[0283] When the linkers of Formulas 1 and 2, and sub-examples thereof are designed, sites for substitution reaction may be specified based on the design of X_1, X_2, and R_2'.

[0284] The linker disclosed in the present invention functions to transfer R_1' to a target molecule by means of a substitution reaction occurring between the activated first and/or second carbonyl groups. Such a substitution reaction is schematically shown as in the following Scheme 1.

[0285] [Scheme 1]



[0286] In Scheme 1, the target molecule is designated "Nu:" for the sake of convenience. The Nu: that serves as a nucleophile because it has an unshared electron pair causes a nucleophilic acyl substitution reaction with the first carbonyl group and/or second carbonyl group to form a bond with a linker. In the nucleophilic acyl substitution reaction, the reactivity of the carbonyl group may be determined by the basicity of the leaving group. Therefore, the reactivity of the carbonyl group is known to increase in the order of the carboxylate, the amide, the carboxylic acid, the ester, the thioester, and the acyl phosphate. Also, the carbonyl group such as an NHS ester, or the like is known to have high reactivity because the carbonyl group forms a very stable leaving group.

[0287] In specific embodiments, X_1 and X_2 may be elements that are more electronegative than carbon. Also, X_1 and X_2 may be NR_1, S, or O, wherein R_1 may be H, a halogen, or a substituted or unsubstituted C_{1-3} alkylene. Because X_1 and X_2 taken together with the residue(s) to which they are attached may form a leaving group, the carbonyl group may be activated.

[0288] In this case, the activation of the carbonyl group may optionally allow Nu: to i) preferentially react with the first carbonyl group or ii) preferentially react with the second carbonyl group. This tendency of reaction may be determined depending on a difference in reactivity between the first carbonyl group and the second carbonyl group. For example, when the basicity of a leaving group including X_1 is lower than that of a leaving group including X_2, the first carbonyl group may react first. In another embodiment, when the basicity of the leaving group including

X_2 is lower than that of the leaving group including X_1, the second carbonyl group may react first.

[0289] In preferred embodiments, the reactivity of the second carbonyl group is preferably higher than that of the first carbonyl group in the linker according to the present invention. According to the present invention, a selective reaction was achieved by allowing X_2-R_2' connected to the second carbonyl group to form a good leaving group, and designing the first carbonyl group to be an amide, a thioester, an ester, and the like, which show mild reactivity. For example, in the case of the linker of Formula 1-3, the second carbonyl group may be an NHS ester, thereby allowing it to react faster than the first carbonyl group (a thioester).

[0290] The prior art disclosed in Publication Nos. US 2018/0141976 A1 and WO 2018/199337 A1 is similar to the present invention in terms of the form of an agent for transferring a first chemical functional group to an antibody (see section 4 below), but is different from the present invention in that the cross-linker includes two NHS esters. The two carbonyl groups of the cross-linker have the same reactivity, and it is difficult to prepare a desired agent for transferring a chemical functional group with high yield. Also, the cross-linker has a high probability of reacting with two SSFIs due to high reactivity of the NHS esters. According to the present invention, such problems have been solved by designing the first carbonyl group to be a thioester, and the like, which have mild reactivity.

[0291] 3. Site-specific antibody interactome (SSAI)

[0292] According to the present invention, there is disclosed a novel peptide for bringing a molecule to be labeled into close contact with a certain site of an antibody. For the sake of convenience, such a peptide is herein referred to as a site-specific antibody interactome, which is indicated by the symbol "SSAI."

[0293] The SSAI provided according to the present invention may have binding activity for a certain site of an antibody.

[0294] In specific embodiments, the SSAI may have binding activity for an Fab domain of the antibody. In this case, the SSAI may preferably have binding activity for a site spaced apart from a paratope of the antibody.

[0295] In specific embodiments, the SSAI may have binding activity for the Fc domain of the antibody. In this case, the SSAI may preferably have binding activity for an FcRn binding site of the antibody or a site spaced apart from residues of the antibody which affects the FcRn binding site of the antibody.

[0296] 3.1: Site-specific Fc interactome (SSFI)

[0297] In the SSAI, a peptide having specific binding activity for the Fc domain is herein

referred to as a "site-specific Fc interactome", which is indicated by the symbol "SSFI."

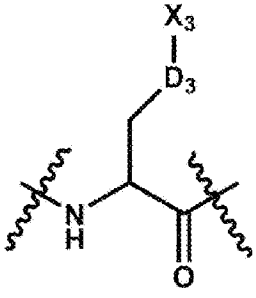
[0298] In specific embodiments, the SSFI may include an amino acid sequence represented by the following Formula 3:

[0299] [Formula 3]

$$(Xaa)_2\text{-}H\text{-}Xa_1\text{-}G\text{-}Xa_2\text{-}L\text{-}V\text{-}Xa_3 \quad \text{(SEQ ID NO: 3)}$$

[0300] wherein each Xaa is independently any amino acid except cysteine,

[0301] H is histidine, G is glycine, Xa_2 is glutamic acid or asparagine, L is leucine, V is valine, Xa_3 is selected from tryptophan, naphthylalanine, and phenylalanine, and

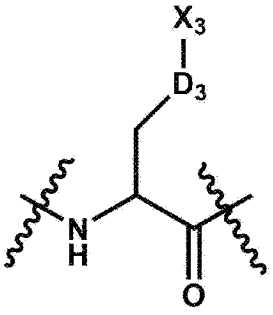
[0302] Xa_1 is  , wherein D_3 is a covalent bond or a C_{1-3} alkylene, and X_3 is NH_2, OH, or SH. As an analogue of the sequence AWHLGELVW (SEQ ID NO: 2), which is a sequence found to have binding activity for the Fc domain in the articles "Dias, R. L. A., *et al* (2006), Protein Ligand Design: From Phage Display to Synthetic Protein Epitope Mimetics in Human Antibody Fc-Binding Peptidomimetics; Journal of the American Chemical Society, 128(8), 2726-2732; and DeLano, W.L., *et al.*, Convergent solutions to binding at a protein-protein interface; Science 2000, 287, 1279-1283," it has binding activity for the Fc domain. The Fc binding activity motif according to the present invention has characteristics as follows: 1) First, key residues having Fc binding activity are specified. 2) The motif is also designed to allow a nucleophilic substitution reaction with a linker by changing the 4th leucine of SEQ ID NO: 2 into Xa_1 having a free electron pair. $(X)_n$ means that it consists of n Xs, and $(X)_{n-m}$ means that it consist of n or more and m or less Xs. Hereinafter, the carbon connected to D_3 is referred to as "beta carbon (β-carbon)."

[0303] Also, the amino acid sequence of Formula 3 may have a structure represented by the following Formula 3-1:

[0304] [Formula 3-1]

$$A\text{-}W\text{-}H\text{-}Xa_1\text{-}G\text{-}Xa_2\text{-}L\text{-}V\text{-}Xa_3 \quad \text{(SEQ ID NO: 4)}$$

[0305] wherein A is alanine, H is histidine, G is glycine, Xa_2 is glutamic acid or asparagine, L is leucine, V is valine, Xa_3 is selected from tryptophan, naphthylalanine, and phenylalanine, and

[0306] Xa_1 is  , wherein D_3 is a covalent bond or a C_{1-3} alkylene, and X_3 is NH_2, OH, or SH. In Formulas 3 and 3-1, X_3 may be NH_2. Alternatively, Xa_2 may be glutamic acid. Alternatively, Xa_3 may be tryptophan.

[0307] When the peptide including each of amino acid sequences of Formula 3 and sub-examples thereof is in a cyclic peptide form in which internal residues are connected to each other, the peptide is known to have better binding activity. The present invention provides a cyclic peptide including the amino acid sequence of Formula 3.

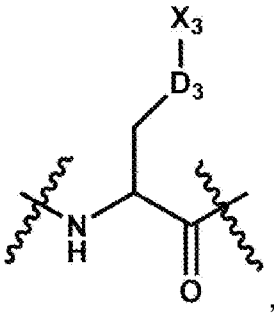
[0308] The present invention provides a cyclic peptide having a structure of the following Formula 4-1:

[0309] [Formula 4-1]

$$^{L}P\text{-}(Xaa)_2\text{-}H\text{-}Xa_1\text{-}G\text{-}Xa_2\text{-}L\text{-}V\text{-}Xa_3\text{-}^{D}P \quad \text{(SEQ ID NO: 5)}$$

[0310] wherein N-terminal ^{L}P and ^{D}P form a D-proline-L-proline template,

[0311] each Xaa is independently any amino acid except cysteine, H is histidine, G is glycine, Xa_2 is glutamic acid or asparagine, L is leucine, V is valine, Xa_3 is selected from tryptophan, naphthylalanine, and phenylalanine, and

[0312] Xa_1 is  , wherein D_3 is a covalent bond or a C_{1-3} alkylene, and X_3 is NH_2, OH, or SH.

[0313] In specific embodiments, X_3 may be NH_2. In specific embodiments, $(X)_2$ may be AW. In specific embodiments, Xa_2 may be glutamic acid. In specific embodiments, Xa_3 may be tryptophan.

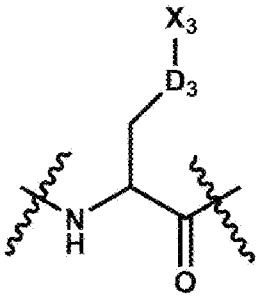
[0314] The present invention provides a cyclic peptide having a structure of the following Formula 4-2:

[0315] [Formula 4-2]

$$(Xaa)_{1-3}\text{-}C\text{-}(Xaa)_2\text{-}H\text{-}Xa_1\text{-}G\text{-}Xa_2\text{-}L\text{-}V\text{-}Xa_3\text{-}C\text{-}(Xaa)_{1-3}$$ (SEQ ID NO: 6)

[0316] wherein each Xaa is independently any amino acid except cysteine,

[0317] C is cysteine, H is histidine, G is glycine, Xa_2 is glutamic acid or asparagine, L is leucine, V is valine, Xa_3 is selected from tryptophan, naphthylalanine, and phenylalanine, and

[0318] Xa_1 is  , wherein D_3 is a covalent bond or a C_{1-3} alkylene, and X_3 is NH_2, OH, or SH.

[0319] In specific embodiments, the peptide may consist of 13 or more and 17 or less amino acid residues.

[0320] In specific embodiments, cysteine located 2 to 4 amino acids from the N-terminus and cysteine located 2 to 4 amino acids from the C-terminus may be optionally connected to each other.

[0321] In specific embodiments, X_3 may be NH_2. In specific embodiments, $(X)_2$ may be AW. In specific embodiments, Xa_2 may be glutamic acid. In specific embodiments, Xa_3 may be tryptophan.

[0322] In specific embodiments, one of the residues constituting the N-terminal $(X)_{1-3}$ and one of the residues constituting the C-terminal $(X)_{1-3}$ may be bound to each other. In one exemplary embodiment, the peptide of Formula 4-2 may have a structure of the following Formula 4-3:

[0323] [Formula 4-3]

$$^{L}P\text{-}D\text{-}C\text{-}(Xaa)_2\text{-}H\text{-}Xa_1\text{-}G\text{-}Xa_2\text{-}L\text{-}V\text{-}Xa_3\text{-}C\text{-}T\text{-}^{D}P$$ (SEQ ID NO: 7)

[0324] wherein N-terminal ^{L}P와 ^{D}P may form a D-proline-L-proline template.

[0325] In another exemplary embodiment, the peptide of Formula 4-2 may have a structure of the following Formula 4-4:

[0326] [Formula 4-4]

$$C\text{-}D\text{-}C\text{-}(Xaa)_2\text{-}H\text{-}Xa_1\text{-}G\text{-}Xa_2\text{-}L\text{-}V\text{-}Xa_3\text{-}C\text{-}T\text{-}C$$ (SEQ ID NO: 8)

[0327] wherein the N-terminal cysteine and the C-terminal cysteine may be connected to each other.

[0328] The present invention provides a cyclic peptide having a structure of the following Formula 4-5:

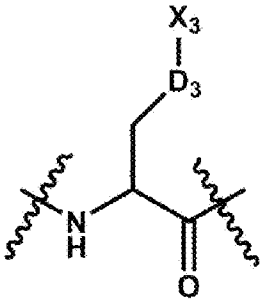
[0329] [Formula 4-5]

$$\mathbf{D - C - (Xaa)_2 - H - Xa_1 - G - Xa_2 - L - V - Xa_3 - C - T} \quad \text{(SEQ ID NO: 9)}$$

[0330] wherein D is aspartic acid, T is threonine,

[0331] each Xaa is independently any amino acid except cysteine,

[0332] C is cysteine, H is histidine, G is glycine, Xa_2 is glutamic acid or asparagine, L is leucine, V is valine, Xa_3 is selected from tryptophan, naphthylalanine, and phenylalanine, and

[0333] Xa_1 is  , wherein D_3 is a covalent bond or a C_{1-3} alkylene, and X_3 is NH_2, OH, or SH.

[0334] In specific embodiments, the peptide may consist of 13 or more and 17 or less amino acid residues.

[0335] In specific embodiments, cysteine located 2 amino acids from the N-terminus and cysteine located 2 amino acids from the C-terminus may be optionally connected to each other. In specific embodiments, X_3 may be NH_2. In specific embodiments, $(X)_2$ may be AW. In specific embodiments, Xa_2 may be glutamic acid. In specific embodiments, Xa_3 may be tryptophan.

[0336] In specific embodiments, the Formula 4-5 may be identical to the following Formula 4-6:

[0337] [Formula 4-6] (SEQ ID NO: 10)

[0338] $$\mathbf{D-C-A-W-H-Xa_1-G-E-L-V-W-C-T} \quad \text{(SEQ ID NO: 10)}$$

[0339] wherein A is alanine, and E is glutamic acid.

[0340] The peptides having the structures of Formulas 4-1 to 4-6 may have binding activity for an antibody. Also, the peptides may have binding activity for immunoglobulin G (IgG). In addition, the peptides may have binding activity for an Fc domain of the antibody.

[0341] In specific embodiments, the N-terminus of the SSFI according to the present

invention may be succinylated. In specific embodiments, the SSFI according to the present invention may include a polar amino acid residue at the N-terminal $(X)_{1-3}$ thereof. In other specific embodiments, the SSFI according to the present invention may include a polar amino acid residue at the C-terminal $(X)_{1-3}$ thereof. In this case, the polar amino acid residue includes an acidic amino acid and a basic amino acid. Also, the polar amino acid residue may include glutamic acid or aspartic acid.

[0342] 3.2. Site-specific Fc interactome according to the present invention may be arranged with an Fc domain of an antibody with a specific topology.

[0343] In the present content, the compound of Formula 4-6 is provided as one example to aid in understanding the present invention, but the scope of the present invention is not limited thereto. It will be noted that the following description also applies to the compounds of Formulas 3, 3-1, and 4-1 to 4-6, and the compound of Formula 4-6 is merely provided as one example for the sake of convenience.

[0344] As previously described above, the SSFI according to the present invention has binding activity for the Fc domain of the antibody. In this case, the SSFI may be arranged with the Fc domain with a specific topology due to the interaction between amino acid residues. The representative interaction between the SSFI sequence according to the present invention and the Fc domain includes (1) a salt linkage of the SSFI with histidine 433 in the Fc domain, (2) a hydrogen bond of the SSFI with asparagine 434, (3) a salt linkage of the SSFI with glutamic acid 380, (4) a salt linkage of the SSFI with arginine 255, and the like. These interactions and specific topologies thus formed may be determined from the research results already known in the art (see DeLano, W.L., *et al.*, Convergent solutions to binding at a protein-protein interface; Science 2000, 287, 1279-1283).

[0345] When the SSFI according to the present invention is designed, it is important for the SSFI to form a stable topology with the Fc domain. This is because an agent for transferring a first chemical functional group to the antibody according to the present invention, and a labeling process using the same are designed based on the topology between the SSFI and the Fc domain found in research (see the following sections 5.2, 5.3, and 5.4). When the interaction between the SSFI and the Fc domain becomes unstable during the design of SSFI to disturb a topology between their molecules, it is unfavorable as the interaction may give a negative effect on the labeling process.

[0346] One embodiment of the design principle will be described with reference to an exemplary compound. The SSFI represented by Formula 4-6 has a structure as follows:

[0347] [Formula 4-6]

[0348] D-C-A-W-H-Xa_1-G-E-L-V-W-C-T (SEQ ID NO: 10)

[0349] The results of simulating the topology between SSFI set forth in SEQ ID NO: 10 and the Fc domain based on the data in the articles, and the like are shown in FIGS. 3 and 4 (see DeLano, W.L., *et al.*, Convergent solutions to binding at a protein-protein interface; Science 2000, 287, 1279-1283). In this case, a histidine residue at position 5 in the SSFI forms a salt linkage with glutamic acid 380 in the Fc domain, indicating that this salt linkage has a significant effect on the topology between the SSFI and the Fc domain (see a dotted line in FIG. 4). Therefore, it is desirable that the histidine residue and its position are not changed during the design of the SSFI (see 'H' next to the residue Xa_1 in Formulas 3, 3-1, and 4-1 to 4-6). In addition, it was confirmed that glutamic acid 8 is a residue that shows electronegativity, and thus forms a salt linkage with arginine 255 in the Fc domain, which shows electropositivity, thereby exerting a significant effect on the topology between the SSFI and the Fc domain (see a dotted line in FIG. 4). Therefore, the corresponding residue is preferably an acidic amino acid that may correspond to glutamic acid, and may be replaced with asparagine (see the residue Xa_2 in Formulas 3, 3-1, and 4-1 to 4-6). When the amino acid residues are replaced with other amino acid residues or are substituted with any functional groups, this may have an effect on the intermolecular interaction, thereby exerting an effect on the topology between the SSFI and the Fc domain.

[0350] In addition, glycine at position 7 in the sequence of SEQ ID NO: 10 is a small amino acid that is required to form a bent structure of the SSFI. Therefore, it is desirable that the glycine residue and its position are not changed during the design of the SSFI (see 'G' between the residues Xa_1 and Xa_2 in Formulas 3, 3-1, and 4-1 to 4-6).

[0351] FIG. 5 shows a topology between lysine residues of the Fc domain and the SSFI. Based on the topology, it can be seen that the lysine residues of the Fc domain located closest to the residue Xa_1 are lysine 246 and 248 (FIG. 5).

[0352] A distance between an amine group of lysine 246 and the beta carbon of Xa_1 was measured. As a result, it was confirmed that the minimum distance is measured to be approximately 11.668 Å (hereinafter referred to as "$D_{246,min}$"), and the maximum distance is measured to be approximately 20.765 Å (hereinafter referred to as "$D_{246,max}$") as the bonds constituting a lysine branch rotate (FIG. 6).

[0353] A distance between an amine group of lysine 248 and the beta carbon of Xa_1 was measured. As a result, it was confirmed that the minimum distance is measured to be approximately 6.723 Å (hereinafter referred to as "$D_{248,min}$"), and the maximum distance is measured to be approximately 16.208 Å (hereinafter referred to as "$D_{248,max}$") as the bonds

constituting a lysine branch rotate (FIG. 7).

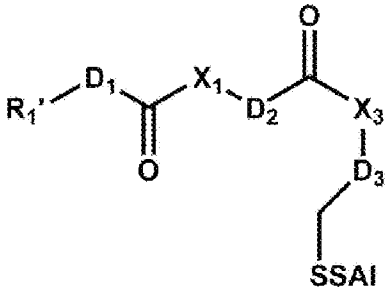
[0354] As will be described in the following section 5.3, the distance relationship may be an important consideration in the design of the linker and the SSFI, as well as the agent for transferring a first chemical functional group to an antibody.

[0355] 4. Agent for transferring first chemical functional group to antibody; Conjugate of R_1'-L_1 and SSAI (R_1'-L_2-SSAI)

[0356] According to the present invention, there is disclosed an agent for transferring a first chemical functional group to an antibody. Such a compound is herein indicated by the symbol "R_1'-L_2-SSAI." The compound is also referred to as a conjugate of R_1'-L_1 and SSAI (an R_1'-L_2-SSAI conjugate) depending on the structure thereof.

[0357] The present invention provides R_1'-L_2-SSAI having a structure of the following Formula 5:

[0358] [Formula 5]



[0359] wherein R_1' is a first chemical functional group,

[0360] D_1 is any alkylene, alkenylene, or alkynylene,

[0361] X_1 is an element that is more electronegative than carbon,

[0362] D_2 is any alkylene, alkenylene, or alkynylene,

[0363] D_3 is a covalent bond or a C_{1-3} alkylene,

[0364] X_3 is NH, O, or S, and

[0365] SSAI is a site-specific antibody interactome.

[0366] In specific embodiments, R_1' may include a click-chemistry functional group. Also, the click-chemistry functional group may include one or more selected from an alkyne, an azide, a strained alkyne, a diene, a dienophile, an alkene, a thiol, and a tetrazine. Furthermore, the click-chemistry functional group may be selected from an azide, or a strained alkyne. Further, the click-chemistry functional group may be selected from an azide, or dibenzocyclooctyne-amine. Additionally, the click-chemistry functional group may be selected from a diene, or a dienophile. Further, the click-chemistry functional group may be selected from a tetrazine, or a norbornene.

Alternatively, the click-chemistry functional group may be selected from a tetrazine, or a trans-cyclooctene. In addition, R_1' may include two or more click-chemistry functional groups.

[0367] In other specific embodiments, R_1' may include a carrier moiety, a fluorescent moiety, a drug moiety, or a radioactive moiety. Also, R_1' may include a drug moiety. In addition, R_1' may include a VC linker. In other specific embodiments, R_1' may include an antibody or an analogue thereof, which includes a paratope.

[0368] In specific embodiments, D_1 may include any one selected from a covalent bond, a C_{1-4} alkylene, a C_{2-4} alkenylene, a C_{2-4} alkynylene, and a C_{3-8} cycloalkylene. Also, D_1 may be $-CH_2OCH_2-$. In addition, D_1 may be a covalent bond.

[0369] In specific embodiments, X_1 may be NR_1, S, or O, wherein R_1 may be H, a halogen, or a substituted or unsubstituted C_{1-3} alkylene. Also, X_1 may be S.

[0370] In specific embodiments, D_2 may include any one selected from a C_{1-7} alkylene, a C_{2-7} alkenylene, a C_{2-7} alkynylene, and a C_{3-8} cycloalkylene. Also, D_2 may be a C_{1-2} alkylene. Furthermore, D_2 may be methylene.

[0371] In specific embodiments, X_3 may be NH.

[0372] In specific embodiments, the SSAI may be a peptide sequence having binding activity for Fab. In other specific embodiments, the SSAI may be a peptide sequence having binding activity for the Fc domain.

[0373] When the SSAI in Formula 5 is SSFI, this is indicated by the symbol "R_1'-L_2-SSFI." The R_1'-L_2-SSFI according to the present invention is produced by means of a nucleophilic substitution reaction of Xa_1 of the SSFI according to the present invention with a second carbonyl group of R_1'-L_1 (see the following section 4-2 and Scheme 2). Therefore, the R_1'-L_2-SSFI according to the present invention includes those in which Xa_1 of the SSFI in Formulas 3, 3-1, and 4-1 to 4-6 is substituted with (Xa_1)', but the present invention is not limited to the following exemplary embodiments thereof.

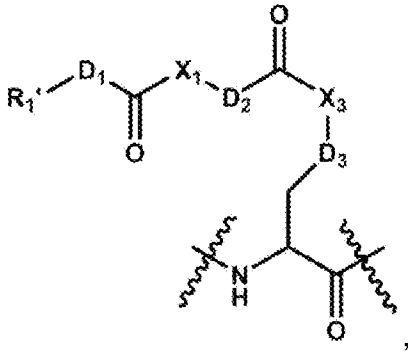
[0374] The present invention provides an R_1'-L_2-SSFI including an amino acid sequence of the following Formula 5-1:

[0375] [Formula 5-1]

[0376] $\mathbf{(Xaa)_2\text{-}H\text{-}(Xa_1)'\text{-}G\text{-}Xa_2\text{-}L\text{-}V\text{-}Xa_3}$ (SEQ ID NO: 11)

[0377] wherein each Xaa is independently any amino acid except cysteine,

[0378] H is histidine, G is glycine, Xa_2 is glutamic acid or asparagine, L is leucine, V is valine, Xa_3 is selected from tryptophan, naphthylalanine, and phenylalanine, and



[0379] $(Xa_1)'$ is ,

[0380] wherein R_1' is a first chemical functional group,

[0381] D_1 is any alkylene, alkenylene, or alkynylene,

[0382] X_1 is an element that is more electronegative than carbon,

[0383] D_2 is any alkylene, alkenylene, or alkynylene,

[0384] D_3 is a covalent bond or a C_{1-3} alkylene, and

[0385] X_3 is NH, O, or S.

[0386] In specific embodiments, R_1' may include a click-chemistry functional group. Also, the click-chemistry functional group may include one or more selected from an alkyne, an azide, a strained alkyne, a diene, a dienophile, an alkene, a thiol, and a tetrazine. Furthermore, the click-chemistry functional group may be selected from an azide, or a strained alkyne. Further, the click-chemistry functional group may be selected from an azide, or dibenzocyclooctyne-amine. Additionally, the click-chemistry functional group may be selected from a diene, or a dienophile. Further, the click-chemistry functional group may be selected from a tetrazine, or a norbornene. Alternatively, the click-chemistry functional group may be selected from a tetrazine, or a trans-cyclooctene. In addition, R_1' may include two or more click-chemistry functional groups.

[0387] In other specific embodiments, R_1' may include a carrier moiety, a fluorescent moiety, or a drug moiety. Also, R_1' may include a VC linker. In addition, R_1' may include a radioactive moiety. Also, R_1' may include a drug moiety. In other specific embodiments, R_1' may include an antibody or an analogue thereof, which includes a paratope.

[0388] In specific embodiments, D_1 may include any one selected from a covalent bond, a C_{1-4} alkylene, a C_{2-4} alkenylene, a C_{2-4} alkynylene, and a C_{3-8} cycloalkylene. Also, D_1 may be $-CH_2OCH_2-$. In addition, D_1 may be a covalent bond.

[0389] In specific embodiments, X_1 may be NR_1, S, or O, wherein R_1 may be H, a halogen, or a substituted or unsubstituted C_{1-3} alkylene.

[0390] In specific embodiments, D_2 may include any one selected from a C_{1-7} alkylene, a C_{2-7} alkenylene, a C_{2-7} alkynylene, and a C_{3-8} cycloalkylene. Also, D_2 may be a C_{1-2} alkylene.

Furthermore, D_2 may be methylene.

[0391] In specific embodiments, X_3 may be NH.

[0392] In specific embodiments, $(X)_2$ may be AW. In specific embodiments, Xa_2 may be glutamic acid. In specific embodiments, Xa_3 may be tryptophan.

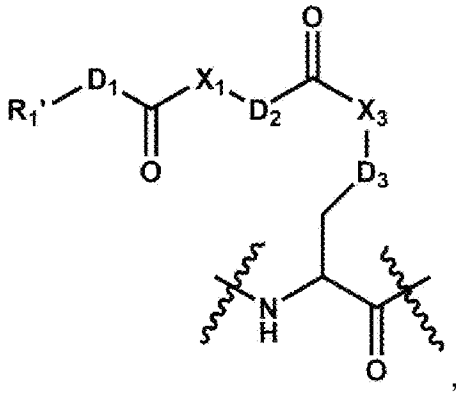
[0393] The present invention provides an R_1'-L_2-SSFI having a structure of the following Formula 5-2:

[0394] [Formula 5-2]

[0395] $\mathbf{(Xaa)_{1-3}\text{-}C\text{-}(Xaa)_2\text{-}H\text{-}(Xa_1)'\text{-}G\text{-}Xa_2\text{-}L\text{-}V\text{-}Xa_3\text{-}C\text{-}(Xaa)_{1-3}}$ (SEQ ID NO: 12)

[0396] wherein each Xaa is independently any amino acid except cysteine,

[0397] C is cysteine, H is histidine, G is glycine, Xa_2 is glutamic acid or asparagine, L is leucine, V is valine, Xa_3 is selected from tryptophan, naphthylalanine, and phenylalanine, and

[0398] (Xa_1)' is 

,

[0399] wherein R_1' is a first chemical functional group,

[0400] D_1 is any alkylene, alkenylene, or alkynylene,

[0401] X_1 is an element that is more electronegative than carbon,

[0402] D_2 is any alkylene, alkenylene, or alkynylene,

[0403] D_3 is a covalent bond or a C_{1-3} alkylene, and

[0404] X_3 is NH, O, or S.

[0405] In specific embodiments, R_1' may include a click-chemistry functional group. Also, the click-chemistry functional group may include one or more selected from an alkyne, an azide, a strained alkyne, a diene, a dienophile, an alkene, a thiol, and a tetrazine. Furthermore, the click-chemistry functional group may be selected from an azide, or a strained alkyne. Further, the click-chemistry functional group may be selected from an azide, or dibenzocyclooctyne-amine. Additionally, the click-chemistry functional group may be selected from a diene, or a dienophile. Further, the click-chemistry functional group may be selected from a tetrazine, or a norbornene.

Alternatively, the click-chemistry functional group may be selected from a tetrazine, or a trans-cyclooctene. In addition, R_1' may include two or more click-chemistry functional groups.

[0406] In other specific embodiments, R_1' may include a carrier moiety, a fluorescent moiety, a drug moiety, or a radioactive moiety. Also, R_1' may include a drug moiety. In addition, R_1' may include a VC linker. In other specific embodiments, R_1' may include an antibody or an analogue thereof, which includes a paratope.

[0407] In specific embodiments, D_1 may include any one selected from a covalent bond, a C_{1-4} alkylene, a C_{2-4} alkenylene, a C_{2-4} alkynylene, and a C_{3-8} cycloalkylene. Also, D_1 may be $-CH_2OCH_2-$. In addition, D_1 may be a covalent bond.

[0408] In specific embodiments, X_1 may be NR_1, S, or O, wherein R_1 may be H, a halogen, or a substituted or unsubstituted C_{1-3} alkylene.

[0409] In specific embodiments, D_2 may include any one selected from a C_{1-7} alkylene, a C_{2-7} alkenylene, a C_{2-7} alkynylene, and a C_{3-8} cycloalkylene. Also, D_2 may be a C_{1-2} alkylene. Furthermore, D_2 may be methylene.

[0410] In specific embodiments, Formula 5-2 may consist of 13 or more and 17 or less amino acid residues (including (Xa_1)').

[0411] In specific embodiments, cysteine located 2 to 4 amino acids from the N-terminus and cysteine located 2 to 4 amino acids from the C-terminus may be optionally connected to each other.

[0412] In specific embodiments, X_3 may be NH. In specific embodiments, $(X)_2$ may be AW. In specific embodiments, Xa_2 may be glutamic acid. In specific embodiments, Xa_3 may be tryptophan.

[0413] In specific embodiments, one of the residues constituting the N-terminal $(X)_{1-3}$ and one of the residues constituting the C-terminal $(X)_{1-3}$ may be bound to each other.

[0414] In specific embodiments, Formula 5-2 may be identical to Formula 5-3:

[0415] [Formula 5-3]

[0416] **D-C-A-W-H-(Xa$_1$)'-G-E-L-V-W-C-T** (SEQ ID NO: 13)

[0417] wherein A is alanine, and E is glutamic acid.

[0418] The R_1'-L_2-SSAI or R_1'-L_2-SSFI having the structures of Formulas 5 and 5-1 to 5-3 may have binding activity for an antibody. Also, the R_1'-L_2-SSAI or R_1'-L_2-SSFI may have binding activity for immunoglobulin G (IgG). In addition, the R_1'-L_2-SSAI or R_1'-L_2-SSFI may have binding activity for an Fc domain of the antibody.

[0419] 4.1. Agent for transferring first click-chemistry functional group to antibody;

Conjugate of H_1-L_1 and SSAI (H_1-L_2-SSAI)

[0420] According to the present invention, there is disclosed an agent for transferring a first click-chemistry functional group to an antibody. Such a compound is herein indicated by the symbol "H_1-L_2-SSAI." The compound is also referred to as a conjugate of H_1-L_1 and SSAI (an H_1-L_2-SSAI conjugate) depending on the structure thereof. In this case, when the SSAI is SSFI, this is indicated by the symbol 'H_1-L_2-SSFI.'

[0421] The H_1-L_2-SSAI or H_1-L_2-SSFI according to the present invention includes those in which R_1' contains a click-chemistry functional group in the section '4. Agent for transferring first chemical functional group to antibody,' but the present invention is not limited to the following exemplary embodiments thereof.

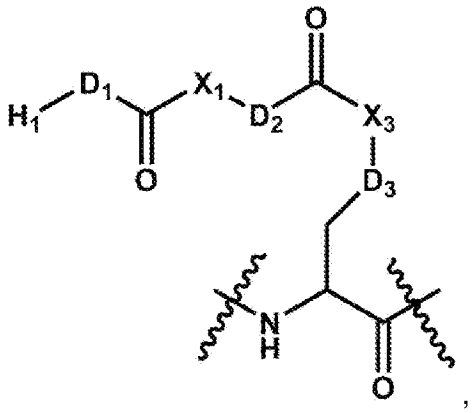
[0422] The present invention provides an H_1-L_2-SSFI including an amino acid sequence of the following Formula 6-1:

[0423] [Formula 6-1]

[0424] $\mathbf{(Xaa)_2\text{-}H\text{-}(Xa_1)'\text{-}G\text{-}Xa_2\text{-}L\text{-}V\text{-}Xa_3}$ (SEQ ID NO: 14)

[0425] wherein each Xaa is independently any amino acid except cysteine,

[0426] H is histidine, G is glycine, Xa_2 is glutamic acid or asparagine, L is leucine, V is valine, Xa_3 is selected from tryptophan, naphthylalanine, and phenylalanine, and

[0427] $(Xa_1)'$ is 

,

[0428] wherein H_1 is a first click-chemistry functional group,

[0429] D_1 is any alkylene, alkenylene, or alkynylene,

[0430] X_1 is an element that is more electronegative than carbon,

[0431] D_2 is any alkylene, alkenylene, or alkynylene,

[0432] D_3 is a covalent bond or a C_{1-3} alkylene, and

[0433] X_3 is NH, O, or S.

[0434] In specific embodiments, H_1 may include any one selected from an alkyne, an

azide, a strained alkyne, a diene, a dienophile, an alkene, a thiol, and a tetrazine. Furthermore, H_1 may be an azide, or a strained alkyne. Further, H_1 may be an azide, or dibenzocyclooctyne-amine. Additionally, H_1 may be a diene, or a dienophile. Further, H_1 may be a tetrazine, or a norbornene. Alternatively, H_1 may be a tetrazine, or a trans-cyclooctene.

[0435] In specific embodiments, D_1 may include any one selected from a covalent bond, a C_{1-4} alkylene, a C_{2-4} alkenylene, a C_{2-4} alkynylene, and a C_{3-8} cycloalkylene. Also, D_1 may be $-CH_2OCH_2-$. In addition, D_1 may be a covalent bond.

[0436] In specific embodiments, X_1 may be NR_1, S, or O, wherein R_1 may be H, a halogen, or a substituted or unsubstituted C_{1-3} alkylene.

[0437] In specific embodiments, D_2 may include any one selected from a C_{1-7} alkylene, a C_{2-7} alkenylene, a C_{2-7} alkynylene, and a C_{3-8} cycloalkylene. Also, D_2 may be a C_{1-2} alkylene. Furthermore, D_2 may be methylene.

[0438] In specific embodiments, X_3 may be NH.

[0439] In specific embodiments, $(X)_2$ may be AW. In specific embodiments, Xa_2 may be glutamic acid. In specific embodiments, Xa_3 may be tryptophan.

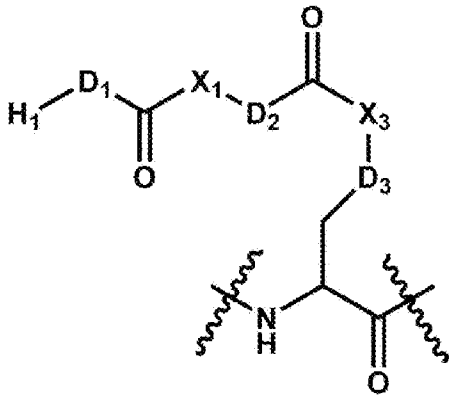
[0440] The present invention provides an H_1-L_2-SSFI having a structure of the following Formula 6-2:

[0441] [Formula 6-2]

[0442] $\mathbf{(Xaa)_{1-3}\text{-}C\text{-}(Xaa)_2\text{-}H\text{-}(Xa_1)'\text{-}G\text{-}Xa_2\text{-}L\text{-}V\text{-}Xa_3\text{-}C\text{-}(Xaa)_{1-3}}$ (SEQ ID NO: 15)

[0443] wherein each Xaa is independently any amino acid except cysteine,

[0444] C is cysteine, H is histidine, G is glycine, Xa_2 is glutamic acid or asparagine, L is leucine, V is valine, Xa_3 is selected from tryptophan, naphthylalanine, and phenylalanine, and

[0445] $(Xa_1)'$ is 

,

[0446] wherein H_1 is a first click-chemistry functional group,

[0447] D_1 is any alkylene, alkenylene, or alkynylene,

[0448] X_1 is an element that is more electronegative than carbon,

[0449] D_2 is any alkylene, alkenylene, or alkynylene,

[0450] D_3 is a covalent bond or a C_{1-3} alkylene, and

[0451] X_3 is NH, O, or S.

[0452] In specific embodiments, H_1 may include any one selected from an alkyne, an azide, a strained alkyne, a diene, a dienophile, an alkene, a thiol, and a tetrazine. Furthermore, H_1 may be an azide, or a strained alkyne. Further, H_1 may be an azide, or dibenzocyclooctyne-amine. Additionally, H_1 may be a diene, or a dienophile. Further, H_1 may be a tetrazine, or a norbornene. Alternatively, H_1 may be a tetrazine, or a trans-cyclooctene.

[0453] In specific embodiments, D_1 may include any one selected from a covalent bond, a C_{1-4} alkylene, a C_{2-4} alkenylene, a C_{2-4} alkynylene, and a C_{3-8} cycloalkylene. Also, D_1 may be $-CH_2OCH_2-$. In addition, D_1 may be a covalent bond.

[0454] In specific embodiments, X_1 may be NR_1, S, or O, wherein R_1 may be H, a halogen, or a substituted or unsubstituted C_{1-3} alkylene.

[0455] In specific embodiments, D_2 may include any one selected from a C_{1-7} alkylene, a C_{2-7} alkenylene, a C_{2-7} alkynylene, and a C_{3-8} cycloalkylene. Also, D_2 may be a C_{1-2} alkylene. Furthermore, D_2 may be methylene.

[0456] In specific embodiments, Formula 5-2 may consist of 13 or more and 17 or less amino acid residues (including $(Xa_1)'$).

[0457] In specific embodiments, cysteine located 2 to 4 amino acids from the N-terminus and cysteine located 2 to 4 amino acids from the C-terminus may be optionally connected to each other.

[0458] In specific embodiments, X_3 may be NH. In specific embodiments, $(X)_2$ may be AW. In specific embodiments, Xa_2 may be glutamic acid. In specific embodiments, Xa_3 may be tryptophan.

[0459] In specific embodiments, one of the residues constituting the N-terminal $(X)_{1-3}$ and one of the residues constituting the C-terminal $(X)_{1-3}$ may be bound to each other.

[0460] In specific embodiments, Formula 6-2 may be identical to Formula 6-3:

[0461] [Formula 6-3] (SEQ ID NO: 16)

[0462] $\mathbf{D\text{-}C\text{-}A\text{-}W\text{-}H\text{-}(Xa_1)'\text{-}G\text{-}E\text{-}L\text{-}V\text{-}W\text{-}C\text{-}T}$ (SEQ ID NO: 16)

[0463] wherein A is alanine, and E is glutamic acid.

[0464] The H_1-L_2-SSAI or H_1-L_2-SSFI having the structures of Formulas 6-1 to 6-3 may have binding activity for an antibody. Also, the H_1-L_2-SSAI or H_1-L_2-SSFI may have binding

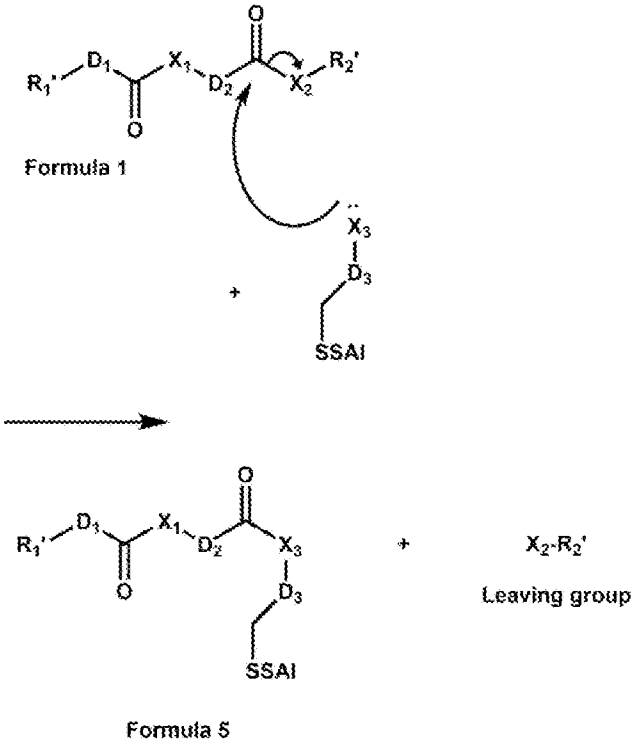
activity for immunoglobulin G (IgG). In addition, the H_1-L_2-SSAI or H_1-L_2-SSFI may have binding activity for an Fc domain of the antibody.

[0465] 4.2. Method of preparing agent for transferring first chemical functional group to antibody

[0466] According to the present invention, there are disclosed methods of preparing the R_1'-L_2-SSAI, the R_1'-L_2-SSFI, the H_1-L_2-SSAI, and the H_1-L_2-SSFI (hereinafter generally referred to as "R_1'-L_2-SSAI"). It will be noted that a description of the following preparation methods is provided to aid in understanding the present invention.

[0467] For example, a method of preparing a compound of Formula 5 will be described. In specific embodiments, the compound of Formula 5 may be prepared through a reaction of the following Scheme 2.

[0468] [Scheme 2]

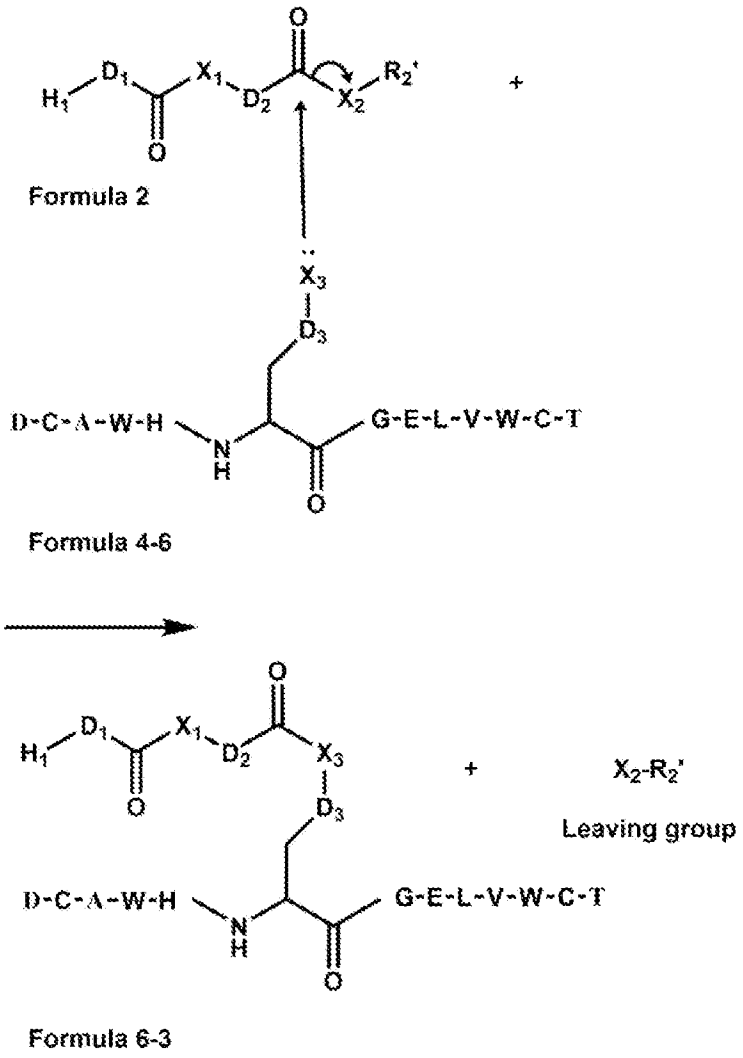


Formula 5

[0469] The R_1'-L_2-SSAI according to the present invention may be prepared by allowing a site-specific antibody interactome (SSAI) to react with the linker (R_1'-L_1) according to the present invention. The SSAI according to the present invention is designed to include a nucleophile X_3. The X_3 may attack an activated carbonyl group included in the linker to cause a nucleophilic substitution reaction. In this case, as the X_3 attacks the second carbonyl carbon, the R_1'-L_2-SSAI according to the present invention is prepared.

[0470] As a more specific example, a method of preparing a compound of Formula 6-3 will be described. In specific embodiments, the compound of Formula 6-3 may be prepared through a reaction of the following Scheme 3.

[0471] [Scheme 3]



[0472] The SSAI including the amino acid sequence of Formula 3 or 3-1 according to the present invention and having each of the structures of Formulas 4-1 to 4-6 includes an Xa_1 residue including a nucleophile X_3. As the X_3 attacks the second carbonyl group included in the linker, the R_1'-L_2-SSAI according to the present invention is prepared.

[0473] In specific embodiments, the basicity of the leaving group including X_2 may be lower than that of the leaving group including X_1 in the linker according to the present invention. In specific embodiments, the linker according to the present invention may allow X_2-R_2' connected to the second carbonyl group to form a good leaving group. Also, X_2 may be O, and R_2' may be

N-succinimide, p-nitrophenyl, or pentafluorophenyl. In this case, the reactivity of the second carbonyl group is higher than that of the first carbonyl group. Therefore, X_3 of the SSAI may specifically attack the second carbonyl group of the linker.

[0474] According to the present invention, there is disclosed a method of preparing an agent for transferring a first chemical functional group to an antibody.

[0475] The present invention provides a method of preparing an R_1'-L_2-SSAI, which comprises:

[0476] reacting a linker according to the present invention with a site-specific antibody interactome according to the present invention.

[0477] In specific embodiments, the linker may be any one selected from Formulas 1, 2, and 2-1 to 2-3.

[0478] In specific embodiments, the site-specific antibody interactome may include or have any one structure selected from Formulas 3, 3-1, and 4-1 to 4-6.

[0479] Also, the linker may have the structure of Formula 2, and the site-specific antibody interactome may have any one structure selected from Formulas 4-2 to 4-6. Furthermore, the site-specific antibody interactome may have the structure of Formula 4-6.

[0480] According to the present invention, there is disclosed a method of preparing an agent for transferring a first click-chemistry functional group to an antibody.

[0481] The present invention provides a method of preparing an H_1-L_2-SSAI, which comprises:

[0482] reacting a linker according to the present invention with a site-specific antibody interactome according to the present invention.

[0483] In specific embodiments, the linker may be any one selected from Formulas 2, and 2-1 to 2-3.

[0484] In specific embodiments, the site-specific antibody interactome may include or have any one structure selected from Formulas 3, 3-1, and 4-1 to 4-6.

[0485] Also, the linker may have the structure of Formula 2, and the site-specific antibody interactome may have any one structure selected from Formulas 4-2 to 4-6. Furthermore, the site-specific antibody interactome may have the structure of Formula 4-6.

[0486] According to the present invention, there is disclosed a kit for preparing an agent for transferring a first chemical functional group to an antibody.

[0487] The present invention provides a kit for preparing an agent for transferring a first chemical functional group to an antibody, which comprises the linker according to the present

invention and the site-specific antibody interactome according to the present invention.

[0488] In specific embodiments, the linker may be any one selected from Formulas 1, 2, and 2-1 to 2-3.

[0489] In specific embodiments, the site-specific antibody interactome may include or have any one structure selected from Formulas 3, 3-1, and 4-1 to 4-6.

[0490] Also, the linker may have the structure of Formula 2, and the site-specific antibody interactome may have any one structure selected from Formulas 4-2 to 4-6. Furthermore, the site-specific antibody interactome may have the structure of Formula 4-6.

[0491] According to the present invention, there is disclosed a kit for preparing an agent for transferring a first click-chemistry functional group to an antibody.

[0492] The present invention provides a kit for preparing an agent for transferring a first click-chemistry functional group to an antibody, which comprises a linker containing the first click-chemistry functional group according to the present invention, and a site-specific antibody interactome.

[0493] In specific embodiments, the linker may be any one selected from Formulas 2, and 2-1 to 2-3.

[0494] In specific embodiments, the site-specific antibody interactome may include or have any one structure selected from Formulas 3, 3-1, and 4-1 to 4-6.

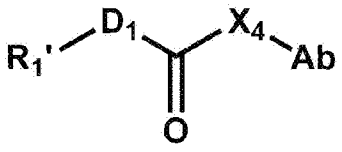
[0495] Also, the linker may have the structure of Formula 2, and the site-specific antibody interactome may have any one structure selected from Formulas 4-2 to 4-6. Furthermore, the site-specific antibody interactome may have the structure of Formula 4-6.

[0496] 5. Antibody containing first chemical functional group (R_1'-Ab)

[0497] According to the present invention, there is disclosed an antibody containing a first chemical functional group. Such a compound is herein indicated by the symbol "R_1'-Ab."

[0498] The present invention provides an R_1'-Ab represented by Formula 7:

[0499] [Formula 7]



[0500] wherein R_1' is a first chemical functional group,

[0501] D_1 is any alkylene, alkenylene, or alkynylene,

[0502] X_4 is NH, O, or S, and

[0503] Ab is an antibody.

[0504] In specific embodiments, R_1' may be a click-chemistry functional group. Also, R_1' may include any one selected from an alkyne, an azide, a strained alkyne, a diene, a dienophile, an alkene, a thiol, and a tetrazine. Furthermore, R_1' may be an azide, or a strained alkyne. Further, R_1' may be an azide, or dibenzocyclooctyne-amine. Additionally, R_1' may be a diene, or a dienophile. Further, R_1' may be a tetrazine, or a norbornene. Alternatively, R_1' may be a tetrazine, or a trans-cyclooctene.

[0505] In other specific embodiments, R_1' may include a carrier moiety, a fluorescent moiety, a drug moiety, or a radioactive moiety. Also, R_1' may include a drug moiety. In addition, R_1' may include a VC linker. In other specific embodiments, R_1' may include an antibody or an analogue thereof, which includes a paratope.

[0506] In specific embodiments, D_1 may include any one selected from a covalent bond, a C_{1-4} alkylene, a C_{2-4} alkenylene, a C_{2-4} alkynylene, and a C_{3-8} cycloalkylene. Also, D_1 may be $-CH_2OCH_2-$. In addition, D_1 may be a covalent bond.

[0507] In one specific embodiment, X_4 may be NH.

[0508] In specific embodiments, Ab may be a human antibody. In other specific embodiments, Ab may be a non-human animal antibody. In specific embodiments, Ab may be immunoglobulin G (IgG). In specific embodiments, Ab may be a whole antibody. In other specific embodiments, Ab may be a fragment of the antibody.

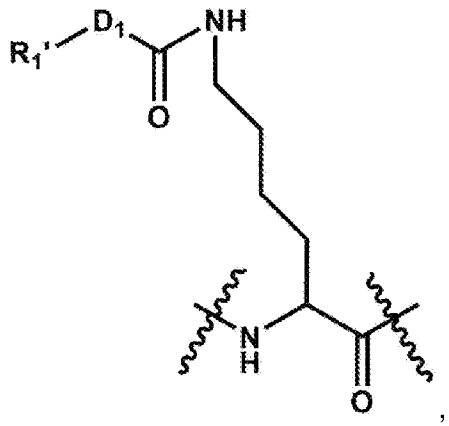
[0509] In specific embodiments, X_4 and Ab may be connected via an Fab domain of Ab. In other specific embodiments, X_4 and Ab may be connected via an Fc domain of Ab. Also, X_4 and Ab may be connected via lysine 246 or 248 in the Fc domain of Ab. Furthermore, X_4 and Ab may be connected via lysine 246 in the Fc domain of Ab. Further, X_4 and Ab may be connected via lysine 248 in the Fc domain of Ab. Alternatively, X_4 and Ab may be connected via lysine 246 and 248 in the Fc domain of Ab. In specific embodiments, X_4 and Ab may be connected via only one of two Fc domains of Ab. In other specific embodiments, X_4 and Ab may be connected via both of the two Fc domains of Ab.

[0510] The present invention provides an antibody or a fragment thereof, which includes an amino acid sequence of the following Formula 7-1:

[0511] [Formula 7-1]

[0512] G—P—S—V—F—L—F—P—P-(K)'-P—K—D—T—L—M—I (SEQ ID NO: 17)

[0513] wherein G is glycine, P is proline, S is serine, V is valine, F is phenylalanine, L is leucine, K is lysine, D is aspartic acid, T is threonine, M is methionine, I is isoleucine, and



[0514] (K)' is ,

[0515] wherein R_1' is a first chemical functional group, and

[0516] D_1 is any alkylene, alkenylene, or alkynylene. The antibody or fragment thereof has R_1' connected via lysine 246 in an Fc domain thereof, or a site corresponding to the lysine 246.

[0517] In specific embodiments, R_1' may include a click-chemistry functional group. Also, the click-chemistry functional group may include one or more selected from an alkyne, an azide, a strained alkyne, a diene, a dienophile, an alkene, a thiol, and a tetrazine. Furthermore, the click-chemistry functional group may be selected from an azide, or a strained alkyne. Further, the click-chemistry functional group may be selected from an azide, or dibenzocyclooctyne-amine. Additionally, the click-chemistry functional group may be selected from a diene, or a dienophile. Further, the click-chemistry functional group may be selected from a tetrazine, or a norbornene. Alternatively, the click-chemistry functional group may be selected from a tetrazine, or a trans-cyclooctene. In addition, R_1' may include two or more click-chemistry functional groups.

[0518] In other specific embodiments, R_1' may include a carrier moiety, a fluorescent moiety, a drug moiety, or a radioactive moiety. Also, R_1' may include a drug moiety. In addition, R_1' may include a VC linker. In other specific embodiments, R_1' may include an antibody or an analogue thereof, which includes a paratope.

[0519] In specific embodiments, D_1 may include any one selected from a covalent bond, a C_{1-4} alkylene, a C_{2-4} alkenylene, a C_{2-4} alkynylene, and a C_{3-8} cycloalkylene. Also, D_1 may be $-CH_2OCH_2-$. In addition, D_1 may be a covalent bond.

[0520] In specific embodiments, the antibody may be a human antibody. In other specific embodiments, the antibody may be a non-human animal antibody. In specific embodiments, the antibody may be immunoglobulin G (IgG). In specific embodiments, the antibody may be a whole antibody. In other specific embodiments, the antibody may be a fragment of the antibody.

[0521] In specific embodiments, the antibody may include the amino acid sequence of

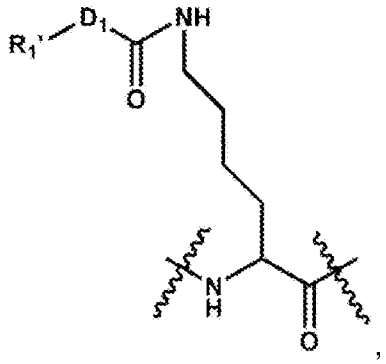
Formula 7-1 in only one of the two Fc domains thereof. In other specific embodiments, the antibody may include the amino acid sequence of Formula 7-1 in both of the two Fc domains thereof.

[0522] The present invention provides an antibody or a fragment thereof, which includes an amino acid sequence of the following Formula 7-2:

[0523] [Formula 7-2]

G—P—S—V—F—L—F—P—P—K—P-(K)'-D—T—L—M—I (SEQ ID NO: 18)

[0524] wherein G is glycine, P is proline, S is serine, V is valine, F is phenylalanine, L is leucine, K is lysine, D is aspartic acid, T is threonine, M is methionine, I is isoleucine, and

[0525] (K)' is 

,

[0526] wherein R_1' is a first chemical functional group, and

[0527] D_1 is any alkylene, alkenylene, or alkynylene. The antibody or fragment thereof has R_1' connected via lysine 248 in an Fc domain thereof, or a site corresponding to the lysine 248.

[0528] In specific embodiments, R_1' may include a click-chemistry functional group. Also, the click-chemistry functional group may include one or more selected from an alkyne, an azide, a strained alkyne, a diene, a dienophile, an alkene, a thiol, and a tetrazine. Furthermore, the click-chemistry functional group may be selected from an azide, or a strained alkyne. Further, the click-chemistry functional group may be selected from an azide, or dibenzocyclooctyne-amine. Additionally, the click-chemistry functional group may be selected from a diene, or a dienophile. Further, the click-chemistry functional group may be selected from a tetrazine, or a norbornene. Alternatively, the click-chemistry functional group may be selected from a tetrazine, or a trans-cyclooctene. In addition, R_1' may include two or more click-chemistry functional groups.

[0529] In other specific embodiments, R_1' may include a carrier moiety, a fluorescent moiety, a drug moiety, or a radioactive moiety. Also, R_1' may include a drug moiety. In addition, R_1' may include a VC linker. In other specific embodiments, R_1' may include an antibody or an analogue thereof, which includes a paratope.

[0530] In specific embodiments, D_1 may include any one selected from a covalent bond, a C_{1-4} alkylene, a C_{2-4} alkenylene, a C_{2-4} alkynylene, and a C_{3-8} cycloalkylene. Also, D_1 may be $-CH_2OCH_2-$. In addition, D_1 may be a covalent bond.

[0531] In specific embodiments, the antibody may be a human antibody. In other specific embodiments, the antibody may be a non-human animal antibody. In specific embodiments, the antibody may be immunoglobulin G (IgG). In specific embodiments, the antibody may be a whole antibody. In other specific embodiments, the antibody may be a fragment of the antibody.

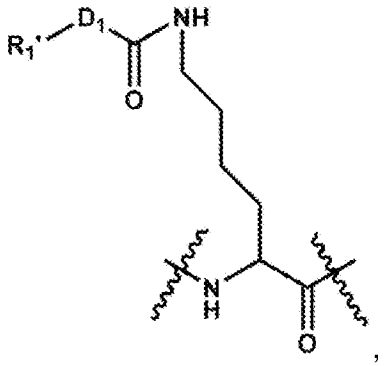
[0532] In specific embodiments, the antibody may include the amino acid sequence of Formula 7-2 in only one of the two Fc domains thereof. In other specific embodiments, the antibody may include the amino acid sequence of Formula 7-2 in both of the two Fc domains thereof.

[0533] The present invention provides an antibody or a fragment thereof, which includes an amino acid sequence of the following Formula 7-3:

[0534] [Formula 7-3] (SEQ ID NO: 19)

G—P—S—V—F—L—F—P—P—(K)'—P—(K)'—D—T—L—M—I (SEQ ID NO: 19)

[0535] wherein G is glycine, P is proline, S is serine, V is valine, F is phenylalanine, L is leucine, K is lysine, D is aspartic acid, T is threonine, M is methionine, I is isoleucine, and

[0536] (K)' is  ,

[0537] wherein R_1' is a first chemical functional group, and

[0538] D_1 is any alkylene, alkenylene, or alkynylene. The antibody or fragment thereof has R_1' connected via lysine 246 and 248 in an Fc domain thereof, or sites corresponding to the lysine 246 and 248.

[0539] In specific embodiments, R_1' may include a click-chemistry functional group. Also, the click-chemistry functional group may include one or more selected from an alkyne, an azide, a strained alkyne, a diene, a dienophile, an alkene, a thiol, and a tetrazine. Furthermore, the click-chemistry functional group may be selected from an azide, or a strained alkyne. Further, the click-chemistry functional group may be selected from an azide, or dibenzocyclooctyne-amine.

Additionally, the click-chemistry functional group may be selected from a diene, or a dienophile. Further, the click-chemistry functional group may be selected from a tetrazine, or a norbornene. Alternatively, the click-chemistry functional group may be selected from a tetrazine, or a trans-cyclooctene. In addition, R_1' may include two or more click-chemistry functional groups.

[0540] In other specific embodiments, R_1' may include a carrier moiety, a fluorescent moiety, a drug moiety, or a radioactive moiety. Also, R_1' may include a drug moiety. In addition, R_1' may include a VC linker. In other specific embodiments, R_1' may include an antibody or an analogue thereof, which includes a paratope.

[0541] In specific embodiments, D_1 may include any one selected from a covalent bond, a C_{1-4} alkylene, a C_{2-4} alkenylene, a C_{2-4} alkynylene, and a C_{3-8} cycloalkylene. Also, D_1 may be $-CH_2OCH_2-$. In addition, D_1 may be a covalent bond.

[0542] In specific embodiments, the antibody may be a human antibody. In other specific embodiments, the antibody may be a non-human animal antibody. In specific embodiments, the antibody may be immunoglobulin G (IgG). In specific embodiments, the antibody may be a whole antibody. In other specific embodiments, the antibody may be a fragment of the antibody.

[0543] In specific embodiments, the antibody may include the amino acid sequence of Formula 7-3 in only one of the two Fc domains thereof. In other specific embodiments, the antibody may include the amino acid sequence of Formula 7-3 in both of the two Fc domains thereof.

[0544] The present invention provides an antibody or a fragment thereof, which includes one or more amino acid sequences selected from Formulas 7-1, 7-2, and 7-3. In this case, the contents of the sequences of Formulas 7-1 to 7-3 are as described above.

[0545] In specific embodiments, D_1 may be a covalent bond.

[0546] In specific embodiments, the antibody or fragment thereof may include only the amino acid sequence of Formula 7-1 and may not include the amino acid sequences of Formulas 7-2 and 7-3. Also, the antibody or fragment thereof may include the amino acid sequence of Formula 7-1 in only one of the two Fc domains thereof. In addition, the antibody may include the amino acid sequence of Formula 7-1 in both of the two Fc domains thereof.

[0547] In other specific embodiments, the antibody or fragment thereof may include only the amino acid sequence of Formula 7-2 and may not include the amino acid sequences of Formulas 7-1 and 7-3. Also, the antibody or fragment thereof may include the amino acid sequence of Formula 7-2 in only one of the two Fc domains thereof. In addition, the antibody may include the amino acid sequence of Formula 7-2 in both of the two Fc domains thereof.

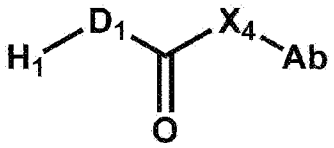
[0548] In specific embodiments, the antibody or fragment thereof may include only the amino acid sequence of Formula 7-3 and may not include the amino acid sequences of Formulas 7-1 and 7-2. Also, the antibody or fragment thereof may include the amino acid sequence of Formula 7-3 in only one of the two Fc domains thereof. In addition, the antibody may include the amino acid sequence of Formula 7-3 in both of the two Fc domains thereof.

[0549] 5.1. Antibody containing first click-chemistry functional group

[0550] According to the present invention, there is disclosed an antibody containing a first click-chemistry functional group. Such a compound is herein indicated by the symbol "H_1-Ab."

[0551] The present invention provides an H_1-Ab represented by Formula 8:

[0552] [Formula 8]



[0553] wherein H_1 is a first click-chemistry functional group,

[0554] D_1 is any alkylene, alkenylene, or alkynylene,

[0555] X_4 is NH, O, or S, and

[0556] Ab is an antibody.

[0557] In specific embodiments, H_1 may include any one selected from an alkyne, an azide, a strained alkyne, a diene, a dienophile, an alkene, a thiol, and a tetrazine. Furthermore, H_1 may be an azide, or a strained alkyne. Further, H_1 may be an azide, or dibenzocyclooctyne-amine. Additionally, H_1 may be a diene, or a dienophile. Further, H_1 may be a tetrazine, or a norbornene. Alternatively, H_1 may be a tetrazine, or a trans-cyclooctene.

[0558] In specific embodiments, D_1 may include any one selected from a covalent bond, a C_{1-4} alkylene, a C_{2-4} alkenylene, a C_{2-4} alkynylene, and a C_{3-8} cycloalkylene. Also, D_1 may be $-CH_2OCH_2-$. In addition, D_1 may be a covalent bond.

[0559] In one specific embodiment, X_4 may be NH.

[0560] In specific embodiments, Ab may be a human antibody. In other specific embodiments, Ab may be a non-human animal antibody. In specific embodiments, Ab may be immunoglobulin G (IgG). In specific embodiments, Ab may be a whole antibody. In other specific embodiments, Ab may be a fragment of the antibody. In specific embodiments, Ab may be a wild-type antibody. In other specific embodiments, Ab may be a manipulated antibody.

[0561] In specific embodiments, X_4 and Ab may be connected via an Fab domain of Ab.

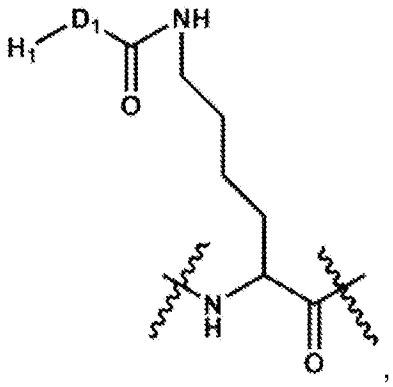
In other specific embodiments, X_4 and Ab may be connected via an Fc domain of Ab. Also, X_4 and Ab may be connected via lysine 246 or 248 in the Fc domain of Ab. Furthermore, X_4 and Ab may be connected via lysine 246 in the Fc domain of Ab. Further, X_4 and Ab may be connected via lysine 248 in the Fc domain of Ab. Alternatively, X_4 and Ab may be connected via lysine 246 and 248 in the Fc domain of Ab. In specific embodiments, X_4 and Ab may be connected via only one of two Fc domains of Ab. In other specific embodiments, X_4 and Ab may be connected via both of the two Fc domains of Ab.

[0562] The present invention provides an antibody or a fragment thereof, which includes an amino acid sequence of the following Formula 8-1:

[0563] [Formula 8-1]

G—P—S—V—F—L—F—P—P—(K)'—P—K—D—T—L—M—I (SEQ ID NO: 20)

[0564] wherein G is glycine, P is proline, S is serine, V is valine, F is phenylalanine, L is leucine, K is lysine, D is aspartic acid, T is threonine, M is methionine, I is isoleucine, and

[0565] (K)' is  ,

[0566] wherein H_1 is a first click-chemistry functional group, and

[0567] D_1 is any alkylene, alkenylene, or alkynylene. The antibody or fragment thereof has H_1 connected via lysine 246 in an Fc domain thereof, or a site corresponding to the lysine 246.

[0568] In specific embodiments, H_1 may include any one selected from an alkyne, an azide, a strained alkyne, a diene, a dienophile, an alkene, a thiol, and a tetrazine. Furthermore, H_1 may be an azide, or a strained alkyne. Further, H_1 may be an azide, or dibenzocyclooctyne-amine. Additionally, H_1 may be a diene, or a dienophile. Further, H_1 may be a tetrazine, or a norbornene. Alternatively, H_1 may be a tetrazine, or a trans-cyclooctene.

[0569] In specific embodiments, D_1 may include any one selected from a covalent bond, a C_{1-4} alkylene, a C_{2-4} alkenylene, a C_{2-4} alkynylene, and a C_{3-8} cycloalkylene. Also, D_1 may be $-CH_2OCH_2-$. In addition, D_1 may be a covalent bond.

[0570] In specific embodiments, the antibody may be a human antibody. In other specific embodiments, the antibody may be a non-human animal antibody. In specific embodiments, the antibody may be immunoglobulin G (IgG). In specific embodiments, the antibody may be a whole antibody. In other specific embodiments, the antibody may be a fragment of the antibody. In specific embodiments, the antibody may be a wild-type antibody. In other specific embodiments, the antibody may be a manipulated antibody.

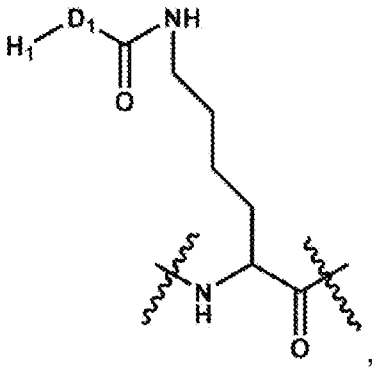
[0571] In specific embodiments, the antibody may include the amino acid sequence of Formula 8-1 in only one of the two Fc domains thereof. In other specific embodiments, the antibody may include the amino acid sequence of Formula 8-1 in both of the two Fc domains thereof.

[0572] The present invention provides an antibody or a fragment thereof, which includes an amino acid sequence of the following Formula 8-2:

[0573] [Formula 8-2]

[0574] G—P—S—V—F—L—F—P—P—K—P-(K)'-D—T—L—M—I (SEQ ID NO: 21)

[0575] wherein G is glycine, P is proline, S is serine, V is valine, F is phenylalanine, L is leucine, K is lysine, D is aspartic acid, T is threonine, M is methionine, I is isoleucine, and

[0576] (K)' is 

,

wherein H_1 is a first click-chemistry functional group, and

[0577] D_1 is any alkylene, alkenylene, or alkynylene. The antibody or fragment thereof has H_1 connected via lysine 248 in an Fc domain thereof, or a site corresponding to the lysine 248.

[0578] In specific embodiments, H_1 may include any one selected from an alkyne, an azide, a strained alkyne, a diene, a dienophile, an alkene, a thiol, and a tetrazine. Furthermore, H_1 may be an azide, or a strained alkyne. Further, H_1 may be an azide, or dibenzocyclooctyne-amine. Additionally, H_1 may be a diene, or a dienophile. Further, H_1 may be a tetrazine, or a norbornene. Alternatively, H_1 may be a tetrazine, or a trans-cyclooctene.

[0579] In specific embodiments, D_1 may include any one selected from a covalent bond, a C_{1-4} alkylene, a C_{2-4} alkenylene, a C_{2-4} alkynylene, and a C_{3-8} cycloalkylene. Also, D_1 may be $-CH_2OCH_2-$. In addition, D_1 may be a covalent bond.

[0580] In specific embodiments, the antibody may be a human antibody. In other specific embodiments, the antibody may be a non-human animal antibody. In specific embodiments, the antibody may be immunoglobulin G (IgG). In specific embodiments, the antibody may be a whole antibody. In other specific embodiments, the antibody may be a fragment of the antibody. In specific embodiments, the antibody may be a wild-type antibody. In other specific embodiments, the antibody may be a manipulated antibody.

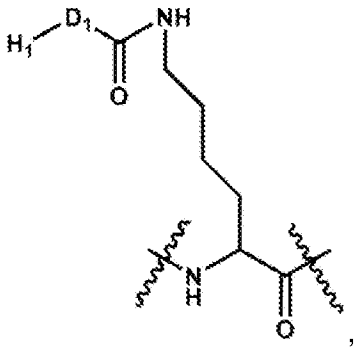
[0581] In specific embodiments, the antibody may include the amino acid sequence of Formula 8-2 in only one of the two Fc domains thereof. In other specific embodiments, the antibody may include the amino acid sequence of Formula 8-2 in both of the two Fc domains thereof.

[0582] The present invention provides an antibody or a fragment thereof, which includes an amino acid sequence of the following Formula 8-3:

[0583] [Formula 8-3]

G—P—S—V—F—L—F—P—P—(K)'-P-(K)'-D—T—L—M—I (SEQ ID NO: 22)

[0584] wherein G is glycine, P is proline, S is serine, V is valine, F is phenylalanine, L is leucine, K is lysine, D is aspartic acid, T is threonine, M is methionine, I is isoleucine, and

[0585] (K)' is 

,

[0586] wherein H_1 is a first click-chemistry functional group, and

[0587] D_1 is any alkylene, alkenylene, or alkynylene. The antibody or fragment thereof has H_1 connected via lysine 246 and 248 in an Fc domain thereof, or sites corresponding to the lysine 246 and 248.

[0588] In specific embodiments, H_1 may include any one selected from an alkyne, an azide, a strained alkyne, a diene, a dienophile, an alkene, a thiol, and a tetrazine. Furthermore, H_1 may be an azide, or a strained alkyne. Further, H_1 may be an azide, or dibenzocyclooctyne-

amine. Additionally, H_1 may be a diene, or a dienophile. Further, H_1 may be a tetrazine, or a norbornene. Alternatively, H_1 may be a tetrazine, or a trans-cyclooctene.

[0589] In specific embodiments, D_1 may include any one selected from a covalent bond, a C_{1-4} alkylene, a C_{2-4} alkenylene, a C_{2-4} alkynylene, and a C_{3-8} cycloalkylene. Also, D_1 may be $-CH_2OCH_2-$. In addition, D_1 may be a covalent bond.

[0590] In specific embodiments, the antibody may be a human antibody. In other specific embodiments, the antibody may be a non-human animal antibody. In specific embodiments, the antibody may be immunoglobulin G (IgG). In specific embodiments, the antibody may be a whole antibody. In other specific embodiments, the antibody may be a fragment of the antibody. In specific embodiments, the antibody may be a wild-type antibody. In other specific embodiments, the antibody may be a manipulated antibody.

[0591] In specific embodiments, the antibody may include the amino acid sequence of Formula 8-3 in only one of the two Fc domains thereof. In other specific embodiments, the antibody may include the amino acid sequence of Formula 8-3 in both of the two Fc domains thereof.

[0592] The present invention provides an antibody or a fragment thereof, which includes one or more amino acid sequences selected from Formulas 8-1, 8-2, and 8-3. In this case, the contents of the sequences of Formulas 8-1 to 8-3 are as described above.

[0593] In specific embodiments, D_1 may be a covalent bond.

[0594] In specific embodiments, the antibody or fragment thereof may include only the amino acid sequence of Formula 8-1 and may not include the amino acid sequences of Formulas 8-2 and 8-3. Also, the antibody or fragment thereof may include the amino acid sequence of Formula 8-1 in only one of the two Fc domains thereof. In addition, the antibody may include the amino acid sequence of Formula 8-1 in both of the two Fc domains thereof.

[0595] In other specific embodiments, the antibody or fragment thereof may include only the amino acid sequence of Formula 8-2 and may not include the amino acid sequences of Formulas 8-1 and 8-3. Also, the antibody or fragment thereof may include the amino acid sequence of Formula 8-2 in only one of the two Fc domains thereof. In addition, the antibody may include the amino acid sequence of Formula 8-2 in both of the two Fc domains thereof.

[0596] In specific embodiments, the antibody or fragment thereof may include only the amino acid sequence of Formula 8-3 and may not include the amino acid sequences of Formulas 8-1 and 8-2. Also, the antibody or fragment thereof may include the amino acid sequence of Formula 8-3 in only one of the two Fc domains thereof. In addition, the antibody may include the amino acid sequence of Formula 8-3 in both of the two Fc domains thereof.

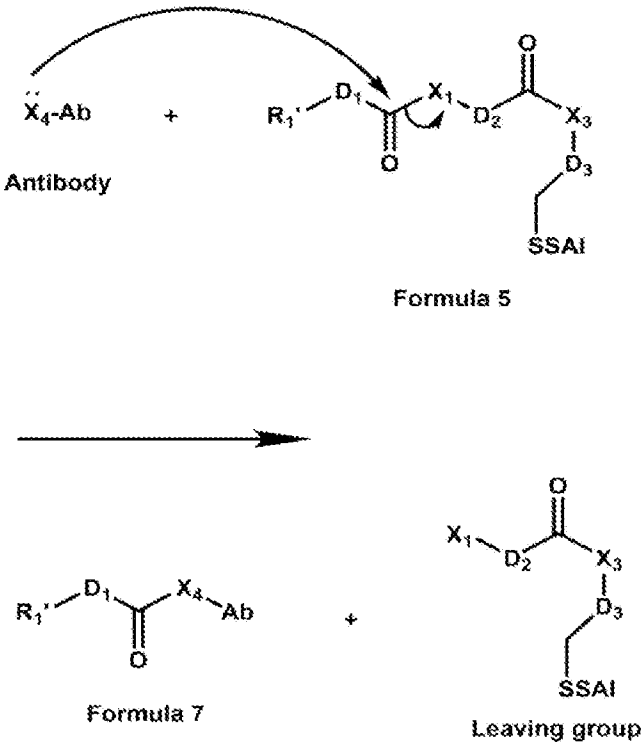
[0597] In specific embodiments, the antibody may be a human antibody. In other specific embodiments, the antibody may be a non-human animal antibody. In specific embodiments, the antibody may be immunoglobulin G (IgG). In specific embodiments, the antibody may be a whole antibody. In other specific embodiments, the antibody may be a fragment of the antibody. In specific embodiments, the antibody may be a wild-type antibody. In other specific embodiments, the antibody may be a manipulated antibody.

[0598] 5.2. Method of preparing antibody containing first chemical functional group

[0599] According to the present invention, there is disclosed a method of preparing the R_1'-Ab, and H_1-Ab (hereinafter generally referred to as "R_1'-Ab"). It will be noted that a description of the following preparation methods is provided to aid in understanding the present invention.

[0600] For example, a method of preparing a compound of Formula 7 will be described. In specific embodiments, the compound of Formula 7 may be prepared through a reaction of the following Scheme 4.

[0601] [Scheme 4]



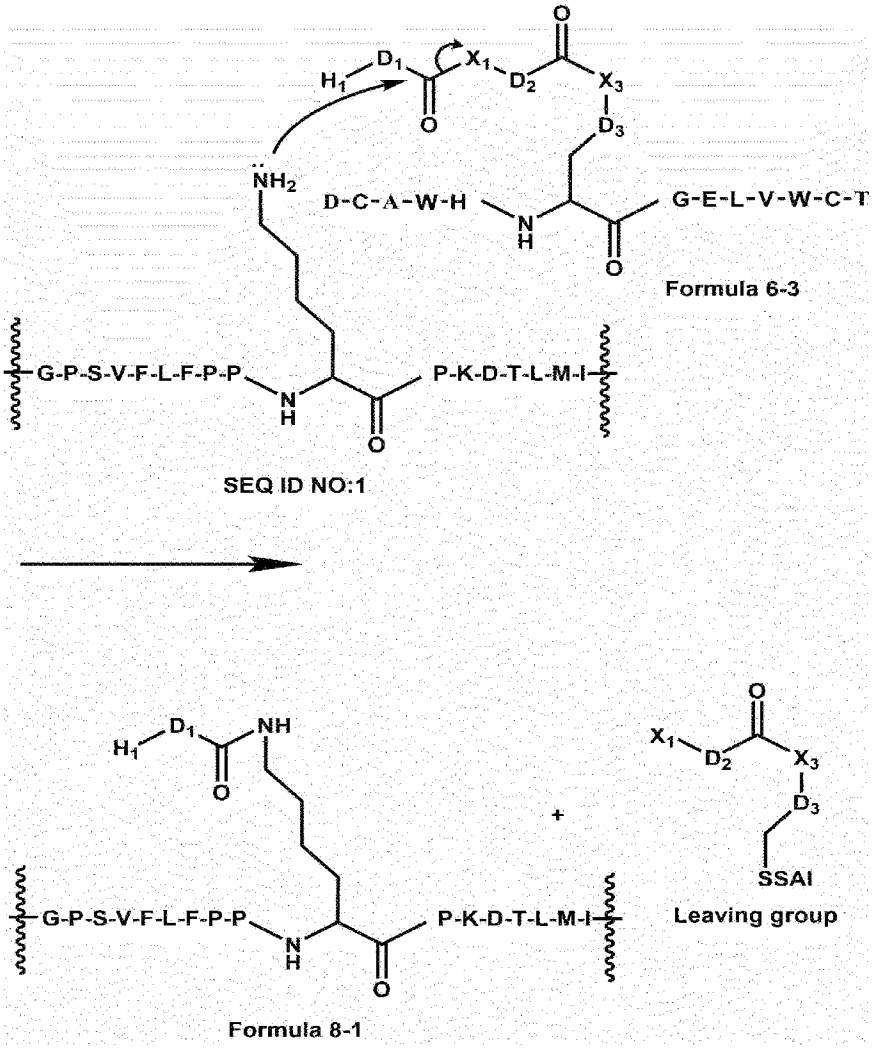
[0602] Because a first carbonyl group of the linker according to the present invention has mild reactivity, cross-linking may be realized when the first carbonyl group of the linker has a close positional relationship with an amine group of an antibody. First, the R_1'-L_2-SSAI represented by

Formula 5 is brought into close contact with a certain site of the antibody to create an environment in which a reaction may occur. Because the R_1'-L_2-SSAI according to the present invention has an activated carbonyl group (i.e., a carbonyl group connected to X_1) corresponding to the first carbonyl group of the linker, the R_1'-L_2-SSAI of the present invention may trigger a nucleophilic substitution reaction. In this case, the compound of Formula 7 may be prepared since an atom (X_4) having a free electron pair present in the antibody serves as a nucleophile to attack the carbonyl group. In this case, by design of the linker, a site-specific antibody interactome (SSAI) leaves while being included in a leaving group and the SSAI is removed from the final product. These characteristics have a positive effect on physical properties of an antibody product, as shown in the section 5.8 below.

[0603] In specific embodiments, the X_4 may be NH_2. Also, the X_4 may be NH_2 of a lysine residue. In other specific embodiments, the X_4 may be SH. Also, the X_4 may be SH of a cysteine residue. In other specific embodiments, the X_4 may be OH.

[0604] As a more specific example, a method of preparing an antibody or a fragment thereof, which includes the amino acid sequence of Formula 8-3, will be described. In specific embodiments, the compound including the amino acid sequence of Formula 8-3 may be prepared through a reaction of the following Scheme 5.

[0605] [Scheme 5]



[0606] The compound having a structure of Formula 6-3 (a structure in which two cysteine residues are optionally connected) is directed towards the Fc domain of the antibody because the compound has an SSFI sequence (see section 3.2). In this case, the antibody including an amino acid sequence of SEQ ID NO: 1 includes lysine residues in an Fc domain thereof, and such a lysine residue may serve as a nucleophile. The exemplary embodiment shows a case where lysine 246 in the Fc domain or a residue corresponding to the lysine 246 (hereinafter referred to as "lysine 246") serves as a nucleophile. In this case, an amine group of the lysine 246 attacks a first carbonyl group of Formula 6-3 to produce a compound of Formula 8-1. In this way, H_1 or R_1 may be transferred to the lysine residue of the Fc domain of the antibody. In this case, to which certain lysine residue the chemical functional group is transferred may depend on the design of the linker, the SSAI, and the R_1'-L_2-SSAI, as described in the sections 5.3 and 5.4 below.

[0607] According to the present invention, there is disclosed a method of preparing an antibody containing a first chemical functional group.

[0608] The present invention provides a method of preparing an R_1'-Ab, which includes:

[0609] reacting an agent for transferring a first chemical functional group to an antibody according to the present invention with an antibody or a fragment thereof.

[0610] In specific embodiments, the agent for transferring a first chemical functional group to an antibody may be any one selected from Formulas 5, 5-1 to 5-3, and 6-1 to 6-3.

[0611] In specific embodiments, the antibody or fragment thereof may be a human antibody. In other specific embodiments, the antibody or fragment thereof may be a non-human animal antibody. In specific embodiments, the antibody or fragment thereof may be immunoglobulin G (IgG). In specific embodiments, the antibody or fragment thereof may be a whole antibody. In other specific embodiments, the antibody or fragment thereof may be a fragment of the antibody. In specific embodiments, the antibody or fragment thereof may be a wild-type antibody. In other specific embodiments, the antibody or fragment thereof may be a manipulated antibody.

[0612] In specific embodiments, the present invention provides a method of preparing an antibody having a first chemical functional group transferred to a specific lysine residue of an Fc domain thereof. In this case, those of aspects as described in the section 5.4 below may be used as the agent for transferring a first chemical functional group to an antibody.

[0613] According to the present invention, there is disclosed a method of preparing an antibody containing a first click-chemistry functional group.

[0614] The present invention provides a method of preparing an H_1-Ab, which includes:

[0615] reacting an agent for transferring a first click-chemistry functional group to an antibody according to the present invention with an antibody or a fragment thereof.

[0616] In specific embodiments, the agent for transferring a first click-chemistry functional group to an antibody may be any one selected from Formulas 6-1 to 6-3. Also, the agent for transferring a first click-chemistry functional group to an antibody may have the structure of Formula 6-3.

[0617] In specific embodiments, the antibody or fragment thereof may be a human antibody. In other specific embodiments, the antibody or fragment thereof may be a non-human animal antibody. In specific embodiments, the antibody or fragment thereof may be immunoglobulin G (IgG). In specific embodiments, the antibody or fragment thereof may be a whole antibody. In other specific embodiments, the antibody or fragment thereof may be a fragment of the antibody. In specific embodiments, the antibody or fragment thereof may be a wild-type antibody. In other specific embodiments, the antibody or fragment thereof may be a

manipulated antibody.

[0618] In specific embodiments, the present invention provides a method of preparing an antibody having a first click-chemistry functional group transferred to a specific lysine residue of an Fc domain thereof. In this case, those of aspects as described in the section 5.4 below may be used as the agent for transferring a first click-chemistry functional group to an antibody.

[0619] According to the present invention, there is disclosed a kit for preparing an antibody or a fragment thereof containing a first chemical functional group.

[0620] The present invention provides a kit for preparing an antibody or a fragment thereof containing a first chemical functional group, which includes an agent for transferring a first chemical functional group to an antibody according to the present invention, and an antibody or a fragment thereof.

[0621] In specific embodiments, the agent for transferring a first chemical functional group to an antibody may be any one selected from Formulas 5, 5-1 to 5-3, and 6-1 to 6-3.

[0622] In specific embodiments, the antibody or fragment thereof may be a human antibody. In other specific embodiments, the antibody or fragment thereof may be a non-human animal antibody. In specific embodiments, the antibody or fragment thereof may be immunoglobulin G (IgG). In specific embodiments, the antibody or fragment thereof may be a whole antibody. In other specific embodiments, the antibody or fragment thereof may be a fragment of the antibody. In specific embodiments, the antibody or fragment thereof may be a wild-type antibody. In other specific embodiments, the antibody or fragment thereof may be a manipulated antibody.

[0623] Also, the present invention provides a kit for preparing an antibody or a fragment thereof containing a first chemical functional group, which includes:

[0624] a linker (R_1'-L_1) according to the present invention;

[0625] a site-specific antibody interactome according to the present invention; and

[0626] an antibody or a fragment thereof.

[0627] In specific embodiments, the linker may be any one selected from Formulas 1, 2, and 2-1 to 2-3.

[0628] In specific embodiments, the site-specific antibody interactome may be any one selected from Formulas 3, 3-1, and 4-1 to 4-6.

[0629] In specific embodiments, the antibody or fragment thereof may be a human antibody. In other specific embodiments, the antibody or fragment thereof may be a non-human animal antibody. In specific embodiments, the antibody or fragment thereof may be

immunoglobulin G (IgG). In specific embodiments, the antibody or fragment thereof may be a whole antibody. In other specific embodiments, the antibody or fragment thereof may be a fragment of the antibody. In specific embodiments, the antibody or fragment thereof may be a wild-type antibody. In other specific embodiments, the antibody or fragment thereof may be a manipulated antibody.

[0630] The present invention provides a kit for preparing an antibody having a first chemical functional group transferred to a specific lysine residue of an Fc domain thereof. In this case, those of aspects as described in the section 5.4 below may be used as the agent for transferring a first chemical functional group to an antibody, the linker, and the site-specific antibody interactome.

[0631] According to the present invention, there is disclosed a kit for preparing an antibody or a fragment thereof containing a first click-chemistry functional group.

[0632] The present invention provides a kit for preparing an antibody or a fragment thereof containing a first click-chemistry functional group, which includes an agent for transferring a first click-chemistry functional group to an antibody according to the present invention, and an antibody or a fragment thereof.

[0633] In specific embodiments, the agent for transferring a first click-chemistry functional group to an antibody may be any one selected from Formulas 6-1 to 6-3. Also, the agent for transferring a first click-chemistry functional group to an antibody may have the structure of Formula 6-3.

[0634] In specific embodiments, the antibody or fragment thereof may be a human antibody. In other specific embodiments, the antibody or fragment thereof may be a non-human animal antibody. In specific embodiments, the antibody or fragment thereof may be immunoglobulin G (IgG). In specific embodiments, the antibody or fragment thereof may be a whole antibody. In other specific embodiments, the antibody or fragment thereof may be a fragment of the antibody. In specific embodiments, the antibody or fragment thereof may be a wild-type antibody. In other specific embodiments, the antibody or fragment thereof may be a manipulated antibody.

[0635] Also, the present invention provides a kit for preparing an antibody or a fragment thereof containing a first click-chemistry functional group, which includes:

[0636] a linker (H_1-L_1) according to the present invention;

[0637] a site-specific antibody interactome according to the present invention; and

[0638] an antibody or a fragment thereof.

[0639] In specific embodiments, the linker may be any one selected from Formulas 2, and 2-1 to 2-3.

[0640] In specific embodiments, the site-specific antibody interactome may be any one selected from Formulas 3, 3-1, and 4-1 to 4-6.

[0641] In specific embodiments, the antibody or fragment thereof may be a human antibody. In other specific embodiments, the antibody or fragment thereof may be a non-human animal antibody. In specific embodiments, the antibody or fragment thereof may be immunoglobulin G (IgG). In specific embodiments, the antibody or fragment thereof may be a whole antibody. In other specific embodiments, the antibody or fragment thereof may be a fragment of the antibody. In specific embodiments, the antibody or fragment thereof may be a wild-type antibody. In other specific embodiments, the antibody or fragment thereof may be a manipulated antibody.

[0642] The present invention provides a kit for preparing an antibody having a first click-chemistry functional group transferred to a specific lysine residue of an Fc domain thereof. In this case, those of aspects as described in the section 5.4 below may be used as the agent for transferring a first click-chemistry functional group to an antibody, the linker, and the site-specific antibody interactome.

[0643] 5.3. Function of $(Xa_1)'$ and design principle of location of $(Xa_1)'$ on R_1'-L_2-SSFI

[0644] In the present content, the compound of Formula 6-3 is provided as one example to aid in understanding the present invention, but the scope of the present invention is not limited thereto. It will be noted that the following description also applies to the compounds of Formulas 5, 5-1 to 5-3, and 6-1 to 6-3, and the compound of Formula 6-3 is merely provided as one example for the sake of convenience.

[0645] As discussed in the section 5.2, $(Xa_1)'$ of the R_1'-L_2-SSFI functions to transfer R_1' to an antibody by means of a nucleophilic substitution reaction. According to the present invention, it is assumed that the conditions for facilitating a nucleophilic substitution reaction satisfy the following requirements: (1) $(Xa_1)'$ is adjacent to a lysine residue of an Fc domain, and (2) a side chain to which R_1' is bound is directed towards the lysine residue. It was expected that the yield and uniformity of a process would drop as the position of $(Xa_1)'$ and the direction of the side chain become farther from the lysine residue of the Fc domain. Also, (3) it will be preferred that a substitution position of $(Xa_1)'$ does not have a great influence on the interaction between the SSFI and the Fc domain (see section 3.2).

[0646] As indirectly seen in FIGS. 3 and 5, it was confirmed that the positions in the SSFI that satisfy the requirement of (1) in relation to lysine 246 and 248 in the Fc domain are positions

5, 6, 7, and 8 based on the following Formula 6-3.

[0647] [Formula 6-3]

$$D\text{-}C\text{-}A\text{-}W\text{-}H\text{-}(Xa_1)'\text{-}G\text{-}E\text{-}L\text{-}V\text{-}W\text{-}C\text{-}T$$

[0648] In this case, as histidine corresponding to position 5 forms a salt linkage with glutamic acid 380 in the Fc domain, replacement of the histidine may affect the interaction between the SSFI and the Fc domain. Therefore, this does not satisfy the requirement of (3). Also, because the direction of this side chain is not close to the lysine 246 and 248, this does not satisfy the requirement of (2). Because glycine corresponding to position 7 helps to form a bent structure of the SSFI, it is not desirable to replace the glycine residue with a large $(Xa_1)'$ residue. As glutamic acid corresponding to position 8 forms a salt linkage with arginine 255 in the Fc domain, replacement of the glutamic acid may affect the interaction between the SSFI and the Fc domain. Therefore, this does not satisfy the requirement of (3) (see section 3.2). It was judged that position 6 is most suitable for the position of $(Xa_1)'$ because position 6 satisfies all the requirements of (1), (2), and (3). Therefore, the R_1'-L_2-SSFI according to the present invention has been completed based on these facts.

[0649] 5.4. The position of R_1' transferred to an antibody may vary depending on the length of D_2 in R_1'-L_1 and D_3 in SSFI.

[0650] The present content is intended to explain a preferred design principle of the R_1'-L_2-SSFI according to the present invention. According to the design of the R1'-L_2-SSFI, it is possible to specifically transfer R_1' to a specific lysine residue of the Fc domain. Also, the present content is intended to explain a design principle of the R_1'-L_1 and SSFI to prepare a preferred R_1'-L_2-SSFI.

[0651] A person of ordinary skill in the art who reads the section 5.2 as described above may recognize that a nucleophilic substitution reaction may occur when the first carbonyl carbon of $(Xa_1)'$ of the R_1'-L_2-SSFI is located adjacent to an amine group of lysine of the Fc domain. Based on the description of the section 3.2 as described above, a person of ordinary skill in the art may also recognize that the R_1'-L_2-SSFI is arranged with the Fc domain with a specific topology, wherein amine groups of lysine 246 and 248 in the Fc domain are spaced apart a certain distance from the beta carbon of $(Xa_1)'$ (FIG. 5). By using this combination to design the R_1'-L_2-SSFI, it was expected that it is possible to specifically label a desired lysine residue when (1) a distance (hereinafter generally referred to as "L_c") between the beta carbon of $(Xa_1)'$ and the first carbonyl carbon is the same as or similar to (2) a distance between the beta carbon of $(Xa_1)'$ and the amine groups of lysine 246 and 248 in the Fc domain in the specific topology.

[0652] The structure of the R_1'-L_2-SSFI according to the present invention and the L_c are shown in FIG. 8. As shown in FIG. 8, D_3, X_3, carbon atoms, D_2, and X_1 are located between the beta carbon of (Xa_1)' and the first carbonyl carbon. Among these, D_3 and X_3 are associated with the design of SSFI, and D_2 and X_1 are associated with the design of L_1-R_1'.

[0653] For the sake of convenience, the invention was embodied on the assumption that D_3 is a C_x alkylene, X_3 is N, D_2 is a C_y alkylene, a C_y alkenylene, or a C_y alkynylene, and X_1 is S, wherein y is an integer greater than or equal to 1. In this case, it was assumed that the alkylene, the alkenylene, and the alkynylene have the same length. The structure of the linker shown in FIG. 8 was modeled using the software Discovery Studio to calculate L_c values. As a result, the L_c values determined with respect to the x + y value are listed in Table 3. In the calculation process, the chain was modeled when it had the longest length.

<Table 3> Lc values with respect to x + y value

x+y	Lc (Å)
1 (x=0, y=1)	6.616
2	7.784
3	9.102
4	10.299
5	11.599
6	12.816
7	14.100
8	15.334
9	16.601
10	17.848
11	19.099
12	20.357
13	21.592
14	22.857

[0654] Hereinafter, the accompanying drawings provided to show a topology between the R_1'-L_2-SSFI and a lysine residue of the Fc domain are provided based on FIG. 9. FIG. 9 shows a topology between an R_1'-L_2-SSFI and an Fc domain so that a direction (a dotted arrow) of a side chain of (Xa_1)' is parallel with the x axis in the drawing. As shown in FIG. 9, it can be seen that the side chain of (Xa_1)' is directed towards the amine groups of the lysine 246 and 248 (see section 5.3). Also, it can be seen from the drawing that the side chain of (Xa_1)' may specifically react with the lysine 246 or 248 depending on the length of L_c. FIGS. 10 to 12 are diagrams

viewing a diagram of FIG. 9 in a direction (a thick solid line arrow) parallel with the y-axis in the drawing along the exemplary length of L_c.

[0655] The distances ($D_{246,min}$, $D_{246,max}$, $D_{248,min}$, and $D_{248,max}$) between the beta carbon of $(Xa_1)'$ and the amine groups of lysine 246 and 248 in the Fc domain are described in the section 3.2. The beta carbon of $(Xa_1)'$ is closer to the lysine 248 in the Fc domain than the lysine residue at position 246. Therefore, it was expected that the first carbonyl carbon would react well with the lysine 248 when the L_c value is shorter than $D_{246,min}$ (FIG. 10), the first carbonyl carbon would react well with the lysine 246 when the L_c value is longer than $D_{248,max}$ (FIG. 11), and the first carbonyl carbon would selectively react with the lysine 246 and 248 when the L_c value is longer than or equal to $D_{246,min}$ and shorter than or equal to $D_{248,max}$ (FIG. 12).

[0656] According to the present invention, there is disclosed an R_1'-L_2-SSFI for specifically transferring a first chemical functional group to lysine 248 in an Fc domain of an antibody (see FIG. 11).

[0657] The present invention provides an agent for transferring a first chemical functional group to an antibody, characterized in that a distance (L_c) between the beta carbon of $(Xa_1)'$ and the first carbonyl carbon is shorter than $D_{246,min}$ (approximately 11.668 Å). In this case, L_c may have a value of approximately 6.5 Å, approximately 7 Å, approximately 8 Å, approximately 9 Å, approximately 10 Å, approximately 11 Å, or approximately 11.5 Å.

[0658] In specific embodiments, when the R_1'-L_2-SSFI has any one structure selected from Formulas 5, 5-1 to 5-3, and 6-1 to 6-3, D_3 is a C_x alkylene, X_3 is N, D_2 is a C_y alkylene, a C_y alkenylene, or a C_y alkynylene, X_1 is S, wherein y may be an integer greater than or equal to 1, and the sum of x and y may be $1 \leq x + y \leq 5$. Also, the sum of x and y may be 1, 2, 3, 4, or 5. For example, x may be 0, and y may be $1 \leq y \leq 5$. In another exemplary embodiment, x may be 1, and y may be $1 \leq y \leq 4$. In still another exemplary embodiment, x may be 2, and y may be $1 \leq y \leq 3$. In yet another exemplary embodiment, x may be 3, and y may be $1 \leq y \leq 2$. The corresponding numerical range is determined based on the value listed in Table 3.

[0659] According to the present invention, there is disclosed an R_1'-L_2-SSFI for specifically transferring a first chemical functional group to lysine 246 in an Fc domain of an antibody.

[0660] According to one aspect of the present invention, the present invention provides an agent for transferring a first chemical functional group to an antibody, characterized in that a distance (L_c) between the beta carbon of $(Xa_1)'$ and the first carbonyl carbon is longer than $D_{248,max}$ (approximately 16.208 Å). For example, L_c may have a value of approximately 16.5 Å, approximately 17 Å, approximately 18 Å, approximately 19 Å, approximately 20 Å, or approximately 20.5 Å.

[0661] In specific embodiments, when the R_1'-L_2-SSFI has any one structure selected from Formulas 5, 5-1 to 5-3, and 6-1 to 6-3, D_3 is a C_x alkylene, X_3 is N, D_2 is a C_y alkylene, a C_y alkenylene, or a C_y alkynylene, X_1 is S, wherein y may be an integer greater than or equal to 1, and the sum of x and y may be greater than or equal to 9. Also, the sum of x and y may be 9, 10, 11, or 12. For example, x may be 0, and y may be $9 \leq y \leq 12$. In another exemplary embodiment, x may be 1, and y may be $8 \leq y \leq 11$. In still another exemplary embodiment, x may be 2, and y may be $7 \leq y \leq 10$. In yet another exemplary embodiment, x may be 3, and y may be $6 \leq y \leq 9$. Optionally, D_2 may be an alkynylene. When D_2 is an alkynylene, the side chain of (Xa_1)' may become stereoscopically rigid, thereby preventing bending of the side chain.

[0662] According to the present invention, there is disclosed an R_1'-L_2-SSFI for selectively transferring a first chemical functional group to lysine 246 or 248 in an Fc domain of an antibody.

[0663] According to one aspect of the present invention, the present invention provides an agent for transferring a first chemical functional group to an antibody, characterized in that a distance (L_c) between the beta carbon of (Xa_1)' and the first carbonyl carbon has a value longer than or equal to $D_{246,min}$ (approximately 11.668 Å) and shorter than or equal to $D_{248,max}$ (approximately 16.208 Å). In this case, L_c may have a value of approximately 11.668 Å, approximately 12 Å, approximately 13 Å, approximately 14 Å, approximately 15 Å, approximately 15.5 Å, approximately 16 Å, or approximately 16.208 Å.

[0664] In specific embodiments, when the R_1'-L_2-SSFI has any one structure selected from Formulas 5, 5-1 to 5-3, and 6-1 to 6-3, D_3 is a C_x alkylene, X_3 is N, D_2 is a C_y alkylene, a C_y alkenylene, or a C_y alkynylene, X_1 is S, wherein y may be an integer greater than or equal to 1, and the sum of x and y may be $6 \leq x + y \leq 8$. In this case, the sum of x and y may be 6, 7, or 8. For example, x may be 0, and y may be $6 \leq y \leq 8$. In another exemplary embodiment, x may be 1, and y may be $5 \leq y \leq 7$. In still another exemplary embodiment, x may be 2, and y may be $4 \leq y \leq 6$. In yet another exemplary embodiment, x may be 3, and y may be $3 \leq y \leq 5$.

[0665] According to the present invention, there is disclosed a method of preparing an R_1'-L_2-SSFI for specifically transferring a first chemical functional group to lysine 248 in an Fc domain of an antibody.

[0666] As one example of the method of preparing an agent for transferring first chemical functional group to an antibody as described in the section 4.2, the present invention provides a method of preparing an R_1'-L_2-SSAI, which is characterized by including:

[0667] reacting a linker according to the present invention with a site-specific antibody interactome according to the present invention,

[0668] wherein D_2 in the linker is a C_y alkylene, a C_y alkenylene, or a C_y alkynylene, and

[0669] D_3 in the site-specific antibody interactome is a C_x alkylene,

[0670] wherein y is an integer greater than or equal to 1, and the sum of x and y is $1 \leq x + y \leq 5$. Also, the R_1'-L_2-SSAI prepared by the method may react with an antibody to specifically transfer a first chemical functional group to lysine 248 in an Fc domain of the antibody.

[0671] According to the present invention, there is disclosed a kit for preparing an R_1'-L_2-SSFI for specifically transferring a first chemical functional group to lysine 248 in an Fc domain of an antibody.

[0672] As one example of the method of preparing an agent for transferring first chemical functional group to an antibody as described in the section 4.2, the present invention provides a kit for preparing an R_1'-L_2-SSAI, which is characterized by including:

[0673] a linker according to the present invention; and

[0674] a site-specific antibody interactome according to the present invention,

[0675] wherein D_2 in the linker is a C_y alkylene, a C_y alkenylene, or a C_y alkynylene, and

[0676] D_3 in the site-specific antibody interactome is a C_x alkylene,

[0677] wherein y is an integer greater than or equal to 1, and the sum of x and y is $1 \leq x + y \leq 5$.

[0678] According to the present invention, there is disclosed a method of preparing an R_1'-L_2-SSFI for specifically transferring a first chemical functional group to lysine 246 in an Fc domain of an antibody.

[0679] As one example of the method of preparing an agent for transferring first chemical functional group to an antibody as described in the section 4.2, the present invention provides a method of preparing an R_1'-L_2-SSAI, which is characterized by including:

[0680] reacting a linker according to the present invention with a site-specific antibody interactome according to the present invention,

[0681] wherein D_2 in the linker is a C_y alkylene, a C_y alkenylene, or a C_y alkynylene, and

[0682] D_3 in the site-specific antibody interactome is a C_x alkylene,

[0683] wherein y is an integer greater than or equal to 1, and the sum of x and y is $9 \leq x + y \leq 12$. Also, the R_1'-L_2-SSAI prepared by the method may react with an antibody to specifically transfer a first chemical functional group to lysine 246 in an Fc domain of the antibody.

[0684] According to the present invention, there is disclosed a kit for preparing an R_1'-L_2-SSFI for specifically transferring a first chemical functional group to lysine 246 in an Fc domain of an antibody.

[0685] As one example of the method of preparing an agent for transferring first chemical

functional group to an antibody as described in the section 4.2, the present invention provides a kit for preparing an R_1'-L_2-SSAI, which is characterized by including:

[0686] a linker according to the present invention; and

[0687] a site-specific antibody interactome according to the present invention,

[0688] wherein D_2 in the linker is a C_y alkylene, a C_y alkenylene, or a C_y alkynylene, and

[0689] D_3 in the site-specific antibody interactome is a C_x alkylene,

[0690] wherein y is an integer greater than or equal to 1, and the sum of x and y is $9 \leq x + y \leq 12$.

[0691] According to the present invention, there is disclosed a method of preparing an R_1'-L_2-SSFI for selectively transferring a first chemical functional group to lysine 246 or 248 in an Fc domain of an antibody.

[0692] As one example of the method of preparing an agent for transferring first chemical functional group to an antibody as described in the section 4.2, the present invention provides a method of preparing an R_1'-L_2-SSAI, which is characterized by including:

[0693] reacting a linker according to the present invention with a site-specific antibody interactome according to the present invention,

[0694] wherein D_2 in the linker is a C_y alkylene, a C_y alkenylene, or a C_y alkynylene, and

[0695] D_3 in the site-specific antibody interactome is a C_x alkylene,

[0696] wherein y is an integer greater than or equal to 1, and the sum of x and y is $6 \leq x + y \leq 8$. Also, the R_1'-L_2-SSAI prepared by the method may react with an antibody to selectively transfer a first chemical functional group to lysine 246 or 248 in an Fc domain of the antibody.

[0697] According to the present invention, there is disclosed a kit for preparing an R_1'-L_2-SSFI for selectively transferring a first chemical functional group to lysine 246 or 248 in an Fc domain of an antibody.

[0698] As one example of the method of preparing an agent for transferring first chemical functional group to an antibody as described in the section 4.2, the present invention provides a kit for preparing an R_1'-L_2-SSAI, which is characterized by including:

[0699] a linker according to the present invention; and

[0700] a site-specific antibody interactome according to the present invention,

[0701] wherein D_2 in the linker is a C_y alkylene, a C_y alkenylene, or a C_y alkynylene, and

[0702] D_3 in the site-specific antibody interactome is a C_x alkylene,

[0703] wherein y is an integer greater than or equal to 1, and the sum of x and y is $6 \leq x + y \leq 8$.

[0704] According to the present invention, there is disclosed a method of preparing an antibody having a first chemical functional group specifically transferred to lysine 248 in an Fc domain thereof.

[0705] As one example of the method of preparing an antibody containing a first chemical functional group as described in the section 5.2, the present invention provides a method of preparing an R_1'-Ab, which is characterized by including:

[0706] reacting an agent for transferring a first chemical functional group to an antibody according to the present invention with an antibody or a fragment thereof,

[0707] wherein, in the agent for transferring a first chemical functional group to an antibody, a distance (L_c) between the beta carbon of (Xa_1)' and the first carbonyl carbon is shorter than approximately 11.668 Å.

[0708] In specific embodiments, in the agent for transferring a first chemical functional group to an antibody, D_3 is a C_x alkylene, and D_2 is a C_y alkylene, a C_y alkenylene, or a C_y alkynylene,

[0709] wherein y may be an integer greater than or equal to 1, and the sum of x and y may be $1 \leq x + y \leq 5$.

[0710] According to the present invention, there is disclosed a kit for preparing an antibody having a first chemical functional group specifically transferred to lysine 248 in an Fc domain thereof.

[0711] As one example of the method of preparing an antibody containing a first chemical functional group as described in the section 5.2, the present invention provides a kit for preparing an R_1'-Ab, which is characterized by including:

[0712] an agent for transferring a first chemical functional group to an antibody according to the present invention; and

[0713] an antibody or a fragment thereof,

[0714] wherein, in the agent for transferring a first chemical functional group to an antibody, a distance (L_c) between the beta carbon of (Xa_1)' and the first carbonyl carbon is shorter than approximately 11.668 Å.

[0715] In specific embodiments, in the agent for transferring a first chemical functional group to an antibody, D_3 is a C_x alkylene, and D_2 is a C_y alkylene, a C_y alkenylene, or a C_y alkynylene,

[0716] wherein y may be an integer greater than or equal to 1, and the sum of x and y may be $1 \leq x + y \leq 5$.

[0717] Optionally, as one example of the method of preparing an antibody containing a first chemical functional group as described in the section 5.2, the present invention provides a kit for preparing an R_1'-Ab, which is characterized by including:

[0718] a linker according to the present invention;

[0719] a site-specific antibody interactome according to the present invention; and

[0720] an antibody or a fragment thereof,

[0721] wherein D_2 in the linker is a C_y alkylene, a C_y alkenylene, or a C_y alkynylene, and

[0722] D_3 in the site-specific antibody interactome is a C_x alkylene,

[0723] wherein y is an integer greater than or equal to 1, and the sum of x and y is $1 \leq x + y \leq 5$.

[0724] According to the present invention, there is disclosed a method of preparing an antibody having a first chemical functional group specifically transferred to lysine 246 in an Fc domain thereof.

[0725] As one example of the method of preparing an antibody containing a first chemical functional group as described in the section 5.2, the present invention provides a method of preparing an R_1'-Ab, which is characterized by including:

[0726] allowing an antibody or a fragment thereof to react with an agent for transferring a first chemical functional group to an antibody according to the present invention,

[0727] wherein, in the agent for transferring a first chemical functional group to an antibody, a distance (L_c) between the beta carbon of (Xa_1)' and the first carbonyl carbon is longer than approximately 16.208 Å.

[0728] In specific embodiments, in the agent for transferring a first chemical functional group to an antibody, D_3 is a C_x alkylene, and D_2 is a C_y alkylene, a C_y alkenylene, or a C_y alkynylene,

[0729] wherein y may be an integer greater than or equal to 1, and the sum of x and y may be $9 \leq x + y \leq 12$.

[0730] According to the present invention, there is disclosed a kit for preparing an antibody having a first chemical functional group specifically transferred to lysine 246 in an Fc domain thereof.

[0731] As one example of the method of preparing an antibody containing a first chemical functional group as described in the section 5.2, the present invention provides a kit for preparing an R_1'-Ab, which is characterized by including:

[0732] an agent for transferring a first chemical functional group to an antibody according

to the present invention; and

[0733] an antibody or a fragment thereof,

[0734] wherein, in the agent for transferring a first chemical functional group to an antibody, a distance (L_c) between the beta carbon of (Xa_1)' and the first carbonyl carbon is longer than approximately 16.208 Å.

[0735] In specific embodiments, in the agent for transferring a first chemical functional group to an antibody, D_3 is a C_x alkylene, and D_2 is a C_y alkylene, a C_y alkenylene, or a C_y alkynylene,

[0736] wherein y may be an integer greater than or equal to 1, and the sum of x and y may be $9 \leq x + y \leq 12$.

[0737] Optionally, as one example of the method of preparing an antibody containing a first chemical functional group as described in the section 5.2, the present invention provides a kit for preparing an R_1'-Ab, which is characterized by including:

[0738] a linker according to the present invention;

[0739] a site-specific antibody interactome according to the present invention; and

[0740] an antibody or a fragment thereof,

[0741] wherein D_2 in the linker is a C_y alkylene, a C_y alkenylene, or a C_y alkynylene, and

[0742] D_3 in the site-specific antibody interactome is a C_x alkylene,

[0743] wherein y is an integer greater than or equal to 1, and the sum of x and y is $9 \leq x + y \leq 12$.

[0744] According to the present invention, there is disclosed a method of preparing an antibody having a first chemical functional group selectively transferred to lysine 246 or 248 in an Fc domain thereof.

[0745] As one example of the method of preparing an antibody containing a first chemical functional group as described in the section 5.2, the present invention provides a method of preparing an R_1'-Ab, which is characterized by including:

[0746] reacting an agent for transferring a first chemical functional group to an antibody according to the present invention with an antibody or a fragment thereof,

[0747] wherein, in the agent for transferring a first chemical functional group to an antibody, a distance (L_c) between the beta carbon of (Xa_1)' and the first carbonyl carbon is longer than or equal to approximately 11.668 Å and shorter than or equal to approximately 16.208 Å.

[0748] In specific embodiments, in the agent for transferring a first chemical functional group to an antibody, D_3 is a C_x alkylene, and D_2 is a C_y alkylene, a C_y alkenylene, or a C_y

alkynylene,

[0749] wherein y may be an integer greater than or equal to 1, and the sum of x and y may be $6 \leq x + y \leq 8$.

[0750] According to the present invention, there is disclosed a kit for preparing an antibody having a first chemical functional group selectively transferred to lysine 246 or 248 in an Fc domain thereof.

[0751] As one example of the method of preparing an antibody containing a first chemical functional group as described in the section 5.2, the present invention provides a kit for preparing an R_1'-Ab, which is characterized by including:

[0752] an agent for transferring a first chemical functional group to an antibody according to the present invention; and

[0753] an antibody or a fragment thereof,

[0754] wherein, in the agent for transferring a first chemical functional group to an antibody, a distance (L_c) between the beta carbon of (Xa_1)' and the first carbonyl carbon is longer than or equal to approximately 11.668 Å and shorter than or equal to approximately 16.208 Å.

[0755] In specific embodiments, in the agent for transferring a first chemical functional group to an antibody, D_3 is a C_x alkylene, and D_2 is a C_y alkylene, a C_y alkenylene, or a C_y alkynylene,

[0756] wherein y may be an integer greater than or equal to 1, and the sum of x and y may be $6 \leq x + y \leq 8$.

[0757] Optionally, as one example of the method of preparing an antibody containing a first chemical functional group as described in the section 5.2, the present invention provides a kit for preparing an R_1'-Ab, which is characterized by including:

[0758] a linker according to the present invention;

[0759] a site-specific antibody interactome according to the present invention; and

[0760] an antibody or a fragment thereof,

[0761] wherein D_2 in the linker is a C_y alkylene, a C_y alkenylene, or a C_y alkynylene, and

[0762] D_3 in the site-specific antibody interactome is a C_x alkylene,

[0763] wherein y is an integer greater than or equal to 1, and the sum of x and y is $6 \leq x + y \leq 8$.

[0764] According to the present invention, there is disclosed a method of preparing an antibody having a first chemical functional group transferred to both lysine 246 and 248 in an Fc domain thereof.

[0765] The present invention provides a method of preparing an R_1'-Ab, which includes:

[0766] reacting a first agent for transferring a first chemical functional group to an antibody with an antibody or a fragment thereof; and

[0767] reacting a second agent for transferring a first chemical functional group to an antibody with the antibody or fragment thereof.

[0768] In specific embodiments, the first agent for transferring a first chemical functional group to an antibody may be an R_1'-L_2-SSFI for specifically transferring a first chemical functional group to lysine 248 in the Fc domain as described above, and the second agent for transferring a first chemical functional group to an antibody may be an R_1'-L_2-SSFI for specifically transferring a first chemical functional group to lysine 246 in the Fc domain as described above.

[0769] In specific embodiments, the first agent for transferring a first chemical functional group to an antibody may be an R_1'-L_2-SSFI for specifically transferring a first chemical functional group to lysine 246 in the Fc domain as described above, and the second agent for transferring a first chemical functional group to an antibody may be an R_1'-L_2-SSFI for specifically transferring a first chemical functional group to lysine 248 in the Fc domain as described above.

[0770] In specific embodiments, the reacting of the first agent for transferring a first chemical functional group to an antibody with the antibody or fragment thereof; and the reacting of the second agent for transferring a first chemical functional group to an antibody with the antibody or fragment thereof may be sequentially performed.

[0771] According to the present invention, there is disclosed a kit for preparing an antibody having a first chemical functional group transferred to both lysine 246 and 248 in an Fc domain thereof.

[0772] The present invention provides a kit for preparing an R_1'-Ab, which includes:

[0773] a first agent for transferring a first chemical functional group to an antibody;

[0774] a second agent for transferring a first chemical functional group to an antibody; and

[0775] an antibody or a fragment thereof.

[0776] In specific embodiments, the first agent for transferring a first chemical functional group to an antibody may be an R_1'-L_2-SSFI for specifically transferring a first chemical functional group to lysine 248 in the Fc domain as described above, and the second agent for transferring a first chemical functional group to an antibody may be an R_1'-L_2-SSFI for specifically transferring a first chemical functional group to lysine 246 in the Fc domain as described above.

[0777] In specific embodiments, the first agent for transferring a first chemical functional

group to an antibody may be an R_1'-L_2-SSFI for specifically transferring a first chemical functional group to lysine 246 in the Fc domain as described above, and the second agent for transferring a first chemical functional group to an antibody may be an R_1'-L_2-SSFI for specifically transferring a first chemical functional group to lysine 248 in the Fc domain as described above.

[0778] 5.5. It is possible to transfer first chemical functional group more site-specifically by modulating the reactivity of the first carbonyl group.

[0779] As described above in the section 5.2, an antibody containing a first chemical functional group may be prepared by a nucleophile of the antibody attacking a first carbonyl group of the R_1'-L_2-SSFI. The first carbonyl group is characterized by having milder reactivity than the second carbonyl group of the linker (see section 2.2). The reactivity of the first carbonyl group may be modulated so that a certain amine group of the antibody can react with the first carbonyl group only when the certain amine group is in the vicinity of the first carbonyl group. In this way, the present invention allows the first carbonyl group not to react well with any amine group through the vicinity conditions for reaction, and enables high positional specificity of the reaction.

[0780] The prior art disclosed in Publication Nos. US 2018/0141976 A1 and WO 2018/199337 A1 aims to site-selectively modulate lysine 246 or 248 in an Fc domain using an analogue of Fc-III. Because the prior art uses a disuccinimidyl cross-linker, the first carbonyl group and the second carbonyl group are equally highly reactive (see section 2.2). Therefore, because the first carbonyl group has high reactivity and has no vicinity conditions for reaction, any lysine residue is highly likely to be labeled.

[0781] 5.6. The antibody containing a first chemical functional group prepared according to the present invention has high uniformity and yield.

[0782] The technical problem and the technical solution to specifically transfer (1) “a desired number” of first chemical functional groups to (2) “certain sites of an antibody” have been described in detail with reference to the sections 5.3 and 5.4. As can be seen from Experimental Examples below, it can be seen that the chemical functional group of the antibody containing a first chemical functional group prepared by means of the technical solution is transferred to a certain lysine residue in the Fc domain thereof with high specificity and yield (see Experimental Example 3.3). Therefore, the present invention has been completed based on these facts.

[0783] As shown in the background art, the conjugate product having high uniformity due to antibody labeling has advantages in that (1) the functions of the antibody conjugate are uniformly guaranteed, (2) the antibody conjugate is safe due to its predictable effects, and (3) a drop in function of an antibody may be avoided because it is possible to label an antibody while

avoiding a functional region of the antibody.

[0784] The prior art disclosed in Publication Nos. US 2018/0141976 A1 and WO 2018/199337 A1 aims to site-selectively modulate lysine 246 or 248 in an Fc domain using an analogue of Fc-III. However, according to the prior art, the uniformity and yield of the antibody may be inevitably low due to two reasons. According to the prior art, first, because a distance (L_c) between the beta carbon of $(Xa_1)'$ and the first carbonyl carbon is approximately 15 Å, it is possible to selectively label lysine at either position 246 or 248, but it is impossible to specifically select one of the lysine 246 and 248 to be labeled. Second, a first carbonyl group of a cross-linker according to the prior art has no vicinity conditions for reaction due to high reactivity (see section 5.5). As a result, the first carbonyl group is highly likely to react with any lysine residue. Therefore, the prior art has a drawback in that the uniformity and yield of an antibody-labeled product are inferior to those of the present invention.

[0785] 5.7. No functions of the antibody are lowered because the antibody containing a first chemical functional group according to the present invention has no FcRn binding site blocked therein.

[0786] The design principle for the antibody labeling site being (1) spaced apart from the paratope; and (2) spaced apart from the recognition site of FcR including FcRn has been described above in the section 1.1. Because the lysine 246 and 248 are included in the Fc domain, the lysine 246 and 248 are spaced apart from the paratope, but the corresponding site may overlap the recognition site of FcRn, which may be problematic.

[0787] It is known that FcRn has various functions, and particularly plays an important role in extending the half-life of the antibody by participating in IgG recycling. When the antibody is used *in vivo*, that is, when the antibody is used as a therapeutic agent, a contrast medium, and the like, the interaction between FcRn and the antibody may not occur smoothly, thereby making it impossible for the antibody to function normally due to the short half-life of the antibody.

[0788] 5.7.1. Lysine 246 and lysine 248 are spaced apart from the FcRn binding site of the antibody

[0789] The spacing between the lysine residues in the Fc domain and the FcRn binding site of Fc was an important consideration to choose the lysine residue in the Fc domain. FIG. 13 shows a binding structure between an Fc domain and FcRn and positions of the FcRn binding site and lysine 246 and 248 in the Fc domain. Papers and computer modeling were employed to show a binding structure between the Fc domain and FcRn (Ying T, Ju TW, Wang Y, Prabakaran P, Dimitrov DS., Interactions of IgG1 CH2 and CH3 Domains with FcRn; Front Immunol. 2014; 5:146., Monnet C, Jorieux S, Urbain R, *et al.*, Selection of IgG Variants with Increased FcRn

Binding Using Random and Directed Mutagenesis: Impact on Effector Functions. Front Immunol. 2015; 6:39.) (see the left image of FIG. 13). The FcRn binding site and the lysine 246 and 248 disclosed in the same article data are indicated in the Fc domain (see the right image of FIG. 13). Referring to the two images, it can be seen that side chains of the lysine 246 and 248 are directed in a direction opposite the FcRn binding site.

[0790] From these facts, it was contemplated that, when an antibody labeling site is chosen, the antibody labeling site does not affect the interaction between the FcRn and Fc domain. In effect, it was confirmed that the half-life of the prepared antibody is not reduced so that the antibody labeling site does not influence binding of FcRn as intended (see Experimental Example 5).

[0791] 5.8. A process of preparing the antibody containing a first chemical functional group according to the present invention is characterized by SSAI leaving,

[0792] As seen from Schemes 4 and 5 in the section 5.2, a process of preparing the R_1'-Ab according to the present invention is characterized in that SSAI leaves the final product R_1'-Ab because the SSAI is included in a leaving group of a nucleophilic substitution reaction. The binding structure between the Fc domain and the SSFI according to the present invention (see section 3.2) was analyzed in comparison with the binding site between Fc and FcRn. As a result, it was confirmed that a portion of the FcRn binding site is covered with the SSFI (FIG. 14). In this regard, it can be expected that, when the SSFI does not leave during the process of preparing the R_1'-Ab, the SSFI has a negative influence on the physical properties (e.g., half-life, and the like) of the antibody.

[0793] The prior art disclosed in Publication No. US 2018/0141976 A1 aims to site-selectively modulate lysine 246 or 248 in an Fc domain using an analogue of Fc-III. However, the corresponding prior art is different from the present invention in that SSFI is included in the final antibody-labeled product because the SSFI does not leave during the preparation process.

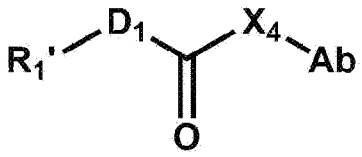
[0794] The prior art disclosed in Publication No. WO 2018/199337 A1 aims to site-selectively modulate lysine 246 or 248 in an Fc domain using an analogue of Fc-III, wherein SSFI is not included in the final antibody-labeled product. However, the corresponding prior art further includes cleaving a "cleavable linker that is a divalent group" in order to remove the SSFI during the preparation process, but the present invention has an advantage in that the SSFI leaves during a conjugation reaction without any additional processes. Also, the cleaving of the cleavable linker that is a divalent group according to the corresponding invention has a drawback in that a disulfide bond forming a structure of the antibody may be cleaved. Because the present invention uses a nucleophilic substitution reaction which occurs easily without any special

conditions, this problem may be dramatically improved.

[0795] 5.9. No side reactions other than a conjugate formation reaction occurs because the antibody containing a first click-chemistry functional group according to the present invention does not contain a highly bioreactive chemical functional group.

[0796] The antibody prepared by the method of preparing an R_1'-Ab according to the present invention has a structure represented by Formula 7:

[0797] [Formula 7]



[0798] When R_1' is transferred to lysine 246 or 248, X_4 may be NH, and D_1 may be any alkylene, alkenylene, or alkynylene. In this case, -NH-(CO)- connected to the antibody is an amide bond that is generally stable *in vivo*. Also, D_1 generally has a structure which is not highly bioreactive. Therefore, it is expected that the R_1'-Ab prepared according to the present invention has high safety because a highly bioreactive structure is not added to molecules other than the labeled molecule (R_1').

[0799] In particular, when the R_1'-Ab is an H_1-Ab having a click-chemistry residue transferred thereto, a highly bioreactive structure is not added to the R_1'-Ab. Therefore, the yield of the click-chemistry reaction may be improved because no secondary reactions other than the click-chemistry reaction occur.

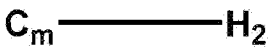
[0800] The prior art disclosed Publication No. WO 2018/199337 A1 aims to site-selectively modulate lysine 246 or 248 in an Fc domain using an analogue of Fc-III and use a biorthogonal functional group. However, the corresponding prior art is different from the present invention in that the antibody product according to the corresponding prior art is allowed to have an additional chemical functional group such as thiol, hydroxy, carboxylic acid, phosphoric acid, amine, and the like during the cleaving of the "cleavable linker that is a divalent group." Such an additional chemical functional group is a bioreactive functional group, and thus may affect the safety of the antibody product.

[0801] 6. Payload (C_m-H_2)

[0802] According to the present invention, there is disclosed a payload. Such a compound is herein indicated by the symbol "C_m-H_2."

[0803] The present invention provides a C_m-H_2 represented by Formula 9:

[0804] [Formula 9]



[0805] wherein C_m is a cargo moiety,

[0806] H_2 is a second click-chemistry functional group.

[0807] In specific embodiments, C_m may include a carrier moiety, a fluorescent moiety, a drug moiety, or a radioactive moiety. Also, R_1' may include a drug moiety. In addition, R_1' may include a VC linker. In other specific embodiments, R_1' may include an antibody or an analogue thereof, which includes a paratope.

[0808] In specific embodiments, when C_m includes a drug moiety, the drug moiety may be an anti-cancer drug. Also, the anti-cancer drug may include one or more selected from DM1, DM3, DM4, Abrin, Ricin A, a *Pseudomonas* exotoxin, a *Cholera* toxin, a *Diphtheria* toxin, a tumor necrosis factor, α interferon, β interferon, a nerve growth factor, a platelet-derived growth factor, a tissue plasminogen activator, a cytokine, an apoptosis-inducing agent, an anti-angiogenic agent, a lymphokine, taxane, a DNA-alkylating agent, anthracyclin, a Tubulysin analogue, a duocarmycin analogue, auristatin E, auristatin F, a maytansinoid, a cytotoxic agent including a reactive polyethylene glycol residue, taxon, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, T. Colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dihydrotestosterone, a glucocorticoid, procaine, tetracaine, lidocaine, propranolol, puromycin, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine, mechlorethamine, thiotepa, chlorambucil, Meiphalan, carmustine, lomustine, cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, cisplatin, dactinomycin, bleomycin, anthramycin, calicheamicin, abiraterone, bendamustine, bortezomib, carboplatin, cabazitaxel, dasatinib, doxetaxel, epirubicin, erlotinib, everolimus, gemcitabine, gefitinib, idarubicin, imatinib, hydroxyurea, lapatinib, leuprorelin, melphalan, nedaplatin, nilotinib, oxaliplatin, pazopanib, pemetrexed, picoplatin, romidepsin, satraplatin, sorafenib, vemurafenib, sunitinib, teniposide, triplatin, and vinorelbine.

[0809] In specific embodiments, C_m may include a plurality of carrier moieties, fluorescent moieties, drug moieties, or radioactive moieties. Also, C_m may include two or more drug moieties. In addition, C_m may include a VC linker.

[0810] In specific embodiments, H_2 may include any one selected from an alkyne, an azide, a strained alkyne, a diene, a dienophile, an alkene, a thiol, and a tetrazine. Furthermore, H_2 may be an azide, or a strained alkyne. Further, H_2 may be an azide, or dibenzocyclooctyne-amine. Additionally, H_2 may be a diene, or a dienophile. Further, H_2 may be a tetrazine, or a

norbornene. Alternatively, H_2 may be a tetrazine, or a trans-cyclooctene.

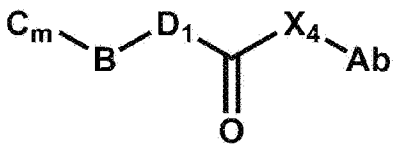
[0811] In specific embodiments, when H_2 is to react with the H_1-Ab according to the present invention, H_2 may be complementary to the first click-chemistry functional group (H_1).

[0812] 7. Antibody-payload conjugate (C_m-Ab)

[0813] According to the present invention, there is disclosed a conjugate of an antibody and a payload (i.e., an antibody-payload conjugate). Such a compound is herein indicated by the symbol "C_m-Ab."

[0814] The present invention provides a C_m-Ab represented by Formula 10:

[0815] [Formula 10]



[0816] wherein C_m is a cargo moiety,

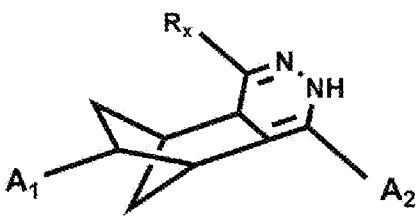
[0817] B is a structure formed by a click-chemistry reaction between a first click-chemistry functional group and a second click-chemistry functional group,

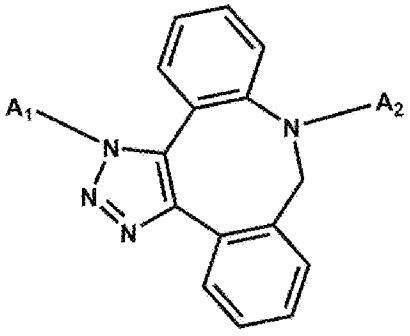
[0818] D_1 is any alkylene, alkenylene, or alkynylene,

[0819] X_4 is NH, O, or S, and

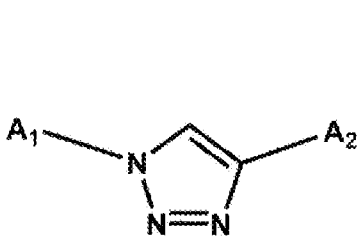
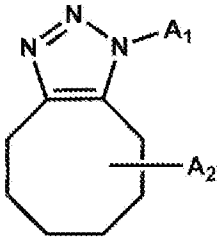
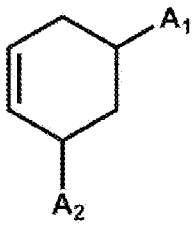
[0820] Ab is an antibody.

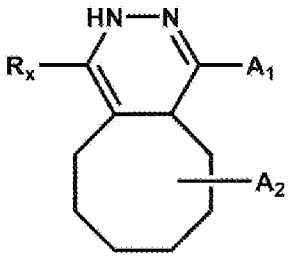
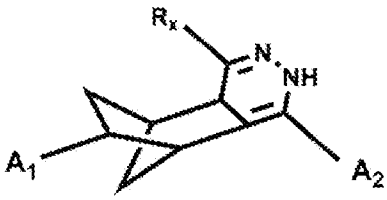
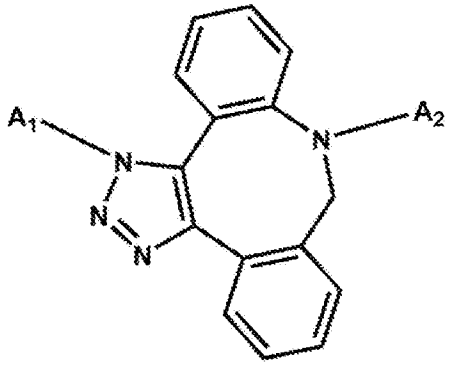
[0821] The contents of the cargo moiety apply to the contents disclosed in the section '6. Payload.'

[0822] In specific embodiments, B may be a structure formed by a click-chemistry reaction between any one selected from an alkyne, an azide, a strained alkyne, a diene, a dienophile, an alkene, a thiol, and a tetrazine and its partner "click-chemistry functional group." Also, B may be any one selected from 

, 

,

, and

, wherein both A_1 and A_2 are connected to the cargo moiety or D_1 so that the A_1 and A_2 cannot be connected to the same moiety, and R_x may be selected from H, a halogen, and a C_{1-3} alkyl. Furthermore, B may be

. Additionally, B may be

.

[0823] In specific embodiments, D_1 may include any one selected from a covalent bond, a C_{1-4} alkylene, a C_{2-4} alkenylene, a C_{2-4} alkynylene, and a C_{3-8} cycloalkylene. Also, D_1 may be $-CH_2OCH_2-$. In addition, D_1 may be a covalent bond.

[0824] In one specific embodiment, X_4 may be NH.

[0825] In specific embodiments, Ab may be a human antibody. In other specific embodiments, Ab may be a non-human animal antibody. In specific embodiments, Ab may be immunoglobulin G (IgG). In specific embodiments, Ab may be a whole antibody. In other specific embodiments, Ab may be a fragment of the antibody.

[0826] In specific embodiments, X_4 and Ab may be connected via an Fab domain of Ab. In other specific embodiments, X_4 and Ab may be connected via an Fc domain of Ab. Also, X_4

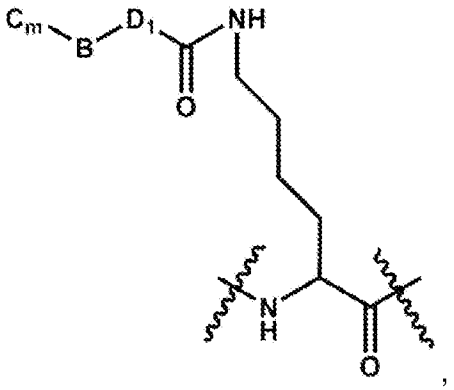
and Ab may be connected via lysine 246 or 248 in the Fc domain of Ab. Furthermore, X_4 and Ab may be connected via lysine 246 in the Fc domain of Ab. Further, X_4 and Ab may be connected via lysine 248 in the Fc domain of Ab. Alternatively, X_4 and Ab may be connected via lysine 246 and 248 in the Fc domain of Ab. In specific embodiments, X_4 and Ab may be connected via only one of two Fc domains of Ab. In other specific embodiments, X_4 and Ab may be connected via both of the two Fc domains of Ab.

[0827] The present invention provides an antibody or a fragment thereof including an amino acid sequence of the following Formula 10-1:

[0828] [Formula 10-1]

[0829] G—P—S—V—F—L—F—P—P-(K)"-P—K—D—T—L—M—I (SEQ ID NO: 23)

[0830] wherein G is glycine, P is proline, S is serine, V is valine, F is phenylalanine, L is leucine, K is lysine, D is aspartic acid, T is threonine, M is methionine, I is isoleucine, and

[0831] (K)" is 

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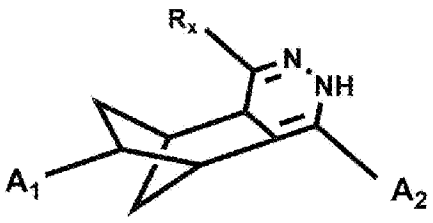
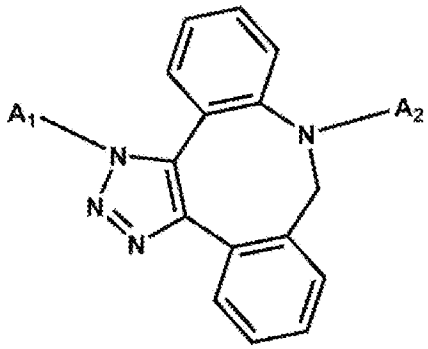
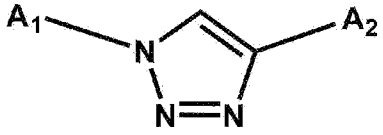
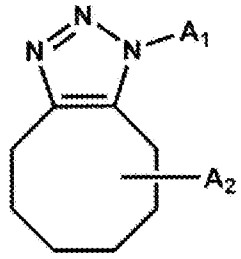
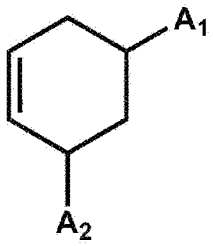
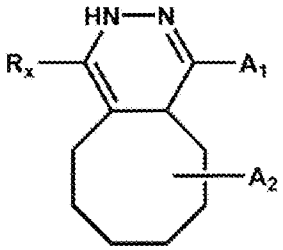
[0832] wherein C_m is a cargo moiety,

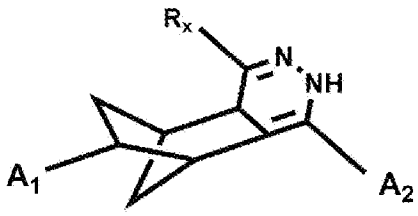
[0833] B is a structure formed by a click-chemistry reaction between a first click-chemistry functional group and a second click-chemistry functional group, and

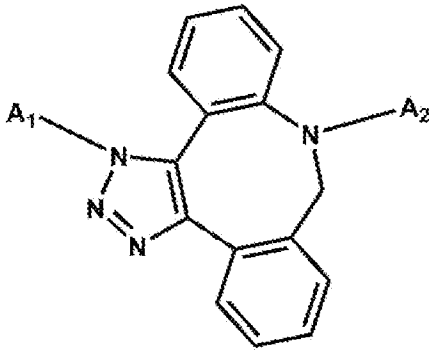
[0834] D_1 is any alkylene, alkenylene, or alkynylene. The antibody or fragment thereof has a cargo moiety conjugated to lysine 246 in an Fc domain thereof, or a site corresponding to the lysine 246.

[0835] The contents of the cargo moiety apply to the contents disclosed in the section '6. Payload.'

[0836] In specific embodiments, B may be a structure formed by a click-chemistry reaction between any one selected from an alkyne, an azide, a strained alkyne, a diene, a dienophile, an alkene, a thiol, and a tetrazine and its partner "click-chemistry functional group." Also, B may be

any one selected from  ,  ,  ,  ,  ,  , 

and , wherein both A_1 and A_2 are connected to the cargo moiety or D_1 so that the A_1 and A_2 cannot be connected to the same moiety, and R_x may be selected from H, a halogen, and a C_{1-3} alkyl. Furthermore, B may be  .

Additionally, B may be  .

[0837] In specific embodiments, D_1 may include any one selected from a covalent bond, a C_{1-4} alkylene, a C_{2-4} alkenylene, a C_{2-4} alkynylene, and a C_{3-8} cycloalkylene. Also, D_1 may be $-CH_2OCH_2-$. In addition, D_1 may be a covalent bond.

[0838] In specific embodiments, the antibody may be a human antibody. In other specific embodiments, the antibody may be a non-human animal antibody. In specific embodiments, the antibody may be immunoglobulin G (IgG). In specific embodiments, the antibody may be a whole antibody. In other specific embodiments, the antibody may be a fragment of the antibody.

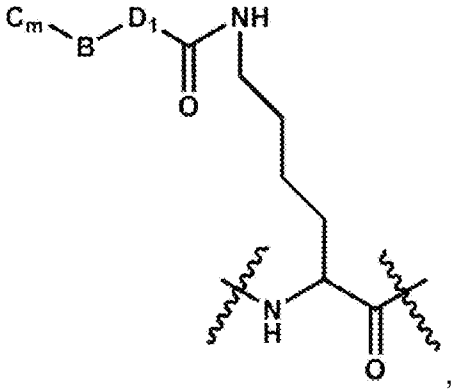
[0839] In specific embodiments, the antibody may include the amino acid sequence of Formula 10-1 in only one of the two Fc domains thereof. In other specific embodiments, the antibody may include the amino acid sequence of Formula 10-1 in both of the two Fc domains thereof.

[0840] The present invention provides an antibody or a fragment thereof including an amino acid sequence of the following Formula 10-2:

[0841] [Formula 10-2]

[0842] G—P—S—V—F—L—F—P—P—K—P-(K)"-D—T—L—M—I (SEQ ID NO: 24)

[0843] wherein G is glycine, P is proline, S is serine, V is valine, F is phenylalanine, L is leucine, K is lysine, D is aspartic acid, T is threonine, M is methionine, I is isoleucine, and

[0844] (K)" is 

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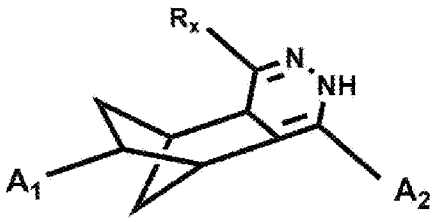
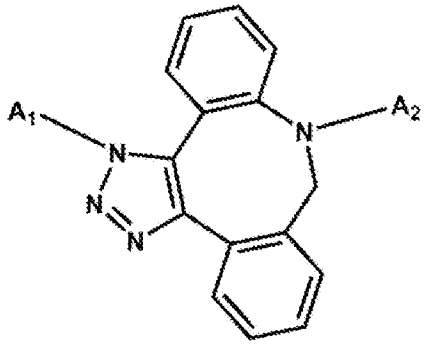
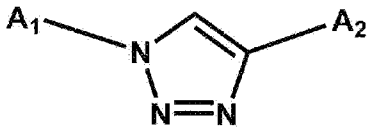
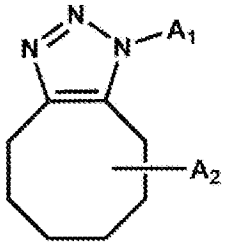
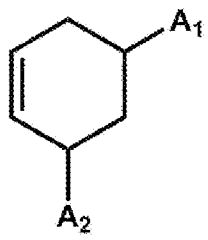
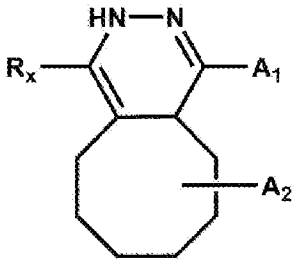
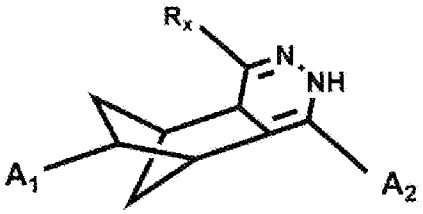
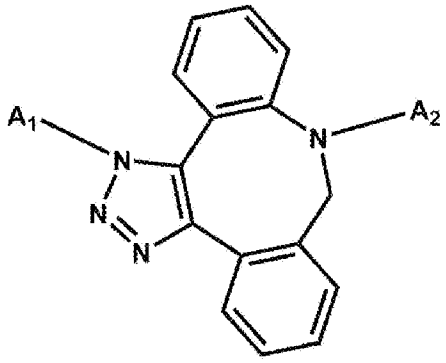
[0845] wherein C_m is a cargo moiety,

[0846] B is a structure formed by a click-chemistry reaction between a first click-chemistry functional group and a second click-chemistry functional group, and

[0847] D_1 is any alkylene, alkenylene, or alkynylene. The antibody or fragment thereof has a cargo moiety conjugated to lysine 246 in an Fc domain thereof, or a site corresponding to the lysine 246.

[0848] The contents of the cargo moiety apply to the contents disclosed in the section '6. Payload.'

[0849] In specific embodiments, B may be a structure formed by a click-chemistry reaction between any one selected from an alkyne, an azide, a strained alkyne, a diene, a dienophile, an

alkene, a thiol, and a tetrazine and its partner "click-chemistry functional group." Also, B may be any one selected from  ,  ,  ,  ,  ,  , and  , wherein both A_1 and A_2 are connected to the cargo moiety or D_1 so that the A_1 and A_2 cannot be connected to the same moiety, and R_x may be selected from H, a halogen, and a C_{1-3} alkyl. Furthermore, B may be  . Additionally, B may be  .

[0850] In specific embodiments, D_1 may include any one selected from a covalent bond,

a C_{1-4} alkylene, a C_{2-4} alkenylene, a C_{2-4} alkynylene, and a C_{3-8} cycloalkylene. Also, D_1 may be $-CH_2OCH_2-$. In addition, D_1 may be a covalent bond.

[0851] In specific embodiments, the antibody may be a human antibody. In other specific embodiments, the antibody may be a non-human animal antibody. In specific embodiments, the antibody may be immunoglobulin G (IgG). In specific embodiments, the antibody may be a whole antibody. In other specific embodiments, the antibody may be a fragment of the antibody.

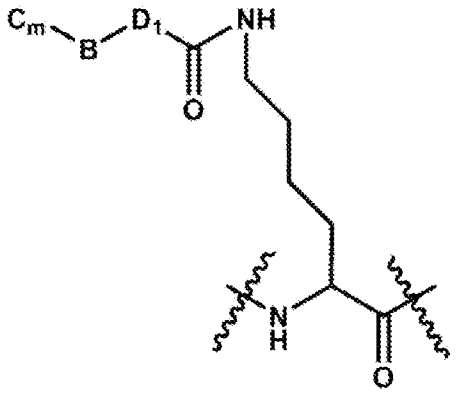
[0852] In specific embodiments, the antibody may include the amino acid sequence of Formula 10-2 in only one of the two Fc domains thereof. In other specific embodiments, the antibody may include the amino acid sequence of Formula 10-2 in both of the two Fc domains thereof.

[0853] The present invention provides an antibody or a fragment thereof including an amino acid sequence of the following Formula 10-3:

[0854] [Formula 10-3]

G—P—S—V—F—L—F—P—P-(K)"-P-(K)"-D—T—L—M—I (SEQ ID NO: 25)

[0855] wherein G is glycine, P is proline, S is serine, V is valine, F is phenylalanine, L is leucine, K is lysine, D is aspartic acid, T is threonine, M is methionine, I is isoleucine, and



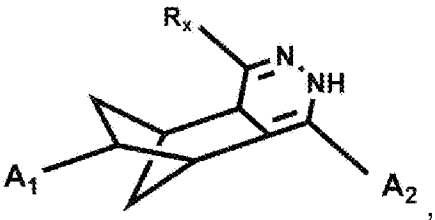
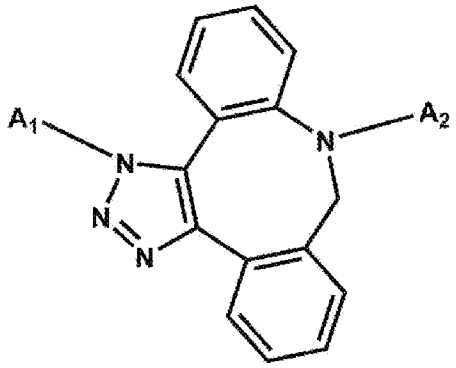
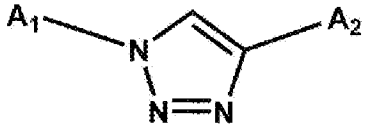
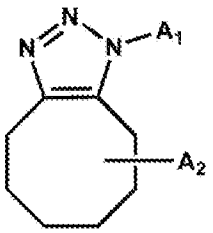
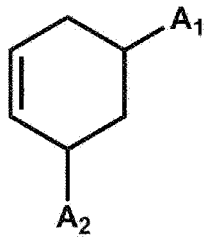
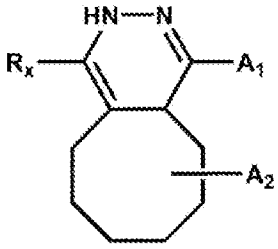
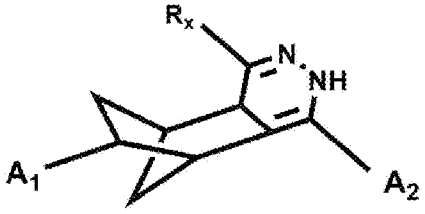
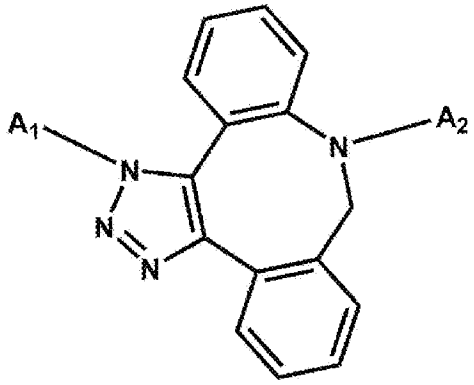
[0856] (K)" is each independently , wherein C_m is a cargo moiety,

[0857] B is a structure formed by a click-chemistry reaction between a first click-chemistry functional group and a second click-chemistry functional group, and

[0858] D_1 is any alkylene, alkenylene, or alkynylene. The antibody or fragment thereof has a cargo moiety conjugated to lysine 246 in an Fc domain thereof, or a site corresponding to the lysine 246.

[0859] The contents of the cargo moiety apply to the contents disclosed in the section "6. Payload."

[0860] In specific embodiments, B may be a structure formed by a click-chemistry reaction

between any one selected from an alkyne, an azide, a strained alkyne, a diene, a dienophile, an alkene, a thiol, and a tetrazine and its partner "click-chemistry functional group." Also, B may be any one selected from  ,  ,  ,  ,  ,  , and  , wherein both A_1 and A_2 are connected to the cargo moiety or D_1 so that the A_1 and A_2 cannot be connected to the same moiety, and R_x may be selected from H, a halogen, and a C_{1-3} alkyl. Furthermore, B may be  . Additionally, B may be  .

[0861] In specific embodiments, D_1 may include any one selected from a covalent bond, a C_{1-4} alkylene, a C_{2-4} alkenylene, a C_{2-4} alkynylene, and a C_{3-8} cycloalkylene. Also, D_1 may be $-CH_2OCH_2-$. In addition, D_1 may be a covalent bond.

[0862] In specific embodiments, the antibody may be a human antibody. In other specific embodiments, the antibody may be a non-human animal antibody. In specific embodiments, the antibody may be immunoglobulin G (IgG). In specific embodiments, the antibody may be a whole antibody. In other specific embodiments, the antibody may be a fragment of the antibody.

[0863] In specific embodiments, the antibody may include the amino acid sequence of Formula 10-3 in only one of the two Fc domains thereof. In other specific embodiments, the antibody may include the amino acid sequence of Formula 10-3 in both of the two Fc domains thereof.

[0864] The present invention provides an antibody or a fragment thereof including one or more amino acid sequences selected from Formulas 10-1, 10-2, and 10-3. In this case, the contents of Formulas 10-1 to 10-3 are as described above.

[0865] In specific embodiments, D_1 may be a covalent bond.

[0866] In specific embodiments, the antibody or fragment thereof may include only the amino acid sequence of Formula 10-1 and may not include the amino acid sequences of Formulas 10-2 and 10-3. Also, the antibody or fragment thereof may include the amino acid sequence of Formula 10-1 in only one of the two Fc domains thereof. In addition, the antibody may include the amino acid sequence of Formula 10-1 in both of the two Fc domains thereof.

[0867] In other specific embodiments, the antibody or fragment thereof may include only the amino acid sequence of Formula 10-2 and may not include the amino acid sequences of Formulas 10-1 and 10-3. Also, the antibody or fragment thereof may include the amino acid sequence of Formula 10-2 in only one of the two Fc domains thereof. In addition, the antibody may include the amino acid sequence of Formula 10-2 in both of the two Fc domains thereof.

[0868] In specific embodiments, the antibody or fragment thereof may include only the amino acid sequence of Formula 10-3 and may not include the amino acid sequences of Formulas 10-1 and 10-2. Also, the antibody or fragment thereof may include the amino acid sequence of Formula 10-3 in only one of the two Fc domains thereof. In addition, the antibody may include the amino acid sequence of Formula 10-3 in both of the two Fc domains thereof.

[0869] 7.1. Antibody-drug conjugate (ADC)

[0870] According to the present invention, there is disclosed a novel antibody-drug conjugate (ADC). The ADC according to the present invention means that a payload in the

antibody-payload conjugate includes a drug moiety.

[0871] The present invention provides a C_m-Ab according to the present invention in which a cargo moiety includes a drug moiety.

[0872] In specific embodiments, the cargo moiety may include two or more drug moieties.

[0873] In specific embodiments, the drug moiety may be an anti-cancer drug. Also, the anti-cancer drug may include one or more selected from DM1, DM3, DM4, Abrin, Ricin A, a *Pseudomonas* exotoxin, a *Cholera* toxin, a *Diphtheria* toxin, a tumor necrosis factor, α interferon, β interferon, a nerve growth factor, a platelet-derived growth factor, a tissue plasminogen activator, a cytokine, an apoptosis-inducing agent, an anti-angiogenic agent, a lymphokine, taxane, a DNA-alkylating agent, anthracyclin, a Tubulysin analogue, a duocarmycin analogue, auristatin E, auristatin F, a maytansinoid, a cytotoxic agent including a reactive polyethylene glycol residue, taxon, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, T. Colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dihydrotestosterone, a glucocorticoid, procaine, tetracaine, lidocaine, propranolol, puromycin, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine, mechlorethamine, thiotepa, chlorambucil, Meiphalan, carmustine, lomustine, cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, cisplatin, dactinomycin, bleomycin, anthramycin, calicheamicin, abiraterone, bendamustine, bortezomib, carboplatin, cabazitaxel, dasatinib, doxetaxel, epirubicin, erlotinib, everolimus, gemcitabine, gefitinib, idarubicin, imatinib, hydroxyurea, lapatinib, leuprorelin, melphalan, nedaplatin, nilotinib, oxaliplatin, pazopanib, pemetrexed, picoplatin, romidepsin, satraplatin, sorafenib, vemurafenib, sunitinib, teniposide, triplatin, and vinorelbine.

[0874] In specific embodiments, the cargo moiety may include a VC linker.

[0875] 7.2. Method of preparing antibody-payload conjugate

[0876] According to the present invention, there is disclosed a method of preparing an antibody-payload conjugate.

[0877] The antibody-payload conjugate of the present invention may be prepared by allowing a C_m-H_2 to react with an H_1-Ab. In this case, the contents of the H_1-Ab apply to the contents disclosed in the section 5.1, and the contents disclosed in the section 6 apply for the C_m-H_2. When the first click-chemistry functional group of H_1-Ab and the second click-chemistry functional group of C_m-H_2 are complementary to each other, that is, when the first click-chemistry functional group of H_1-Ab and the second click-chemistry functional group of C_m-H_2 function as partner click-chemistry functional groups, a click-chemistry reaction may occur to prepare an antibody-payload conjugate according to the present invention. The contents of the click-

chemistry reaction were sufficiently described above in the section "Definition." The click-chemistry reaction is a biorthogonal reaction, and has an advantage in that the reaction occurs at a very rapid reaction rate and is used to form a strong binding structure. Therefore, the click-chemistry reaction has the same advantages as in the following sections 7.3 and 7.4.

[0878] The present invention provides a method of preparing an antibody-payload conjugate, characterized in that the method includes reacting an antibody containing a first click-chemistry functional group according to the present invention with a payload according to the present invention, wherein H_2 of the payload is a second click-chemistry functional group complementary to the first click-chemistry functional group.

[0879] In this case, the antibody provided in the section 5.1 applies to the antibody containing the first click-chemistry functional group, and the payload provided in the section 6 applies to the payload. Because all the antibodies containing a first click-chemistry functional group in the lysine 246 and/or the lysine 248 are disclosed in the section 5.1, it is desirable that the antibody-payload conjugates in which a cargo moiety is conjugated to the lysine 246 and/or the lysine 248 are disclosed to a fully reproducible extent (see Formulas 10 and 10-1 to 10-3 in the section 7).

[0880] The present invention provides a kit for preparing an antibody-payload conjugate, characterized in that the kit includes an antibody containing a first click-chemistry functional group according to the present invention; and a payload according to the present invention, wherein H_2 of the payload is a second click-chemistry functional group complementary to the first click-chemistry functional group.

[0881] In this case, the antibody provided in the section 5.1 applies to the antibody containing the first click-chemistry functional group, and the payload provided in the section 6 applies to the payload.

[0882] Optionally, the present invention provides a kit for preparing an antibody-payload conjugate, which includes:

[0883] an antibody;

[0884] an agent for transferring a first click-chemistry functional group to an antibody according to the present invention;

[0885] a payload according to the present invention.

[0886] In specific embodiments, the agent for transferring a first click-chemistry functional group to an antibody may be an H_1-L_2-SSFI for specifically transferring a first click-chemistry functional group to lysine 246 or 248 in an Fc domain of an antibody according to the present

invention. In other specific embodiments, the agent for transferring a first click-chemistry functional group to an antibody may be an H_1-L_2-SSFI for specifically transferring a first click-chemistry functional group to lysine 246 in an Fc domain of an antibody according to the present invention. In still another specific embodiment, the agent for transferring a first click-chemistry functional group to an antibody may be an H_1-L_2-SSFI for specifically transferring a first click-chemistry functional group to lysine 248 in an Fc domain of an antibody according to the present invention.

[0887] Optionally, the present invention provides a kit for preparing an antibody-payload conjugate, which includes:

[0888] an antibody;

[0889] a linker according to the present invention;

[0890] a site-specific antibody interactome according to the present invention; and

[0891] a payload according to the present invention.

[0892] In specific embodiments, D_2 in the linker is a C_y alkylene, a C_y alkenylene, or a C_y alkynylene, and D_3 in the site-specific antibody interactome is a C_x alkylene, wherein y may be an integer greater than or equal to 1. Also, the sum of x and y may be $1 \leq x + y \leq 5$. In addition, the sum of x and y may be $6 \leq x + y \leq 8$. Further, the sum of x and y may be $9 \leq x + y \leq 12$.

[0893] 7.3. The antibody-payload conjugate according to the present invention uses a biorthogonal reaction to form a stable bond.

[0894] The antibody-payload conjugate according to the present invention is formed through a click-chemistry reaction. The click-chemistry reaction is a biorthogonal reaction that does not influence biochemical phenomena naturally occurring *in vivo*. Also, a bond formed by the biorthogonal reaction is not recognized by *in vivo* lyases. Therefore, the antibody-payload conjugate according to the present invention has an advantage in that the antibody and the cargo moiety form a very stable bond *in vivo*.

[0895] 7.4. The antibody-payload conjugate prepared according to the present invention has high uniformity and yield.

[0896] As described above in the section 5.6, the antibody containing a first click-chemistry functional group according to the present invention has high uniformity and yield. Therefore, the antibody-payload conjugate prepared from the antibody according to the present invention has an advantage in that it also has high uniformity and yield. The uniformity of C_m-Ab has to be high in order that the antibody conjugate has uniform performance.

[0897] In the case of the antibody conjugates prepared by the prior art disclosed in

Publication Nos. US 2018/0141976 A1 and WO 2018/199337 A1, since the antibody conjugates have poor yield and uniformity, as described above in the section 5.6, there may be a negative effect on the uniformity of performance.

[0898] 7.5. No functions of the antibody are lowered because the antibody-payload conjugate according to the present invention has no FcRn binding site blocked therein.

[0899] The C_m-Ab according to present invention has the same advantages as described in the sections 5.7 and 5.8. In particular, pharmacokinetic (PK) characteristics of the ADC used *in vivo* may be greatly improved because the ADC has an increased half-life.

[0900] An FcRn binding site of the ADC prepared by the prior art disclosed in Publication No. US 2018/0141976 A1 is blocked because SSFI is included in the final antibody-labeled product. Therefore, the ADC according to the present invention has superior PK characteristics, compared to the ADC according to the corresponding prior art.

[0901] 7.6. The antibody-drug conjugate according to the present invention is stable because the antibody-drug conjugate does not contain a highly bioreactive chemical functional group.

[0902] The C_m-Ab according to the present invention has the same advantages as described in the section 5.9. In particular, pharmacodynamic (PD) characteristics of the ADC used *in vivo* may be greatly improved because the ADC enables a smooth antibody-drug action.

[0903] In the case of the ADC prepared by the prior art disclosed in Publication No. WO 2018/199337 A1, an additional chemical functional group such as thiol, hydroxy, a carboxylic acid, phosphoric acid, an amine, and the like are included in the final antibody-labeled product. Therefore, the ADC according to the present invention has superior PD characteristics, compared to the ADC according to the corresponding prior art.

[0904] 8. Composition

[0905] According to the present invention, there is disclosed a composition including an antibody. In this case, the antibody may be an antibody containing a first chemical functional group according to the present invention. Alternatively, the antibody may be an antibody-payload conjugate according to the present invention. Also, the antibody may be an antibody-drug conjugate according to the present invention.

[0906] The composition according to the present invention may be used for various applications depending on the function of an antibody included in the composition. For example, when a first chemical functional group or a cargo moiety includes a radioactive moiety, the corresponding composition may be used as a radioactive contrast medium, and the like.

Alternatively, when the first chemical functional group or the cargo moiety includes a fluorescent moiety, the corresponding composition may be used as a label used in an enzyme-linked immunosorbent assay (ELISA), and the like. Alternatively, when the first chemical functional group or the cargo moiety includes a drug moiety, the corresponding composition may be used as a pharmaceutical composition. In this case, components of the composition generally used in the related art fall within the scope of the present invention. Also, compositional ratios of the corresponding components generally used in the art also fall within the scope of the present invention.

[0907] The present invention provides a composition including an antibody containing a first chemical functional group according to the present invention. In specific embodiments, the antibody containing the first chemical functional group may be at least one selected from Formulas 7, 7-1 to 7-3, 8, and 8-1 to 8-3.

[0908] Also, the present invention provides a composition including an antibody containing a first click-chemistry functional group according to the present invention. In specific embodiments, the antibody containing the first click-chemistry functional group may be at least one selected from Formulas 8, and 8-1 to 8-3.

[0909] In addition, the present invention provides a composition including an antibody-payload conjugate according to the present invention. In this case, the contents described in the section 7 apply to the contents of the antibody-payload conjugate.

[0910] Optionally, the present invention provides a composition including an antibody-drug conjugate according to the present invention. In this case, the contents described in the section 7.1 apply to the contents of the antibody-drug conjugate.

[0911] Pharmaceutical composition

[0912] The following content relates to the where the composition is a pharmaceutical composition used for diagnostic, prophylactic and/or therapeutic purposes. Only within the content "pharmaceutical composition" here, all the R_1'-Ab, H_1-Ab, C_m-Ab, and ADC according to the present invention are used interchangeably with the term "antibody or fragment thereof."

[0913] The present invention provides a pharmaceutical composition including an antibody or a fragment thereof according to the present invention. Also, the pharmaceutical composition may be a composition for treating cancer. Furthermore, the cancer may include any one selected from bladder cancer, bone cancer, brain cancer, breast cancer, heart cancer, cervical cancer, colon cancer, rectal cancer, esophageal cancer, fibrosarcoma, gastric cancer, stomach cancer, head and neck cancer, Kaposi's sarcoma, renal cancer, leukemia, liver cancer, lung cancer, lymphoma, melanoma, myeloma, ovarian cancer, pancreatic cancer, penile cancer,

prostate cancer, testicular germ cell cancer, thymoma, and thymic carcinoma. Further, the cancer may be breast cancer.

[0914] The present invention provides a therapeutic method which includes administering a pharmaceutical composition including an antibody or a fragment thereof according to the present invention into a subject. Also, the therapeutic method may be a method of treating cancer. Furthermore, the cancer may include any one selected from bladder cancer, bone cancer, brain cancer, breast cancer, heart cancer, cervical cancer, colon cancer, rectal cancer, esophageal cancer, fibrosarcoma, gastric cancer, stomach cancer, head and neck cancer, Kaposi's sarcoma, renal cancer, leukemia, liver cancer, lung cancer, lymphoma, melanoma, myeloma, ovarian cancer, pancreatic cancer, penile cancer, prostate cancer, testicular germ cell cancer, thymoma, and thymic carcinoma. Further, the cancer may be breast cancer.

[0915] To prepare a pharmaceutical or sterilized composition including an antibody or a fragment thereof, the antibody or fragment thereof according to the present invention may be mixed with a pharmaceutically acceptable carrier or excipient. The composition may further include one or more other therapeutic agents which are suitable for treating or preventing cancer (e.g., breast cancer, colorectal cancer, lung cancer, multiple myeloma, ovarian cancer, liver cancer, gastric cancer, pancreatic cancer, acute myelogenous leukemia, chronic myelogenous leukemia, osteosarcoma, squamous cell carcinoma, peripheral nerve sheath tumors, schwannoma, head and neck cancer, bladder cancer, esophageal cancer, Barrett's esophageal cancer, glioblastoma, clear cell sarcoma of soft tissue, malignant mesothelioma, neurofibromatosis, kidney cancer, melanoma, prostate cancer, benign prostate hypertrophy (BPH), gynecomastia, rhabdomyosarcoma, and endometriosis).

[0916] A formulation of therapeutic and diagnostic agents may be prepared by mixing with a physiologically acceptable carrier, an excipient, or a stabilizing agent, for example, in the form of a freeze-dried powder, slurry, an aqueous solution, a lotion, or a suspension (see, for example, Hardman *et al.*, Goodman and Gilman's The Pharmacological Basis of Therapeutics, McGraw-Hill, New York, N.Y., 2001; Gennaro, Remington: The Science and Practice of Pharmacy, Lippincott, Williams, and Wilkins, New York, N.Y., 2000; Avis, *et al.* (eds.), Pharmaceutical Dosage Forms: Parenteral Medications, Marcel Dekker, NY, 1993; Lieberman, *et al.* (eds.), Pharmaceutical Dosage Forms: Tablets, Marcel Dekker, NY, 1990; Lieberman, *et al.* (eds.) Pharmaceutical Dosage Forms: Disperse Systems, Marcel Dekker, NY, 1990; Weiner and Kotkoskie, Excipient Toxicity and Safety, Marcel Dekker, Inc., New York, N.Y., 2000).

[0917] In specific embodiments, the clinical service form (CSF) of the antibody-drug conjugate according to the present invention is a lyophilisate present in a vial containing ADC,

sodium succinate, and polysorbate 20. The lyophilisate may be reconstituted with water for injection, and the solution includes ADC, sodium succinate, sucrose and polysorbate 20 at approximately pH 5.0. For sequential intravenous administration, the resulting solution will be usually further diluted into a carrier solution.

[0918] The choice of a therapeutic dosing regimen depends on various factors including a rate of serum or tissue replacement of a substance, a level of symptoms, the immunogenicity of the substance, and the accessibility of target cells in a biological matrix. In specific embodiments, the dosing regimens maximize an amount of a therapeutic agent to be delivered to a patient so as to satisfy acceptable levels of side effects. Therefore, an amount of a biological agent to be administered depends in part on the certain substance and the severity of the condition being treated. Guidance is available for selection of the appropriate doses of antibodies, cytokines, and small molecules (see, for example, Wawrzynczak, Antibody Therapy, Bios Scientific Pub. Ltd, Oxfordshire, UK, 1996; Kresina (ed.), Monoclonal Antibodies, Cytokines and Arthritis, Marcel Dekker, New York, N.Y., 1991; Bach (ed.), Monoclonal Antibodies and Peptide Therapy in Autoimmune Diseases, Marcel Dekker, New York, N.Y., 1993; Baert *et al.*, New Engl. J. Med. 348:601-608, 2003; Milgrom *et al.*, New Engl. J. Med. 341:1966-1973, 1999; Slamon *et al.*, New Engl. J. Med. 344:783-792, 2001; Beniaminovitz *et al.*, New Engl. J. Med. 342:613-619, 2000; Ghosh *et al.*, New Engl. J. Med. 348:24-32, 2003; Lipsky *et al.*, New Engl. J. Med. 343:1594-1602, 2000).

[0919] The appropriate dose is, for example, determined by a clinical practitioner using parameters or factors known in the related art or suspected to affect treatment, or using parameters or factors expected to affect treatment. In general, the dose begins with an amount somewhat smaller than the optimal dose and then increases in small increments until the desirable or optimal effects are achieved relative to any negative side effects. Important diagnostic measures, for example, include symptoms of inflammation or levels of inflammatory cytokines produced.

[0920] An actual dose level of the active ingredient in the pharmaceutical composition according to the present invention may vary in order to achieve an effective amount of the active ingredient to realize desired therapeutic responses in a certain patient, a composition, and a mode of administration without causing any toxicity in patients. The chosen dose level may be determined depending on various pharmacokinetic factors including the activities of a certain composition of the present invention used, or esters, salts or amides thereof, a route of administration, an administration time, a secretion rate of a certain compound used, a duration of treatment, other drugs, compounds and/or substances used in combination with the certain

compound used, the age, sex, weight, condition, overall health, and previous medical history of a patient to be treated, and other factors known in the medical field.

[0921] The composition including an antibody or a fragment thereof according to the present invention may be given by continuous infusion, or given in a single dose, for example, daily, weekly, 1 to 7 times per week, bi-weekly, once every three weeks, once every four weeks, once every five weeks, once every six weeks, once every seven weeks, or once every eight weeks. The dose may be given intravenously, subcutaneously, topically, orally, intranasally, intrarectally, intramuscularly, intracerebrally, or by inhalation. A certain dosing protocol involves the maximum dose or administration frequency that avoids significant unwanted side effects.

[0922] For the antibody or fragment thereof according to the present invention, the dose to be administered to a patient may be in a range of 0.0001 mg/kg to 100 mg/kg (patient weight). The dose may be in a range of 0.0001 mg/kg to 20 mg/kg, 0.0001 mg/kg to 10 mg/kg, 0.0001 mg/kg to 5 mg/kg, 0.0001 to 2 mg/kg, 0.0001 to 1 mg/kg, 0.0001 mg/kg to 0.75 mg/kg, 0.0001 mg/kg to 0.5 mg/kg, 0.0001 mg/kg to 0.25 mg/kg, 0.0001 to 0.15 mg/kg, 0.0001 to 0.10 mg/kg, 0.001 to 0.5 mg/kg, 0.01 to 0.25 mg/kg, or 0.01 to 0.10 mg/kg (patient weight). The dose of the antibody or fragment thereof according to the present invention may be calculated by multiplying the weight (kilogram (kg)) of a patient by the dose (mg/kg) to be administered.

[0923] The antibody or fragment thereof according to the present invention may be repeatedly administered, and the administration may be performed at intervals of at least a day, two days, three days, five days, 10 days, 15 days, 30 days, 45 days, 2 months, 75 days, 3 months, or at least 6 months. In specific embodiments, the antibody or fragment thereof according to the present invention may be repeatedly administered every three weeks.

[0924] An effective amount to be administered to a certain patient may vary depending on factors such as the condition to be treated, the overall health of a patient, the mode, route and dosage of administration, and the severity of side effects (see, for example, Maynard *et al.*, A Handbook of SOPs for Good Clinical Practice, Interpharm Press, Boca Raton, Fla., 1996; Dent, Good Laboratory and Good Clinical Practice, Urch Publ., London, UK, 2001).

[0925] The route of administration may be, for example, by topical or skin application, by intravenous, intraperitoneal, intracerebral, intramuscular, intraocular, intra-arterial, intramedullary, intralesional injection or infusion, or by a sustained release system or an implant (see, for example, Sidman *et al.*, Biopolymers 22:547-556, 1983; Langer *et al.*, J. Biomed. Mater. Res. 15:167-277, 1981; Langer, Chem. Tech. 12:98-105, 1982; Epstein *et al.*, Proc. Natl. Acad. Sci. USA 82:3688-3692, 1985; Hwang *et al.*, Proc. Natl. Acad. Sci. USA 77:4030-4034, 1980; US Patent Nos. 6,350,466 and 6,316,024). When necessary, the composition may also include a solubilizing

agent and a topical anesthetic (for example, lidocaine) for relieving pain at a site of injection. For example, the composition may also be formulated using an inhaler or a nebulizer, or may be used for pulmonary administration by formulation using an aerosolizing agent. See, for example, US Patent Nos. 6,019,968, 5,985,320, 5,985,309, 5,934,272, 5,874,064, 5,855,913, 5,290,540, and 4,880,078; and PCT Publication Nos. WO 92/19244, WO 97/32572, WO 97/44013, WO 98/31346, and WO 99/66903, the entire contents of which are hereby incorporated by reference.

[0926] Also, the composition of the present invention may be administered through one or more routes of administration using one or more various methods known in the related art. As recognized by a person of ordinary skill in the art, the route and/or mode of administration will depend on the desired results. The route of administration selected for the antibody or fragment thereof according to the present invention includes intravenous, intramuscular, intradermal, intraperitoneal, subcutaneous, intraspinal, or other parenteral routes of administration, for example, routes of administration by injection or infusion. Parenteral administration generally represents a mode of administration by injection other than the enteral and topical administration, and includes intravenous, intramuscular, intra-arterial, intrathecal, intracapsular, intraorbital, intracardial, intradermal, intraperitoneal, transtracheal, subcutaneous, subepidermal, intra-articular, subcapsular, subarachnoid, intraspinal, epidural, and intrasternal injections and infusions, but the present invention is not limited thereto. Alternatively, the composition of the present invention may be administered through a non-parenteral route of administration, for example, a topical, intraepidermal or intramucosal route of administration, and may be, for example, administered intranasally, orally, vaginally, intrarectally, sublingually, or topically. In one exemplary embodiment, the antibody or fragment thereof according to the present invention may be administered by infusion. In another exemplary embodiment, the antibody or fragment thereof according to the present invention may be subcutaneously administered.

[0927] When the antibody or fragment thereof according to the present invention is administered using a controlled or delayed release system, a pump may be used to achieve the controlled or delayed release (see Langer, supra; Sefton, CRC Crit. Ref Biomed. Eng. 14:20, 1987; Buchwald *et al.*, Surgery 88:507, 1980; Saudek *et al.*, N. Engl. J. Med. 321:574, 1989). A polymeric material may be used to achieve the controlled or delayed release in the therapy of the present invention (see, for example, Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Fla., 1974; Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York, 1984; Ranger and Peppas, J. Macromol. Sci. Rev. Macromol. Chem. 23:61, 1983; also see Levy *et al.*, Science 228:190, 1985; During *et al.*, Ann. Neurol. 25:351, 1989; Howard *et al.*, J. Neurosurg. 7 1:105, 1989; US Patent

No. 5,679,377; US Patent No. 5,916,597; US Patent No. 5,912,015; US Patent No. 5,989,463; US Patent No. 5,128,326; PCT Publication No. WO 99/15154; and PCT Publication No. WO 99/20253). Examples of polymers used for a sustained release formulation include poly(2-hydroxyethyl methacrylate), poly(methyl methacrylate), poly(acrylic acid), poly(ethylene-co-vinyl acetate), poly(methacrylic acid), polyglycolide (PLG), a polyanhydride, poly(N-vinyl pyrrolidone), poly(vinyl alcohol), polyacrylamide, poly(ethylene glycol), polylactide (PLA), poly(lactide-co-glycolide) (PLGA), and a polyorthoester, but the present invention is not limited thereto. In one exemplary embodiment, the polymer used for a sustained release formulation is inert, has no filterable impurities, and is stable upon storage, sterilization, and biodegradability. The controlled or delayed release system may be located adjacent to a prophylactic or therapeutic target, and thus requires a portion of the systemic dose (see, for example, Goodson, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138, 1984).

[0928] The controlled release system is discussed in the review of the article "Langer, Science 249:1527-1533, 1990." Any prior art known to a person of ordinary skill in the art may be used to produce a sustained release formulation including one or more antibodies or a fragments thereof according to the present invention. See, for example, US Patent No. 4,526,938, PCT Publication No. WO 91/05548, PCT Publication No. WO 96/20698, Ning *et al.*, Radiotherapy & Oncology 39:179-189, 1996; Song *et al.*, PDA Journal of Pharmaceutical Science & Technology 50:372-397, 1995; Cleek *et al.*, Pro. Int'l. Symp. Control. Rel. Bioact. Mater. 24:853-854, 1997; and Lam *et al.*, Proc. Int'l. Symp. Control Rel. Bioact. Mater. 24:759-760, 1997, the entire contents of which are hereby incorporated by reference.

[0929] When the antibody or fragment thereof according to the present invention is topically administered, the antibody or fragment thereof may be formulated into forms of an ointment, a cream, a transdermal patch, a lotion, a gel, a shampoo, a spray, an aerosol, a solution, or an emulsion, or other forms well known to a person of ordinary skill in the art. See, for example, Remington's Pharmaceutical Sciences and Introduction to Pharmaceutical Dosage Forms, 19th ed., Mack Pub. Co., Easton, Pa. (1995). A non-sprayable topical dosage form includes a carrier or one or more excipients suitable for topical application. In some cases, viscous, semisolid, or solid forms having a higher kinematic viscosity than water may be generally used as the topical dosage form. Suitable preparations include a solution, a suspension, an emulsion, a cream, an ointment, a powder, a liniment, a salve, and the like, all of which are sterilized, or mixed with an adjuvant (for example, a preservative, a stabilizing agent, a wetting agent, a buffer, or a salt) influencing various characteristics, for example, such as osmotic pressure, when necessary, but the present invention is not limited thereto. In some cases, other suitable topical dosage forms

include a sprayable aerosol formulation filled in a mixture of compressed volatile substances (for example, a gaseous propellant such as freon) or a squeeze bottle after the active ingredient is combined with a solid or liquid inert carrier. When necessary, a moisturizing agent or a humectant may be also added to the pharmaceutical composition and the dosage form. Examples of such an additional component are known in the related art.

[0930] When the composition including an antibody or a fragment thereof is intranasally administered, the composition may be formulated into forms of an aerosol, a spray, mist, or drops. In particular, a prophylactic or therapeutic agent for use in the present invention may be conveniently delivered in an aerosol spray dosage form from a pressurized pack or an atomizer using a suitable propellant (for example, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide, or other suitable gases). In the case of the pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. A capsule and cartridge (for example, consisting of gelatin) for use in an inhaler or an insufflator may be formulated to include a powder mixture of compounds, and a suitable powder base, for example, lactose or starch.

[0931] Methods for co-administration or treatment with a second therapeutic agent, for example, a cytokine, a steroid, a chemotherapeutic agent, an antibiotic, or radiation are known in the related art (see, for example, Hardman *et al.*, (eds.) (2001) Goodman and Gilman's The Pharmacological Basis of Therapeutics, 10th ed., McGraw-Hill, New York, N.Y.; Poole and Peterson (eds.) (2001) Pharmacotherapeutics for Advanced Practice:A Practical Approach, Lippincott, Williams & Wilkins, Phila., Pa.; Chabner and Longo (eds.) (2001) Cancer Chemotherapy and Biotherapy, Lippincott, Williams & Wilkins, Phila., Pa.). An effective amount of the therapeutic agent may reduce symptoms by at least 10%; at least 20%; at least approximately 30%; at least 40%, or at least 50%.

[0932] An additional therapy (for example, a prophylactic or therapeutic agent) that may be administered in combination with the antibody or fragment thereof according to the present invention may be administered together with the antibody or fragment thereof according to the present invention at intervals of less than 5 minutes, intervals of less than 30 minutes, intervals of an hour, intervals of approximately an hour, intervals of approximately 1 to approximately 2 hours, intervals of approximately 2 hours to approximately 3 hours, intervals of approximately 3 hours to approximately 4 hours, intervals of approximately 4 hours to approximately 5 hours, intervals of approximately 5 hours to approximately 6 hours, intervals of approximately 6 hours to approximately 7 hours, intervals of approximately 7 hours to approximately 8 hours, intervals of approximately 8 hours to approximately 9 hours, intervals of approximately 9 hours to

approximately 10 hours, intervals of approximately 10 hours to approximately 11 hour, intervals of approximately 11 hour to approximately 12 hours, intervals of approximately 12 hours to 18 hours, intervals of 18 hours to 24 hours, intervals of 24 hours to 36 hours, intervals of 36 hours to 48 hours, intervals of 48 hours to 52 hours, intervals of 52 hours to 60 hours, intervals of 60 hours to 72 hours, intervals of 72 hours to 84 hours, intervals of 84 hours to 96 hours, or intervals of 96 hours to 120 hours. Two or more therapeutic agents may be administered during the same visit by a patient.

[0933] In specific embodiments, the antibody or fragment thereof according to the present invention may be formulated to insure a proper distribution *in vivo*. For example, the blood-brain barrier (BBB) excludes a number of highly hydrophilic compounds. To ensure therapeutic compounds of the invention cross the BBB (when necessary), they can be, for example, formulated in liposomes. For a method of preparing liposomes, for example, see US Patent Nos. 4,522,811; 5,374,548; and 5,399,331. The liposomes may include one or more moieties that are selectively transported into specific cells or organs, thereby enhancing targeted drug delivery (see, for example, Ranade, (1989) J. Clin. Pharmacol. 29:685). Exemplary targeting moieties include folate or biotin (see, for example, US Patent No. 5,416,016 (to Low *et al.*)); mannosides (see Umezawa *et al.*, (1988) Biochem. Biophys. Res. Commun. 153:1038); antibodies (see Bloeman *et al.*, (1995) FEBS Lett. 357:140; Owais *et al.*, (1995) Antimicrob. Agents Chemother. 39:180); a surfactant protein A receptor (see Briscoe *et al.*, (1995) Am. J. Physiol. 1233:134); p 120 (see Schreier *et al.*, (1994) J. Biol. Chem. 269:9090), and the like. Also, see K. Keinanen; M. L. Laukkanen (1994) FEBS Lett. 346:123; J. J. Killion; I. J. Fidler (1994) Immunomethods 4:273.

[0934] The present invention provides a protocol for administering a pharmaceutical composition, which includes the antibody or fragment thereof according to the present invention, to a subject in need thereof alone or in combination with other therapies. The therapeutic agent (for example, a prophylactic or therapeutic agent) for combination therapy of the present invention may be simultaneously or sequentially administered to the subject. The therapeutic agent (for example, a prophylactic or therapeutic agent) for combination therapy of the present invention may also be administered periodically. A periodic therapy includes administering a first therapeutic agent (for example, a first prophylactic or therapeutic agent) for a predetermined period of time, administering a second therapeutic agent (for example, a second prophylactic or therapeutic agent) for a predetermined period of time, and then sequentially repeating the steps of administration, that is, a predetermined cycle for reducing the occurrence of resistance to one of the therapeutic agents (for example, agonists), and/or preventing or reducing side effects of one of the therapeutic agents (for example, agonists), and/or improving efficacy of the therapeutic

agents.

[0935] The therapeutic agent (for example, a prophylactic or therapeutic agent) for combination therapy of the present invention may be simultaneously administered to a subject.

[0936] The term "simultaneously" means that therapies (for example, prophylactic or therapeutic agents) need not be administered at exactly the same time without limitation, but are rather administered to a subject sequentially with the pharmaceutical composition including an antibody or a fragment thereof according to the present invention, and are administered at time intervals that may serve to provide higher benefits compared to when the antibody of the present invention is administered at a different time from other therapy(ies). For example, the respective therapeutic agents may be sequentially administered to a subject at the same time or different points of time in any order; but should be administered at sufficiently short time intervals to provide a desire therapeutic or prophylactic effect when they are not administered at the same time. The respective therapeutic agents may be administered to a subject in any adequate form through any suitable route of administration. In various exemplary embodiments, the therapeutic agent (for example, a prophylactic or therapeutic agent) may be administered to a subject at intervals of less than 15 minutes, intervals of less than 30 minutes, intervals of less than an hour, intervals of approximately an hour, intervals of approximately 1 to approximately 2 hours, intervals of approximately 2 hours to approximately 3 hours, intervals of approximately 3 hours to approximately 4 hours, intervals of approximately 4 hours to approximately 5 hours, intervals of approximately 5 hours to approximately 6 hours, intervals of approximately 6 hours to approximately 7 hours, intervals of approximately 7 hours to approximately 8 hours, intervals of approximately 8 hours to approximately 9 hours, intervals of approximately 9 hours to approximately 10 hours, intervals of approximately 10 hours to approximately 11 hour, intervals of approximately 11 hour to approximately 12 hours, intervals of 24 hours, 48 hours, 72 hours, or intervals of a week. In other exemplary embodiments, two or more therapeutic agents (for example, prophylactic or therapeutic agents) may be administered during the same visit by a patient.

[0937] In the same pharmaceutical composition, the prophylactic or therapeutic agent for combination therapy may be administered to a subject. Alternatively, in the individual pharmaceutical compositions, the prophylactic or therapeutic agent for combination therapy may be simultaneously administered to a subject. The prophylactic or therapeutic agent may be administered to a subject through the same or different routes of administration.

Experimental Examples

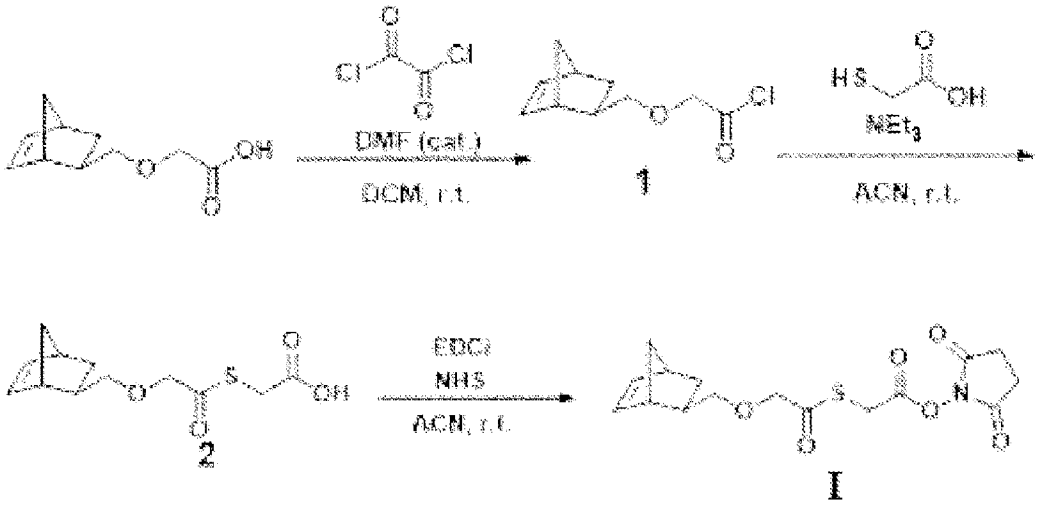
[0938] 1. Preparation Examples of compounds for preparing an agent for transferring a

site-specific first click-chemistry functional group to an antibody

[0939] 1.1. Synthesis and confirmation of structure of linker (H_1-L_1)

[0940] 1.1.1.: Compound I (SO1 Linker: NHS & Norbornene)

[0941] [Scheme 6]



[0942] Synthesis of Compound I (Scheme 6, FIG. 15)

[0943] Synthesis of Compound 1

[0944] 2 g (10.98 mmol, 1.0 eq.) of 2-(bicyclo[2.2.1]hept-5-ene-2-ylmethoxy)acetic acid was dissolved in 50 mL of DCM, and 0.085 mL (1.098 mmol, 0.1 eq.) of DMF and 1.91 mL (21.96 mmol, 2.0 eq.) of oxalyl chloride were then added dropwise while stirring at room temperature. The reaction solution was stirred for 3 hours, and then concentrated under reduced pressure to obtain 1.97 g of a target compound (yield: 90%).

[0945] Synthesis of Compound 2

[0946] 1.97 g (9.88 mmol, 1.0 eq.) of Compound 1 was dissolved in 20 mL of acetonitrile (ACN), and 0.68 mL (9.88 mmol, 1.0 eq.) of thioglycolic acid and 2.06 mL (14.82 mmol, 1.5 eq.) of triethylamine were then added dropwise while stirring at room temperature. The reaction solution was stirred for 18 hours, and then concentrated under reduced pressure. Subsequently, water was added to the reaction solution, and the reaction solution was extracted three times with ethyl acetate (EA). An organic layer was dried over magnesium sulfate, concentrated under reduced pressure, and then purified by column chromatography (DCM:MeOH = 10:1) to obtain 2.05 g (yield: 79%) of a target compound.

[0947] ^{1}H NMR (500 MHz, $CDCl_3$) δ ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.15 - 6.01 (m, 2H), 4.20 (t, J = 5.0 Hz, 1H), 3.77 - 3.71 (m, 2H), 3.68 - 3.57 (m, 1H), 3.56 - 3.45 (m, 1H), 2.84 (s, 2H),

1.81 - 1.69 (m, 2H), 1.30 (ddd, J = 19.8, 14.5, 8.6 Hz, 4H), 1.15 (ddd, J = 16.0, 7.6, 3.3 Hz, 1H).

[0948] Synthesis of Compound I

[0949] 2.05 g (7.81 mmol, 1.0 eq.) of Compound 2 was dissolved in 50 mL of ACN, and 2.76 g (14.44 mmol, 1.8 eq.) of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCi) and 2.21 g (19.25 mmol, 2.46 eq.) of N-hydroxysuccinimide (NHS) were then added while stirring at room temperature. The reaction solution was stirred for 18 hours, and then concentrated under reduced pressure. Subsequently, water was added to the reaction solution, and the reaction solution was extracted three times with EA. An organic layer was recovered, dried over magnesium sulfate, and then concentrated under reduced pressure. Then, the residue was purified on a silica gel column using chromatography (EA:Hex = 2:1) to obtain 2.7 g (yield: 98%) of a target compound.

[0950] ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.15 - 6.03 (m, 2H), 4.45 (s, 1H), 4.21 (d, J = 5.0 Hz, 1H), 3.97 (s, 1H), 3.65 (dd, J = 12.0, 7.3 Hz, 1H), 3.52 (t, J = 8.9 Hz, 1H), 2.83 (dd, J = 24.4, 11.8 Hz, 6H), 1.81 - 1.67 (m, 1H), 1.30 (dq, J = 27.0, 9.5 Hz, 4H), 1.16 (ddd, J = 15.3, 7.4, 3.8 Hz, 1H).

[0951] Confirmation of structure of Compound I

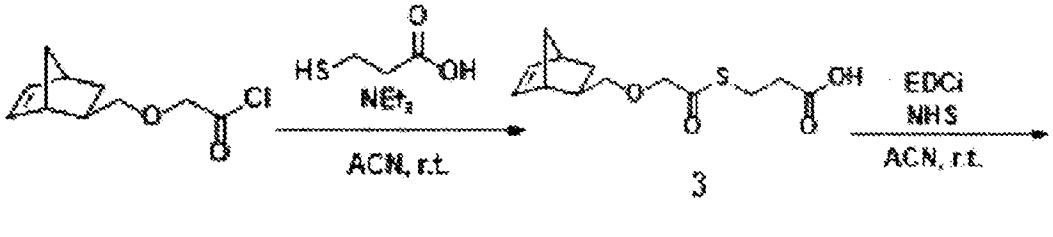
[0952] ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.15 - 6.03 (m, 2H), 4.45 (s, 1H), 4.21 (d, J = 5.0 Hz, 1H), 3.97 (s, 1H), 3.65 (dd, J = 12.0, 7.3 Hz, 1H), 3.52 (t, J = 8.9 Hz, 1H), 2.83 (dd, J = 24.4, 11.8 Hz, 6H), 1.81 - 1.67 (m, 1H), 1.30 (dq, J = 27.0, 9.5 Hz, 4H), 1.16 (ddd, J = 15.3, 7.4, 3.8 Hz, 1H).

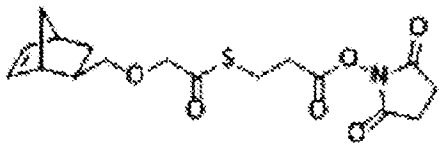
[0953] LRMS (ESI): m/z 371.2 [M+ NH_4^+]

[0954] The structure of Compound I was confirmed by mass spectrometry. The results are shown in FIG. 16.

[0955] 1.1.2. Compound II (SO2 Linker: NHS & Norbornene)

[0956] [Scheme 7]





II

[0957] Synthesis of Compound II (Scheme 7, FIG. 17)

[0958] Synthesis of Compound 3

[0959] 0.55 g (2.33 mmol, 1.0 eq.) of Compound 1 was dissolved in 5 mL of acetonitrile (ACN), and 0.2 mL (2.33 mmol, 1.0 eq.) of 3-mercaptopropionic acid and 0.49 mL (3.49 mmol, 1.5 eq.) of triethylamine were then added dropwise while stirring at room temperature. The reaction solution was stirred for 11 hours, and then concentrated under reduced pressure. Subsequently, water was added to the reaction solution, and the reaction solution was extracted three times with ethyl acetate (EA). An organic layer was dried over magnesium sulfate, concentrated under reduced pressure, and then purified by silica gel column chromatography (DCM:MeOH = 10:1) to obtain 0.56 g (yield: 89%) of a target compound.

[0960] Synthesis of Compound II

[0961] 0.56 g (2.07 mmol, 1.0 eq.) of Compound 3 was dissolved in 10 mL of acetonitrile (ACN), and 0.81 g (4.21 mmol, 2.0 eq.) of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCi) and 0.65 g (5.61 mmol, 2.7 eq.) of N-hydroxysuccinimide (NHS) were added while stirring at room temperature. The reaction solution was stirred for 12 hours, and then concentrated under reduced pressure. Subsequently, water was added to the reaction solution, and the reaction solution was extracted three times with EA. An organic layer was dried over magnesium sulfate, and concentrated under reduced pressure. Then, the residue was purified by silica gel column chromatography (EA:Hex = 2:1) to obtain 0.63 g (yield: 83%) of a target compound.

[0962] Confirmation of structure of Compound II

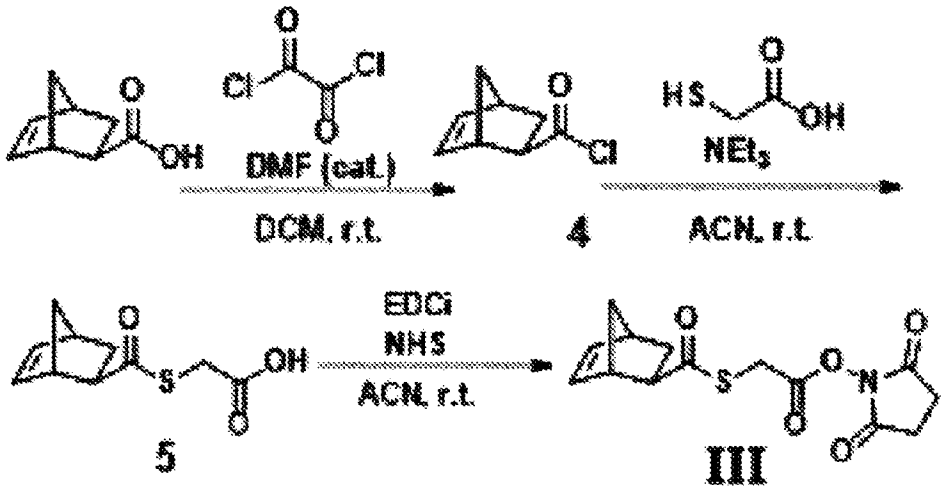
[0963] ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.16-6.14 (m, 2H), 4.45 (s, 1H), 4.21 - 4.08 (m, 1H), 3.69 - 3.58 (m, 1H), 3.51 (dt, J = 14.5, 8.9 Hz, 1H), 3.22 (t, J = 7.1 Hz, 2H), 2.96 (t, J = 7.1 Hz, 2H), 2.79-2.83 (m, 6H), 1.81 - 1.67 (m, 1H), 1.37 - 1.21 (m, 4H), 1.13-1.18 (m, 1H).

[0964] LRMS (ESI): m/z 385.1 [M+ NH_4^+]

[0965] The structure of Compound II was confirmed by mass spectrometry. The results are shown in FIG. 18.

[0966] 1.1.3. Compound III (SO3 Linker: NHS & Norbornene)

[0967] [Scheme 8]



[0968] Synthesis of Compound III (Scheme 8, FIG. 19)

[0969] Synthesis of Compound 4

[0970] 0.5 g (3.62 mmol, 1.0 eq.) of exo-5-norbornenecarboxylic acid was dissolved in 20 mL of DCM, and 0.028 mL (0.37 mmol, 0.94 eq.) of DMF and 0.63 mL (7.24 mmol, 2.0 eq.) of oxalyl chloride were then added dropwise while stirring at room temperature. The reaction solution was stirred for 3 hours, and then concentrated under reduced pressure to obtain 0.47 g (yield: 83%) of a target compound.

[0971] Synthesis of Compound 5

[0972] 0.47 g (3.0 mmol, 1.0 eq.) of Compound 4 was dissolved in 15 mL of acetonitrile (ACN), and 0.21 mL (3.0 mmol, 1.0 eq.) of thioglycolic acid and 0.63 mL (4.5 mmol, 1.5 eq.) of triethylamine were then added dropwise while stirring at room temperature. The reaction solution was stirred for 18 hours, and then concentrated under reduced pressure. Subsequently, water was added to the reaction solution, and the reaction solution was extracted three times with ethyl acetate (EA). An organic layer was dried over magnesium sulfate, concentrated under reduced pressure, and then purified by column chromatography (DCM:MeOH = 10:1) to obtain 0.3 g (yield: 47%) of a target compound.

[0973] ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.82 (brs, 1H), 6.24 - 6.07 (m, 2H), 3.76 (s, 2H), 3.12 (s, 1H), 2.97 (s, 1H), 2.54 (dd, J = 9.0, 4.7 Hz, 1H), 2.04 - 1.87 (m, 1H), 1.57 (d, J = 8.5 Hz, 1H), 1.48 - 1.35 (m, 2H).

[0974] Synthesis of Compound III

[0975] 0.26 g (1.22 mmol, 1.0 eq.) of Compound 5 was dissolved in 15 mL of acetonitrile (ACN), and 0.35 g (1.83 mmol, 1.5 eq.) of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCi) and 0.28g (2.44 mmol, 2.0 eq.) of N-hydroxysuccinimide (NHS) were added while stirring at room temperature. The reaction solution was stirred for 3 hours, and then concentrated under reduced

pressure. Subsequently, water was added to the reaction solution, and the reaction solution was extracted three times with EA. An organic layer was recovered, dried over magnesium sulfate, and then concentrated under reduced pressure. Then, the residue was purified on a silica gel column using chromatography (EA:Hex = 2:1) to obtain 0.32 g (yield: 85%) of a target compound.

[0976] Confirmation of structure of Compound III

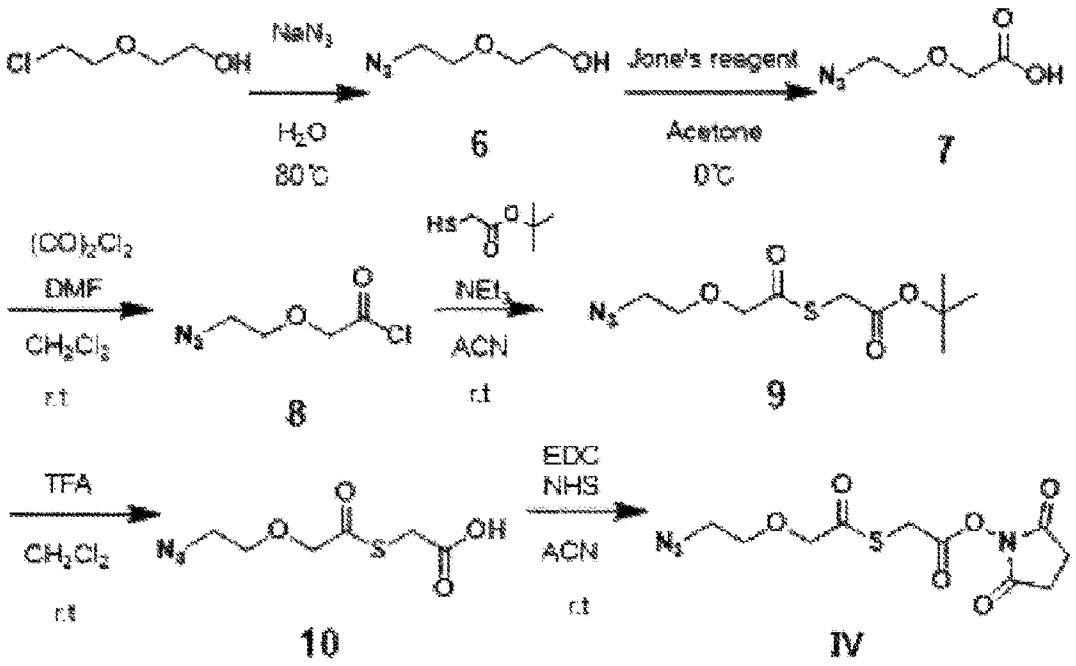
[0977] ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.21 (dd, J = 5.5, 3.0 Hz, 1H), 6.15 (dd, J = 5.5, 3.1 Hz, 1H), 4.01 (s, 2H), 3.15 (s, 1H), 2.98 (s, 1H), 2.86 (s, 4H), 2.54 (dd, J = 9.2, 4.6 Hz, 1H), 2.02 (dt, J = 11.9, 4.0 Hz, 1H), 1.59 (d, J = 8.6 Hz, 1H), 1.46 - 1.36 (m, 2H).

[0978] LCMS (ESI): m/z 332.16 [M+Na$^+$]

[0979] The structure of Compound III was confirmed by mass spectrometry. The results are shown in FIG. 20.

[0980] 1.1.4. Compound IV (SO4 Linker: NHS & azide)

[0981] [Scheme 9]



[0982] Synthesis of Compound IV (Scheme 9, FIGS. 21 and 22)

[0983] Synthesis of Compound 6

[0984] 2-(2-Chloroethoxy)ethanol (2 mL, 18.94 mmol) was dissolved in distilled water (12 mL), and NaN_3 (3.08 g, 47.35 mmol, 2.5 eq.) was added thereto. The resulting mixture was stirred at 80°C for 16 hours. The reaction mixture was cooled to room temperature, and poured into 5% NaOH (aq.) (20 mL), and then stirred for approximately 10 minutes. The reaction mixture was extracted three times with diethyl ether, and an organic layer was dried over magnesium

sulfate, and filtered. The filtrate was concentrated under reduced pressure to obtain 2.47 g (yield: 99%) of a target compound.

[0985] Synthesis of Compound 7

[0986] 2.47 g (18.84 mmol) of Compound 6 was dissolved in acetone (50 mL), and a 1 M Jone's reagent (75.36 mL, 75.36 mmol, 4 eq.) was then slowly added at 0°C. The reaction mixture was stirred at 0°C for 3 hours, warmed to room temperature, and then stirred for approximately 10 minutes. The reaction mixture was extracted three times with ethyl acetate (EA), and an organic layer was dried over magnesium sulfate, and filtered. The filtrate was concentrated under reduced pressure to obtain 2.69 g (yield: 98%) of a target compound.

[0987] Synthesis of Compound 8

[0988] 2.69 g (18.57 mmol, 1.0 eq.) of Compound 7 was dissolved in 50 mL of DCM, and 0.1 mL (1.29 mmol, 0.07 eq.) of DMF and 2.43 mL (27.86 mmol, 1.5 eq.) of oxalyl chloride were then added dropwise while stirring at room temperature. The reaction solution was stirred for 3 hours, and then concentrated under reduced pressure to obtain 2.42 g (yield: 80%) of a target compound.

[0989] Synthesis of Compound 9

[0990] 0.88 g (5.40 mmol, 1.0 eq.) of Compound 8 was dissolved in 30 mL of DCM, and 0.8 g (5.40 mmol, 1.0 eq.) of tert-butyl 2-mercaptoacetate and 1.41 mL (8.10 mmol, 1.5 eq.) of N,N-diisopropylethylamine were then added dropwise while stirring at room temperature. The reaction mixture was stirred for 2 hours, and then concentrated under reduced pressure. Subsequently, water was added to the reaction solution, and the reaction solution was extracted three times with dichloromethane (DCM). An organic layer was dried over magnesium sulfate, concentrated under reduced pressure, and then purified by column chromatography (Hex:EA = 5:1) to obtain 0.73 g (yield: 49%) of a target compound.

[0991] Synthesis of Compound 10

[0992] 0.73 g (2.66 mmol, 1 eq.) of Compound 9 was dissolved in 10 mL of DCM, and 10 mL (129.8 mmol, 48 eq.) of trifluoroacetic acid was then added dropwise while stirring at room temperature. The reaction solution was stirred for 8 hours, and then concentrated under reduced pressure to obtain 0.40 g (yield: 70%) of a target compound.

[0993] Synthesis of Compound IV

[0994] 1.97 g (9.0 mmol, 1.0 eq.) of Compound 10 was dissolved in 25 mL of acetonitrile (ACN), and 2.58 g (13.5 mmol, 1.5 eq.) of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCi) and 2.07 g (18.0 mmol, 2.0 eq.) of N-hydroxysuccinimide (NHS) were added while stirring at room

temperature. The reaction solution was stirred for 3 hours, and then concentrated under reduced pressure. Subsequently, water was added to the reaction solution, and the reaction solution was extracted three times with EA. An organic layer was recovered, dried over magnesium sulfate, and then concentrated under reduced pressure. Then, the residue was purified on a silica gel column using chromatography (EA:Hex = 2:1) to obtain 1.03 g (yield: 36%) of a target compound.

[0995] Confirmation of structure of Compound IV

[0996] ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.30 (s, 2H), 4.00 (s, 2H), 3.87 - 3.75 (m, 2H), 3.55 - 3.47 (m, 2H), 2.86 (s, 4H).

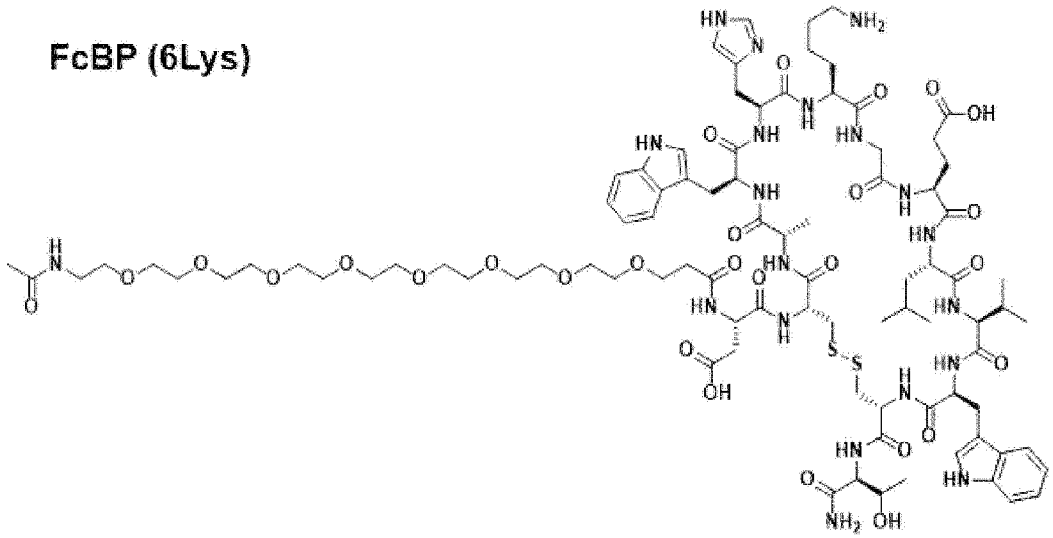
[0997] LRMS (ESI): m/z 334.0 [M+ NH_4^+]

[0998] The structure of Compound IV was confirmed by mass spectrometry. The results are shown in FIG. 23.

[0999] 1.2. Synthesis and confirmation of structure of site-specific Fc interactome (SSFI)

[1000] 1.2.1. Synthesis and confirmation of structure of SSFI (where Xa_1 is lysine)

[1001] Synthesis of SSFI (6Lys)



[1002] List and order of introduction of Fmoc amino acids used

[1003] Fmoc-L-Thr(tBu)-OH, Fmoc-Cys(Trt)-OH, Fmoc-L-Trp(Boc)-OH, Fmoc-L-Val-OH, Fmoc-L-Leu-OH, Fmoc-L-Glu(OtBu)-OH, Fmoc-Gly-OH, Fmoc-Lys(Boc)-OH, Fmoc-L-His(Trt)-OH, Fmoc-L-Trp(Boc)-OH, Fmoc-Ala-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(tBu)-OH.

[1004] Preparation method

[1005] (a) Introduction of amino acids

[1006] Amounts of reagents used in the following process were based on 0.25 mmole. 0.5 g (0.48 mmole/g) of a clear amide resin (Peptide International Inc., USA) was put into a

synthesis reactor, and 1 mmole of each Fmoc-amino acid block was weighed to prepare a peptide amino acid sequence from the C-terminus to the N-terminus in the above order.

[1007] A reaction for activating an Fmoc-amino acid to attach the activated residue to a clear amide resin was performed sequentially from the C-terminal amino acid.

[1008] Removal of Fmoc was performed in 20% piperidine-containing DMF, and activation and introduction of the residue was performed by mixing amino acids prepared to correspond to the sequence with 2 mL of a 0.5 M HOBt-containing DMF solution, 2 mL of a 0.5 M HBTU-containing DMF solution, and 174 μL of DIPEA for 5 minutes, and mixing the resulting mixture for 2 hours in a reactor containing the resin.

[1009] Confirmation of an introduction reaction was carried out using a Kaiser test method. When the introduction reaction was confirmed to be incomplete, the introduction reaction was repeated once more, or capping was performed using a 20% Ac_2O-containing DMF solution. In each of the introduction reaction and the Fmoc removal, the resin was thoroughly washed with DMF and DCM before moving to the next step. This process was repeatedly performed until the targeted peptide sequence was completed.

[1010] (b) Introduction of H-PEG8-OH

[1011] To introduce H-PEG_8-OH to the N-terminus of the sequence after the introduction of the amino acids was completed, 1 mL of a 0.5 M Fmoc-N-amido-dPEG8-acid-in-DMF solution, 1 mL of a 0.5 M HBTU-containing DMF solution, 1 mL of a 0.5 M HOBt-containing DMF solution, and 87 μL of DIPEA were mixed for 5 minutes, and the resulting mixture was mixed for 2 hours in a reactor containing a reactive resin.

[1012] Progression of the reaction was monitored by the Kaiser test method. When the unreacted amine was found to remain, the reaction time was extended for another 1 to 3 hours, or the reaction solution was discarded, and the aforementioned reaction process was repeated again. Removal of the N-terminal Fmoc protective group was performed using 20% piperidine-containing DMF, and the resin to which the peptide was attached was then dried and weighed.

[1013] (c) 250 mg of the resin to which the peptide was attached as prepared in the step (b) was stirred with 2 mL of a mixed solution of TFA, TIS, water and EDT (94:1.0:2.5:2.5) at room temperature for 120 minutes to cleave the peptide from the resin. The cleaved mixture was filtered, and the filtrate was concentrated to about half its volume using nitrogen gas. Thereafter, ether was poured into the mixture to precipitate the peptide. The precipitated peptide was washed three times with ether, and dried under nitrogen gas. The dried precipitate was dissolved in 0.1% TFA-30% ACN-containing water, stirred for 6 hours, and then concentrated.

[1014] The concentrate was dissolved at a concentration of 0.1 mg/mL in a 5%-DMSO-

20%-ACN-containing 0.01 M ammonium acetate buffer (pH 6.5) solution, and then stirred for 3 days while being exposed to air. Progression of a disulfide bond-forming reaction was monitored by HPLC. When the reaction was found not to progress any more, the reaction solution was freeze-dried to obtain a peptide precipitate.

[1015] (d) Purification

[1016] The peptide precipitate obtained by freeze-drying in the step (c) was separated under the prep-LC primary purification conditions listed in the following Table 4, further purified under the prep-LC secondary purification conditions listed in the following Table 5, and then freeze-dried. The resulting peptide was confirmed to have a purity of 90% or more by analytical HPLC, and the molecular weight of the synthesized peptide was confirmed using an LC/MS mass spectrometer.

[1017] H-PEG8-Asp-Cys*-Ala-Trp-His-Lys-Gly-Glu-Leu-Val-Trp-Cys*-Thr-NH_2 (Cys*: disulfide bonding sites)

<Table 4> Prep-LC primary purification condition

Prep-LC primary purification condition			
Equipment name	Waters 2525 pump, Waters 2487 UV detector		
Column	Waters, XBridge Prep C18 5 μm OBD 19 x 250 mm		
Mobile phase A/B	0.1% TFA-20% ACN / 0.1% TFA in 80% ACN		
Gradient	0 min → 30 min : B 0% → B 100%		
Flow rate	17 mL/min	Detection wavelength	UV 280 nm

<Table 5> Prep-LC secondary purification condition

Prep-LC secondary purification condition			
Equipment name	Waters 2525 pump, Waters 2487 UV detector		
Column	Waters, XBridge Prep C18 5 μm OBD 19 x 250 mm		
Mobile phase A/B	0.1% TFA-30% ACN / 0.1% TFA in 70% ACN		
Gradient	0 min → 30 min : B 0% → B 100%		
Flow rate	15 mL/min	Detection wavelength	UV 280 nm

[1018] Confirmation of structure of SSFI (6Lys)

[1019] Synthesis of SSFI (6Lys) was confirmed by molecular weight measurement by matrix-assisted laser desorption/ionization (MALDI) mass spectrometry.

[1020] Measuring equipment: Ultraflextreme (Bruker)

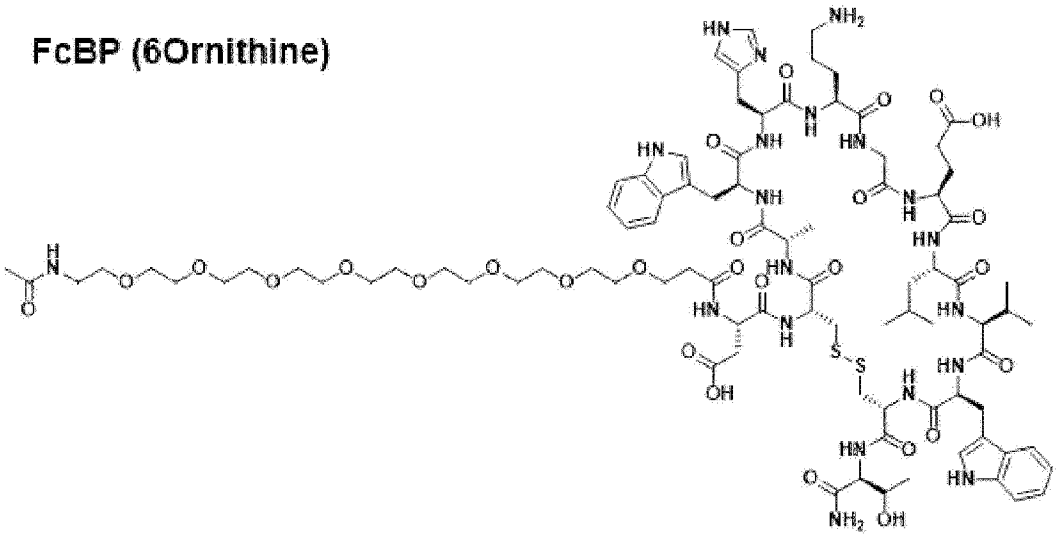
[1021] Measuring matrix: CHCA (α-Cyano-4-hydroxycinnamic acid) & DHB (2,5-Dihydroxybenzoic acid)

[1022] Calculated molecular weight: 2010.29 g/mol

[1023] Measured molecular weight $(M+H)^+$: 2011.89 g/mol

[1024] The mass spectrometry results of SSFI (6Lys) are shown in FIG. 24.

[1025] 1.2.2. Synthesis and confirmation of structure of SSFI (where Xa_1 is ornithine)



[1026] Synthesis of SSFI (6Orn)

[1027] List and order of introduction of Fmoc amino acids used

[1028] Fmoc-L-Thr(tBu)-OH, Fmoc-Cys(Trt)-OH, Fmoc-L-Trp(Boc)-OH, Fmoc-L-Val-OH, Fmoc-L-Leu-OH, Fmoc-L-Glu(OtBu)-OH, Fmoc-Gly-OH, Fmoc-Orn(Boc)-OH, Fmoc-L-His(Trt)-OH, Fmoc-L-Trp(Boc)-OH Fmoc-Ala-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(tBu)-OH.

[1029] Preparation method

[1030] (a) Introduction of amino acids

[1031] Amounts of reagents used in the following process were based on 0.25 mmole. 0.5 g (0.48 mmole/g) of a clear amide resin (Peptide International Inc., USA) was put into a synthesis reactor, and 1 mmole of each Fmoc-amino acid block was weighed to prepare a peptide

amino acid sequence from the C-terminus to the N-terminus in the above.

[1032] A reaction for activating an Fmoc-amino acid to attach the activated residue to a clear amide resin was performed sequentially from the C-terminal amino acid.

[1033] Removal of Fmoc was performed in 20% piperidine-containing DMF, and activation and introduction of the residue was performed by mixing amino acids prepared to correspond to the sequence with 2 mL of a 0.5 M HOBt-containing DMF solution, 2 mL of a 0.5 M HBTU-containing DMF solution, and 174 μL of DIPEA for 5 minutes, and mixing the resulting mixture for 2 hours in a reactor containing the resin.

[1034] Confirmation of an introduction reaction was carried out using a Kaiser test method. When the introduction reaction was confirmed to be incomplete, the introduction reaction was repeated once more, or capping was performed using a 20% Ac_2O-containing DMF solution. In each of the introduction reaction and the Fmoc removal, the resin was thoroughly washed with DMF and DCM before moving to the next step. This process was repeatedly performed until the targeted peptide sequence was completed.

[1035] (b) Introduction of H-PEG8-OH

[1036] To introduce H-PEG_8-OH to the N-terminus of the sequence after the introduction of the amino acids was completed, 1 mL of a 0.5 M Fmoc-N-amido-dPEG8-acid-in-DMF solution, 1 mL of a 0.5 M HBTU-containing DMF solution, 1 mL of a 0.5 M HOBt-containing DMF solution, and 87 μL of DIPEA were mixed for 5 minutes, and the resulting mixture was mixed for 2 hours in a reactor containing a reactive resin.

[1037] Progression of the reaction was monitored by the Kaiser test method. When the unreacted amine was found to remain, the reaction time was extended for another 1 to 3 hours, or the reaction solution was discarded, and the aforementioned reaction process was repeated again. Removal of the N-terminal Fmoc protective group was performed using 20% piperidine-containing DMF, and the resin to which the peptide was attached was then dried and weighed.

[1038] (c) 250 mg of the resin to which the peptide was attached as prepared in the step (b) was stirred with 2 mL of a mixed solution of TFA, TIS, water and EDT (94:1.0:2.5:2.5) at room temperature for 120 minutes to cleave the peptide from the resin. The cleaved mixture was filtered, and the filtrate was concentrated to about half its volume using nitrogen gas. Thereafter, ether was poured into the mixture to precipitate the peptide. The precipitated peptide was washed three times with ether, and dried under nitrogen gas. The dried precipitate was dissolved in 0.1% TFA-30% ACN-containing water, stirred for 6 hours, and then concentrated.

[1039] The concentrate was dissolved at a concentration of 0.1 mg/mL in a 5%-DMSO-20%-ACN-containing 0.01 M ammonium acetate buffer (pH 6.5) solution, and then stirred for 3

days while being exposed to air. Progression of a disulfide bond-forming reaction was monitored by HPLC. When the reaction was found not to progress any more, the reaction solution was freeze-dried to obtain a peptide precipitate.

[1040] (d) Purification

[1041] The peptide precipitate obtained by freeze-drying in the step (c) was separated under the prep-LC primary purification conditions listed in the following Table 6, further purified under the prep-LC secondary purification conditions listed in the following Table 7, and then freeze-dried. The resulting peptide was confirmed to have a purity of 90% or more by analytical HPLC, and the molecular weight of the synthesized peptide was confirmed using an LC/MS mass spectrometer.

[1042] H-PEG8-Asp-Cys*-Ala-Trp-His-Orn-Gly-Glu-Leu-Val-Trp-Cys*-Thr-NH_2 (Cys*: disulfide bonding sites)

<Table 6> Prep-LC primary purification condition

Prep-LC primary purification condition			
Equipment name	Waters 2525 pump, Waters 2487 UV detector		
Column	Waters, XBridge Prep C18 5 μm OBD 19 x 250 mm		
Mobile phase A/B	0.1% TFA-20% ACN / 0.1% TFA in 80% ACN		
Gradient	0 min → 30 min : B 0% → B 100%		
Flow rate	17 mL/min	Detection wavelength	UV 280 nm

<Table 7> Prep-LC secondary purification condition

Prep-LC secondary purification condition			
Equipment name	Waters 2525 pump, Waters 2487 UV detector		
Column	Waters, XBridge Prep C18 5 μm OBD 19 x 250 mm		
Mobile phase A/B	0.1% TFA-30% ACN / 0.1% TFA in 70% ACN		
Gradient	0 min → 30 min : B 0% → B 100%		
Flow rate	15 mL/min	Detection wavelength	UV 280 nm

[1043] Confirmation of structure of SSFI (6Orn)

[1044] Synthesis of SSFI (6Ornithine) was confirmed by molecular weight measurement

by matrix-assisted laser desorption/ionization (MALDI) mass spectrometry.

[1045] Measuring equipment: Ultraflextreme (Bruker)

[1046] Measuring matrix: CHCA (α-Cyano-4-hydroxycinnamic acid) & DHB (2,5-Dihydroxybenzoic acid)

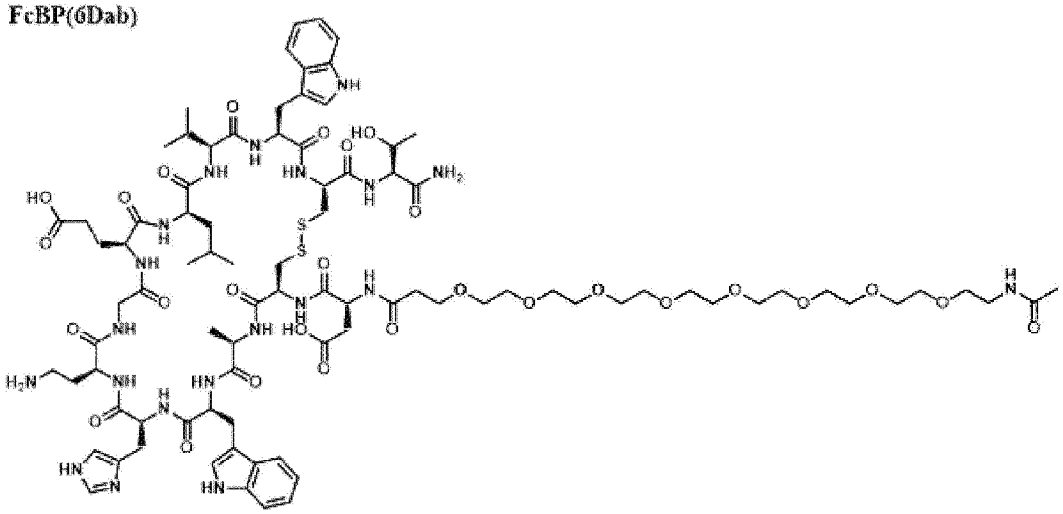
[1047] Calculated molecular weight: 1996.26 g/mol

[1048] Measured molecular weight $(M+2H)^{2+}$: 999.13 g/mol

[1049] The mass spectrometry results of SSFI (6Orn) are shown in FIG. 25.

[1050] 1.2.3. Synthesis and confirmation of structure of SSFI (where Xa_1 is diaminobutyric acid (Dab))

FcBP(6Dab)



[1051] Synthesis of SSFI (6Dab)

[1052] List and order of introduction of Fmoc amino acids used

[1053] Fmoc-L-Thr(tBu)-OH, Fmoc-Cys(Trt)-OH, Fmoc-L-Trp(Boc)-OH, Fmoc-L-Val-OH, Fmoc-L-Leu-OH, Fmoc-L-Glu(OtBu)-OH, Fmoc-Gly-OH, Fmoc-Dab(Boc)-OH, Fmoc-L-His(Trt)-OH, Fmoc-L-Trp(Boc)-OH Fmoc-Ala-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(tBu)-OH.

[1054] Preparation method

[1055] (a) Introduction of amino acids

[1056] Amounts of reagents used in the following process were based on 0.25 mmole. 0.5 g (0.48 mmole/g) of a clear amide resin (Peptide International Inc., USA) was put into a synthesis reactor, and 1 mmole of each Fmoc-amino acid block was weighed to prepare a peptide amino acid sequence from the C-terminus to the N-terminus in the above order.

[1057] A reaction for activating an Fmoc-amino acid to attach the activated residue to a clear amide resin was performed sequentially from the C-terminal amino acid.

[1058] Removal of Fmoc was performed in 20% piperidine-containing DMF, and activation

and introduction of the residue was performed by mixing amino acids prepared to correspond to the sequence with 2 mL of a 0.5 M HOBt-containing DMF solution, 2 mL of a 0.5 M HBTU-containing DMF solution, and 174 μL of DIPEA for 5 minutes, and mixing the resulting mixture for 2 hours in a reactor containing the resin.

[1059] Confirmation of an introduction reaction was carried out using a Kaiser test method. When the introduction reaction was confirmed to be incomplete, the introduction reaction was repeated once more, or capping was performed using a 20% Ac_2O-containing DMF solution. In each of the introduction reaction and the Fmoc removal, the resin was thoroughly washed with DMF and DCM before moving to the next step. This process was repeatedly performed until the targeted peptide sequence was completed.

[1060] (b) Introduction of H-PEG8-OH

[1061] To introduce H-PEG_8-OH to the N-terminus of the sequence after the introduction of the amino acids was completed, 1 mL of a 0.5 M Fmoc-N-amido-dPEG8-acid-in-DMF solution, 1 mL of a 0.5 M HBTU-containing DMF solution, 1 mL of a 0.5 M HOBt-containing DMF solution, and 87 μL of DIPEA were mixed for 5 minutes, and the resulting mixture was mixed for 2 hours in a reactor containing a reactive resin.

[1062] Progression of the reaction was monitored by the Kaiser test method. When the unreacted amine was found to remain, the reaction time was extended for another 1 to 3 hours, or the reaction solution was discarded, and the aforementioned reaction process was repeated again. Removal of the N-terminal Fmoc protective group was performed using 20% piperidine-containing DMF, and the resin to which the peptide was attached was then dried and weighed.

[1063] (c) 250 mg of the resin to which the peptide was attached as prepared in the step (b) was stirred with 2 mL of a mixed solution of TFA, TIS, water and EDT (94:1.0:2.5:2.5) at room temperature for 120 minutes to cleave the peptide from the resin. The cleaved mixture was filtered, and the filtrate was concentrated to about half its volume using nitrogen gas. Thereafter, ether was poured into the mixture to precipitate the peptide. The precipitated peptide was washed three times with ether, and dried under nitrogen gas. The dried precipitate was dissolved in 0.1% TFA-30% ACN-containing water, stirred for 6 hours, and then concentrated.

[1064] The concentrate was dissolved at a concentration of 0.1 mg/mL in a 5%-DMSO-20%-ACN-containing 0.01 M ammonium acetate buffer (pH 6.5) solution, and then stirred for 3 days while being exposed to air. Progression of a disulfide bond-forming reaction was monitored by HPLC. When the reaction was found not to progress any more, the reaction solution was freeze-dried to obtain a peptide precipitate.

[1065] (d) Purification

[1066] The peptide precipitate obtained by freeze-drying in the step (c) was separated under the prep-LC primary purification conditions listed in the following Table 8, further purified under the prep-LC secondary purification conditions listed in the following Table 9, and then freeze-dried. The resulting peptide was confirmed to have a purity of 90% or more by analytical HPLC, and the molecular weight of the synthesized peptide was confirmed using an LC/MS mass spectrometer.

[1067] H-PEG8-Asp-Cys*-Ala-Trp-His-Dab-Gly-Glu-Leu-Val-Trp-Cys*-Thr-NH_2 (Cys*: disulfide bonding sites)

<Table 8> Prep-LC primary purification condition

Prep-LC primary purification condition			
Equipment name	Waters 2525 pump, Waters 2487 UV detector		
Column	Waters, XBridge Prep C18 5 μm OBD 19 x 250 mm		
Mobile phase A/B	0.1% TFA-20% ACN / 0.1% TFA in 80% ACN		
Gradient	0 min → 30 min : B 0% → B 100%		
Flow rate	17 mL/min	Detection wavelength	UV 280 nm

<Table 9> Prep-LC secondary purification condition

Prep-LC secondary purification condition			
Equipment name	Waters 2525 pump, Waters 2487 UV detector		
Column	Waters, XBridge Prep C18 5 μm OBD 19 x 250 mm		
Mobile phase A/B	0.1% TFA-30% ACN / 0.1% TFA in 70% ACN		
Gradient	0 min → 30 min : B 0% → B 100%		
Flow rate	15 mL/min	Detection wavelength	UV 280 nm

[1068] Confirmation of structure of SSFI (6Dab)

[1069] Synthesis of SSFI (6Dab) was confirmed by molecular weight measurement by matrix-assisted laser desorption/ionization (MALDI) mass spectrometry.

[1070] Measuring equipment: Ultraflextreme (Bruker)

[1071] Measuring matrix: CHCA (α-Cyano-4-hydroxycinnamic acid) & DHB (2,5-Dihydroxybenzoic acid)

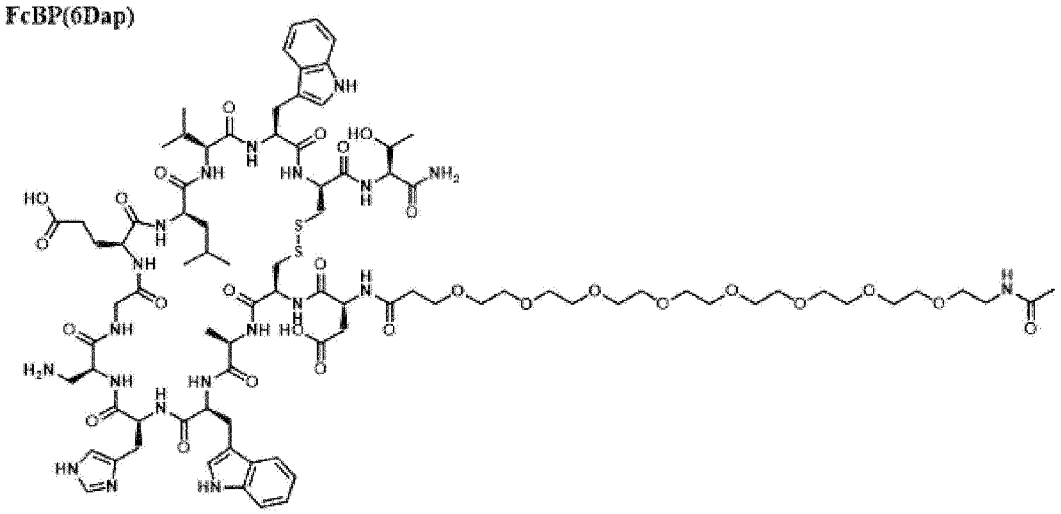
[1072] Calculated molecular weight: 1982.24 g/mol

[1073] Measured molecular weight $(M+2H)^{2+}$: 992.33 g/mol

[1074] The mass spectrometry results of SSFI (6Dab) are shown in FIG. 26.

[1075]

[1076] 1.2.4. Synthesis and confirmation of structure of SSFI (where Xa_1 is diaminopropionic acid (Dap))



[1077] Synthesis of SSFI (6Dap)

[1078] Sequence: Nor.-PEG8-DCAWHA(beta amino alanine, Dap)GELVWCT-$CONH_2$

[1079] List and order of introduction of Fmoc amino acids used

[1080] Fmoc-L-Thr(tBu)-OH, Fmoc-Cys(Trt)-OH, Fmoc-L-Trp(Boc)-OH, Fmoc-L-Val-OH, Fmoc-L-Leu-OH, Fmoc-L-Glu(OtBu)-OH, Fmoc-Gly-OH, Fmoc-Dap(Boc)-OH, Fmoc-L-His(Trt)-OH, Fmoc-L-Trp(Boc)-OH Fmoc-Ala-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asp(tBu)-OH.

[1081] Preparation method

[1082] (a) Introduction of amino acids

[1083] Amounts of reagents used in the following process were based on 0.25 mmole. 0.5 g (0.48 mmole/g) of a clear amide resin (Peptide International Inc., USA) was put into a synthesis reactor, and 1 mmole of each Fmoc-amino acid block was weighed to prepare a peptide amino acid sequence from the C-terminus to the N-terminus in the above order.

[1084] A reaction for activating an Fmoc-amino acid to attach the activated residue to a clear amide resin was performed sequentially from the C-terminal amino acid.

[1085] Removal of Fmoc was performed in 20% piperidine-containing DMF, and activation and introduction of the residue was performed by mixing amino acids prepared to correspond to the sequence with 2 mL of a 0.5 M HOBt-containing DMF solution, 2 mL of a 0.5 M HBTU-

containing DMF solution, and 174 μL of DIPEA for 5 minutes, and mixing the resulting mixture for 2 hours in a reactor containing the resin.

[1086] Confirmation of an introduction reaction was carried out using a Kaiser test method. When the introduction reaction was confirmed to be incomplete, the introduction reaction was repeated once more, or capping was performed using a 20% Ac_2O-containing DMF solution. In each of the introduction reaction and the Fmoc removal, the resin was thoroughly washed with DMF and DCM before moving to the next step. This process was repeatedly performed until the targeted peptide sequence was completed.

[1087] (b) Introduction of H-PEG8-OH

[1088] To introduce H-PEG_8-OH to the N-terminus of the sequence after the introduction of the amino acids was completed, 1 mL of a 0.5 M Fmoc-N-amido-dPEG8-acid-in-DMF solution, 1 mL of a 0.5 M HBTU-containing DMF solution, 1 mL of a 0.5 M HOBt-containing DMF solution, and 87 μL of DIPEA were mixed for 5 minutes, and the resulting mixture was mixed for 2 hours in a reactor containing a reactive resin.

[1089] Progression of the reaction was monitored by the Kaiser test method. When the unreacted amine was found to remain, the reaction time was extended for another 1 to 3 hours, or the reaction solution was discarded, and the aforementioned reaction process was repeated again. Removal of the N-terminal Fmoc protective group was performed using 20% piperidine-containing DMF, and the resin to which the peptide was attached was then dried and weighed.

[1090] (c) 250 mg of the resin to which the peptide was attached as prepared in the step (b) was stirred with 2 mL of a mixed solution of TFA, TIS, water and EDT (94:1.0:2.5:2.5) at room temperature for 120 minutes to cleave the peptide from the resin. The cleaved mixture was filtered, and the filtrate was concentrated to about half its volume using nitrogen gas. Thereafter, ether was poured into the mixture to precipitate the peptide. The precipitated peptide was washed three times with ether, and dried under nitrogen gas. The dried precipitate was dissolved in 0.1% TFA-30% ACN-containing water, stirred for 6 hours, and then concentrated.

[1091] The concentrate was dissolved at a concentration of 0.1 mg/mL in a 5%-DMSO-20%-ACN-containing 0.01 M ammonium acetate buffer (pH 6.5) solution, and then stirred for 3 days while being exposed to air. Progression of a disulfide bond-forming reaction was monitored by HPLC. When the reaction was found not to progress any more, the reaction solution was freeze-dried to obtain a peptide precipitate.

[1092] (d) Purification

[1093] The peptide precipitate obtained by freeze-drying in the step (c) was separated under the prep-LC primary purification conditions listed in the following Table 10, further purified

under the prep-LC secondary purification conditions listed in the following Table 11, and then freeze-dried. The resulting peptide was confirmed to have a purity of 90% or more by analytical HPLC, and the molecular weight of the synthesized peptide was confirmed using an LC/MS mass spectrometer.

[1094] H-PEG8-Asp-Cys*-Ala-Trp-His-Dap-Gly-Glu-Leu-Val-Trp-Cys*-Thr-NH_2 (Cys*: disulfide bonding sites)

<Table 10> Prep-LC primary purification condition

Prep-LC primary purification condition			
Equipment name	Waters 2525 pump, Waters 2487 UV detector		
Column	Waters, XBridge Prep C18 5 μm OBD 19 x 250 mm		
Mobile phase A/B	0.1% TFA-20% ACN / 0.1% TFA in 80% ACN		
Gradient	0 min → 30 min : B 0% → B 100%		
Flow rate	17 mL/min	Detection wavelength	UV 280 nm

<Table 12> Prep-LC secondary purification condition

Prep-LC secondary purification condition			
Equipment name	Waters 2525 pump, Waters 2487 UV detector		
Column	Waters, XBridge Prep C18 5 μm OBD 19 x 250 mm		
Mobile phase A/B	0.1% TFA-30% ACN / 0.1% TFA in 70% ACN		
Gradient	0 min → 30 min : B 0% → B 100%		
Flow rate	15 mL/min	Detection wavelength	UV 280 nm

[1095] Confirmation of structure of SSFI (6Dap)

[1096] Measuring equipment: Quattro Premier Xe (Waters)

[1097] Calculated molecular weight: 1968.21 g/mol

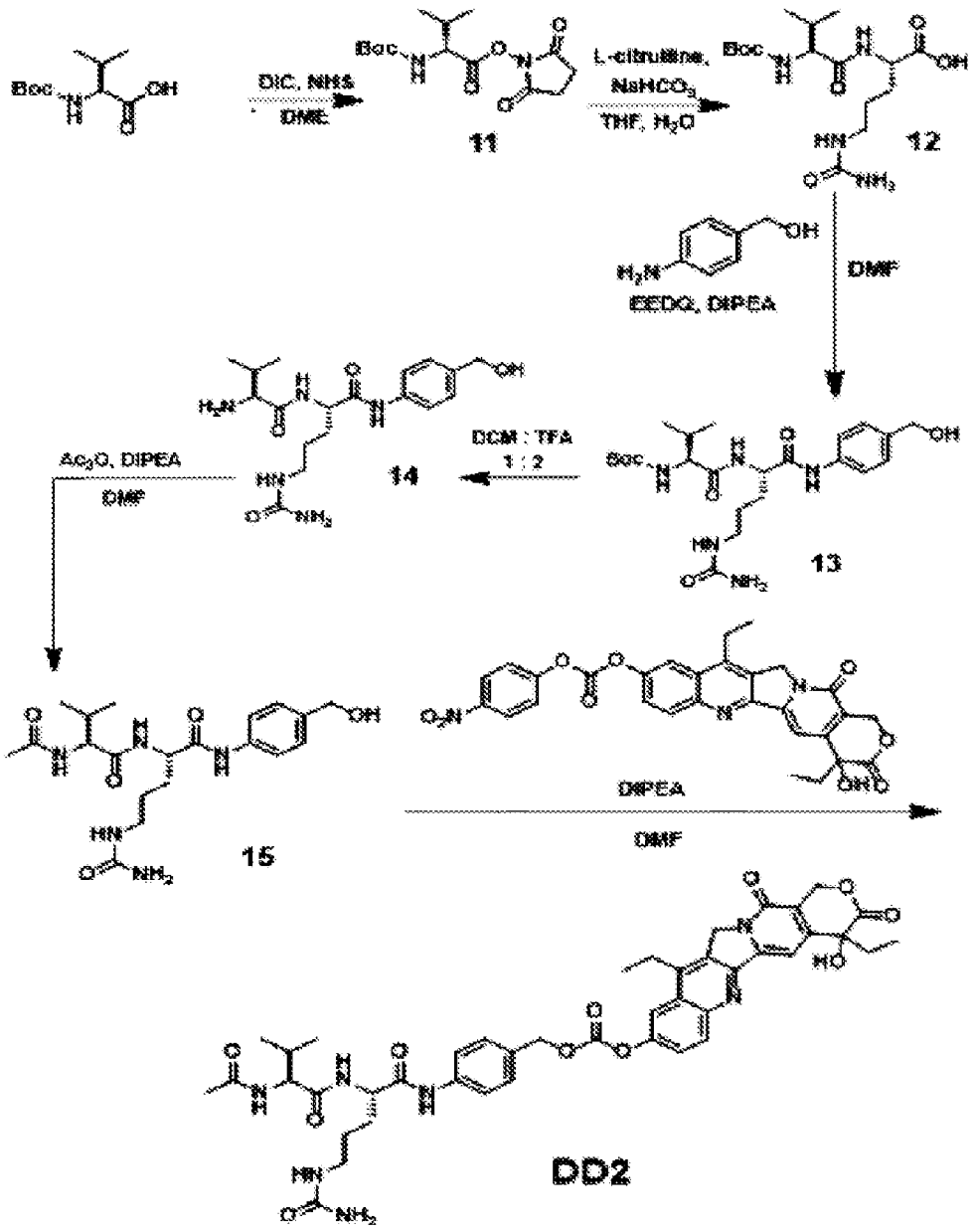
[1098] Measured molecular weight $(M+2H)^{2+}$: 984.71 g/mol

[1099] The mass spectrometry results of SSFI (6Dap) are shown in FIG. 27.

[1100] 1.3. Synthesis and confirmation of structure of VC linker

[1101] 1.3.1. Synthesis and confirmation of structure of VC linker (DD2)

[1102] [Scheme 10]



[1103] Synthesis of VC linker (DD2)

[1104] Synthesis of Compound 11

[1105] 5 g (14.7 mmol, 1.0 eq.) of Fmoc-Val-OH and 1.7 g (14.7 mmol, 1.0 eq.) of N-hydroxysuccinimide (NHS) were dissolved in 140 mL of dimethoxyethane (DME), and stirred. 2.5 mL (16.2 mmol, 1.1 eq.) of N,N'-diisopropylcarbodiimide (DIC) was added dropwise at 0°C, and stirred for 16 hours. The reaction solution was filtered under reduced pressure to remove floating matter, and the filtrate was concentrated under reduced pressure. The residue was dissolved in acetone, and stored at a low temperature for 4 hours in a refrigerator. Thereafter, the resulting solution was filtered under reduced pressure to remove the re-formed floating matter,

and used in the next reaction without any purification (crude yield: 5.5 g, 86%). TLC (EA:Hex = 1:1); R_f = 0.5.

[1106] Synthesis of Compound 12

[1107] 2.0 g (11.5 mmol, 1.0 eq.) of L-citrulline was dissolved in 100 mL of a 1:1 mixed solution of tetrahydrofuran (THF) and water, and stirred. 988 mg of sodium hydrogen carbonate was added thereto, and stirred. Thereafter, 5.0 g (11.5 mmol, 1.0 eq.) of Compound 8 was dissolved in 80 mL of acetone, added dropwise to the reaction solution, and then stirred. The resulting mixture was stirred for 21 hours, and then concentrated under reduced pressure to remove the organic solvent. An aqueous layer was washed with ethyl acetate (EA), and then titrated to pH 3 by slow dropwise addition of 2 N HCl. EA was added thereto so that the precipitate in an organic layer was extracted. Then, the organic layer was dried over a saturated saline solution and sodium sulfate, and used in the next reaction without any purification (crude yield: 5.6 g, 98%). TLC (DCM:MeOH = 10:1, one drop of formic acid); R_f = 0.1.

[1108] Synthesis of Compound 13

[1109] 50 mL of 10% piperidine in N,N-dimethylformamide (DMF) was added dropwise to 2.84 g (5.72 mmol, 1.0 eq.) of Compound 12, and stirred. After 4 hours, the resulting mixture was concentrated under reduced pressure to remove a reaction solution, and the residue was dissolved in water, and filtered under reduced pressure to remove the formed floating matter. An aqueous layer was concentrated to obtain a target compound, which was used in the next reaction without any purification. TLC (DCM:MeOH = 10:1); R_f = 0.01.

[1110] Synthesis of Compound 14

[1111] 54 mg (0.22 mmol, 1.0 eq.) of Compound 10 was dissolved in 2 mL of DMF, and 0.05 mL (0.264 mmol, 1.2 eq.) of N,N-diisopropylethylamine (DIPEA) was added dropwise thereto. Compound 13 was completely dissolved by adding 2 mL of water, and 0.05 mL (0.528 mmol, 2.4 eq.) of an acetic anhydride was added dropwise thereto at room temperature. After 3 hours, the resulting mixture was concentrated under reduced pressure to remove a reaction solution, and the concentrate was purified by reversed-phase column chromatography to obtain a target compound. TLC (DCM:MeOH = 10:1); R_f = 0.05.

[1112] Synthesis of Compound 15

[1113] 1 g (3.16 mmol, 1.0 eq.) of Compound 14 was dissolved in 30 mL of a 2:1 mixed solution of DCM and methanol, and 868 mg (3.48 mmol, 1.1 eq.) of N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) was added thereto, and stirred. 451 mg (3.66 mmol, 1.16 eq.) of 4-aminobenzyl alcohol was added thereto, and stirred for 5 hours. The resulting mixture was concentrated under reduced pressure to remove a reaction solution, and the concentrate was

purified by column chromatography (10% MeOH in DCM) to obtain 419 mg of a target compound (yield: 32%). TLC (DCM:MeOH = 10:1); R_f = 0.1.

[1114] Synthesis of Compound DD2

[1115] 109 mg (0.3 mmol, 1.2 eq.) of phenol-activated SN38 and 0.06 mL (0.33 mmol, 1.3 eq.) of DIPEA were added dropwise to a solution obtained by dissolving 105 mg (0.25 mmol, 1.0 eq.) of Compound 15 in 10 mL of DMF. The resulting mixture was stirred for 20 hours, and then concentrated under reduced pressure to remove a reaction solution. The concentrate was purified by column chromatography (10% MeOH in DCM) to obtain a target compound. TLC (DCM:MeOH = 10:1); R_f = 0.2.

[1116] Confirmation of structure of VC linker (DD2)

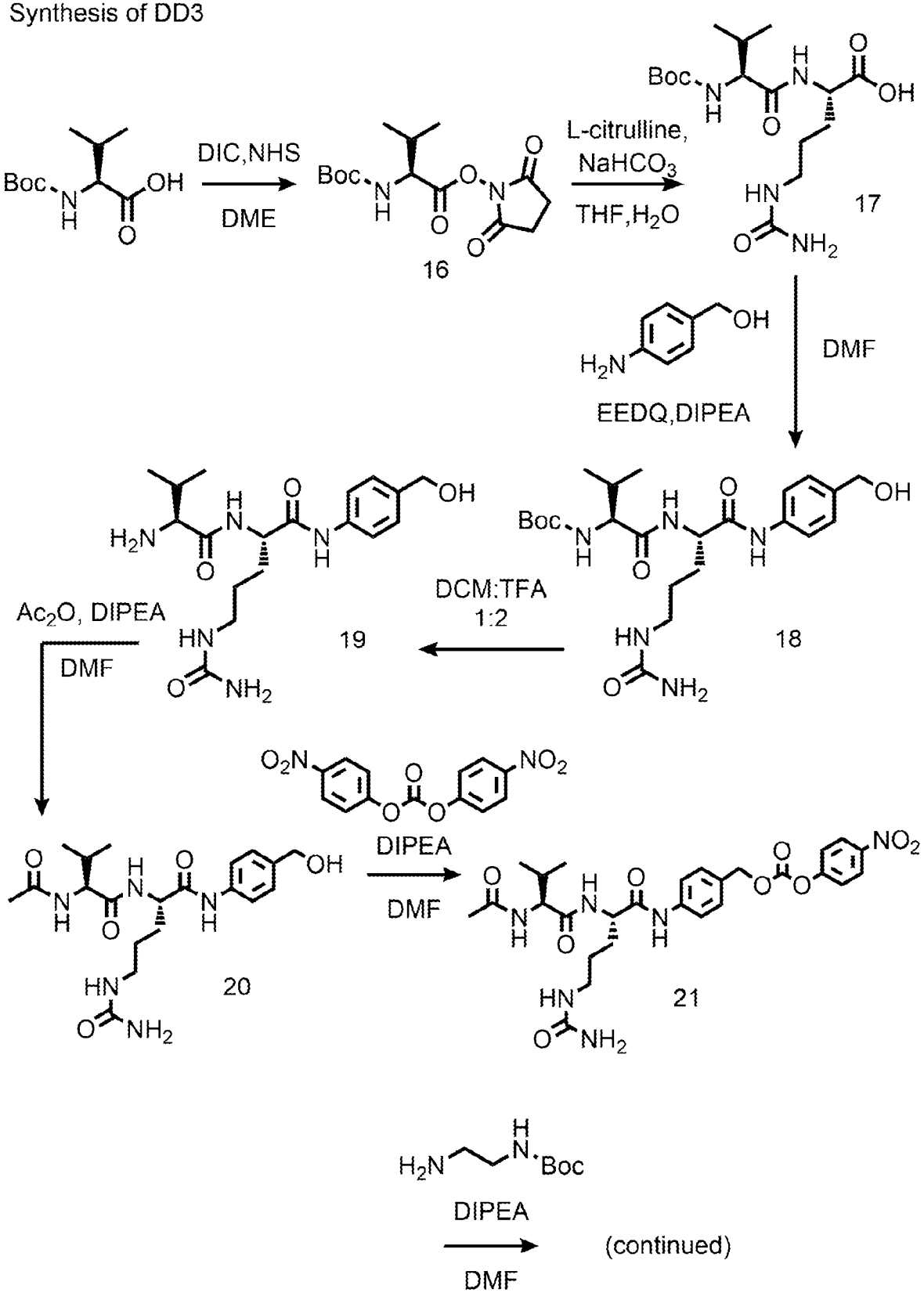
[1117] LRMS (ESI): m/z 840.4 [$M+H^+$]

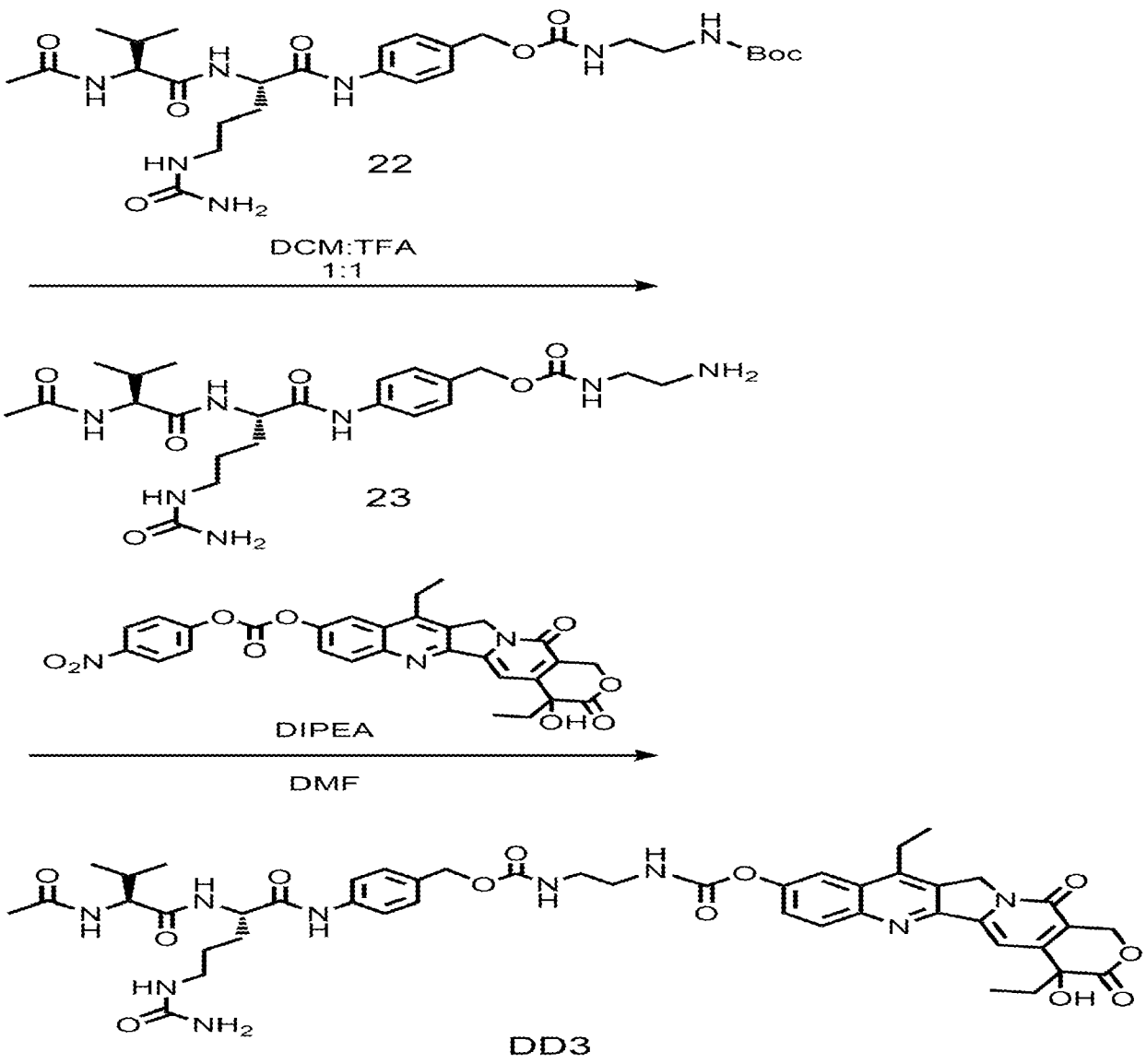
[1118] The results are shown in FIG. 28.

[1119] 1.3.2. Synthesis and confirmation of structure of VC linker (DD3)

[1120] [Scheme 11]

Synthesis of DD3





[1121] Synthesis of VC linker (DD3)

[1122] Synthesis of Compound 16

[1123] 5 g (14.7 mmol, 1.0 eq.) of Fmoc-Val-OH and 1.7 g (14.7 mmol, 1.0 eq.) of N-hydroxysuccinimide (NHS) were dissolved in 140 mL of dimethoxyethane (DME), and stirred. 2.5 mL (16.2 mmol, 1.1 eq.) of N,N'-diisopropylcarbodiimide (DIC) was added dropwise at 0°C, and stirred for 16 hours. The reaction solution was filtered under reduced pressure to remove floating matter, and the filtrate was concentrated under reduced pressure. The residue was dissolved in acetone, and stored at a low temperature for 4 hours in a refrigerator. Thereafter, the resulting solution was filtered under reduced pressure to remove the re-formed floating matter, and used in the next reaction without any purification (crude yield: 5.5 g, 86%). TLC (EA:Hex = 1:1); R_f = 0.5.

[1124] Synthesis of Compound 17

[1125] 2.0 g (11.5 mmol, 1.0 eq.) of L-citrulline was dissolved in 100 mL of a 1:1 mixed solution of tetrahydrofuran (THF) and water, and stirred. 988 mg of sodium hydrogen carbonate was added thereto, and stirred. Thereafter, 5.0 g (11.5 mmol, 1.0 eq.) of Compound 16 was dissolved in 80 mL of acetone, added dropwise to the reaction solution, and then stirred. The resulting mixture was stirred for 21 hours, and then concentrated under reduced pressure to remove the organic solvent. An aqueous layer was washed with ethyl acetate (EA), and then titrated to pH 3 by dropwise addition of 2 N HCl. EA was added thereto so that the precipitate in an organic layer was extracted. Then, the organic layer was dried over a saturated saline solution and sodium sulfate, and used in the next reaction without any purification (crude yield: 5.6 g, 98%). TLC (DCM:MeOH = 10:1, one drop of formic acid); R_f = 0.1.

[1126] Synthesis of Compound 18

[1127] 50 mL of 10% piperidine in N,N-dimethylformamide (DMF) was added dropwise to 2.84 g (5.72 mmol, 1.0 eq.) of Compound 17, and stirred. After 4 hours, the resulting mixture was concentrated under reduced pressure to remove a reaction solution, and the residue was dissolved in water, and filtered under reduced pressure to remove the formed floating matter. An aqueous layer was concentrated to obtain a target compound, which was used in the next reaction without any purification. TLC (DCM:MeOH = 10:1); R_f = 0.01.

[1128] Synthesis of Compound 19

[1129] 54 mg (0.22 mmol, 1.0 eq.) of Compound 18 was dissolved in 2 mL of DMF, and 0.05 mL (0.264 mmol, 1.2 eq.) of N,N-diisopropylethylamine (DIPEA) was added dropwise thereto. Compound 15 was completely dissolved by adding 2 mL of water, and 0.05 mL (0.528 mmol, 2.4 eq.) of an acetic anhydride was added dropwise thereto at room temperature. After 3 hours, the resulting mixture was concentrated under reduced pressure to remove a reaction solution, and the concentrate was purified by reversed-phase column chromatography to obtain a target compound. TLC (DCM:MeOH = 10:1); R_f = 0.05.

[1130] Synthesis of Compound 20

[1131] 1 g (3.16 mmol, 1.0 eq.) of Compound 19 was dissolved in 30 mL of a 2:1 mixed solution of DCM and methanol, and 868 mg (3.48 mmol, 1.1 eq.) of N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) was added thereto, and stirred. 451 mg (3.66 mmol, 1.16 eq.) of 4-aminobenzyl alcohol was added thereto, and stirred for 5 hours. The resulting mixture was concentrated under reduced pressure to remove a reaction solution, and the concentrate was purified by column chromatography (10% MeOH in DCM) to obtain 419 mg of a target compound (yield: 32%). TLC (DCM:MeOH = 10:1); R_f = 0.1.

[1132] Synthesis of Compound 21

[1133] 240 mg (0.55 mmol, 1.0 eq.) of Compound 20 was dissolved in 10 mL of DMF, and 0.3 mL (1.65 mmol, 3.0 eq.) of DIPEA was added dropwise thereto, and stirred. Thereafter, a reaction was carried out by adding 509 mg (1.65 mmol, 3.0 eq.) of bis-(4-aminophenyl) carbonate. After 3 hours, the resulting mixture was concentrated under reduced pressure to remove a reaction solution, and diethyl ether was added thereto to precipitate a target compound, which was filtered under reduced pressure. The obtained target compound was used in the next reaction without any purification.

[1134] Synthesis of Compound 22

[1135] 33 mg (0.06 mmol, 1.0 eq.) of Compound 21 was dissolved in 5 mL of DMF, and 16 mg (0.08 mmol, 1.3 eq.) of 1-[(tert-butoxycarbonyl)amino]-2-aminoethane and 0.02 mL (0.08 mmol, 1.3 eq.) of DIPEA were added dropwise thereto, and stirred. The resulting mixture was stirred for 7 hours, and then concentrated under reduced pressure to remove a reaction solution, and diethyl ether was added thereto to precipitate a target compound, which was filtered under reduced pressure. The obtained target compound was used in the next reaction without any purification. TLC (DCM:MeOH = 10:1); R_f = 0.1.

[1136] Synthesis of Compound 23

[1137] 17 mg (0.03 mmol, 1.0 eq.) of Compound 22 was dissolved in 1 mL of DCM, and 1 mL of trifluoroacetic acid (TFA) was then added dropwise thereto at 0°C. A reaction was carried out by stirring the resulting mixture at 0°C for 30 minutes, followed by stirring at room temperature for 30 minutes. Thereafter, the mixture was concentrated under reduced pressure to remove a reaction solution such as DCM, and this concentration was then repeated three times. The concentrated matter was dried under high vacuum, and purified by reversed-phase column chromatography to obtain 13 mg of a target compound. TLC (DCM:MeOH = 10:1); R_f = 0.01.

[1138] Synthesis of Compound DD3

[1139] 0.01 mL (0.04 mmol, 1.5 eq.) of DIPEA was added dropwise to a solution obtained by dissolving 13 mg (0.03 mmol, 1.0 eq.) of Compound 23 in 3 mL of DMF, and 22 mg (0.04 mmol, 1.5 eq.) of phenol-activated SN38 was added thereto. The resulting mixture was stirred for an hour, and then concentrated under reduced pressure to remove a reaction solution. The concentrate was purified by column chromatography (10% MeOH in DCM) to obtain a target compound. TLC (DCM:MeOH = 10:1); R_f = 0.2.

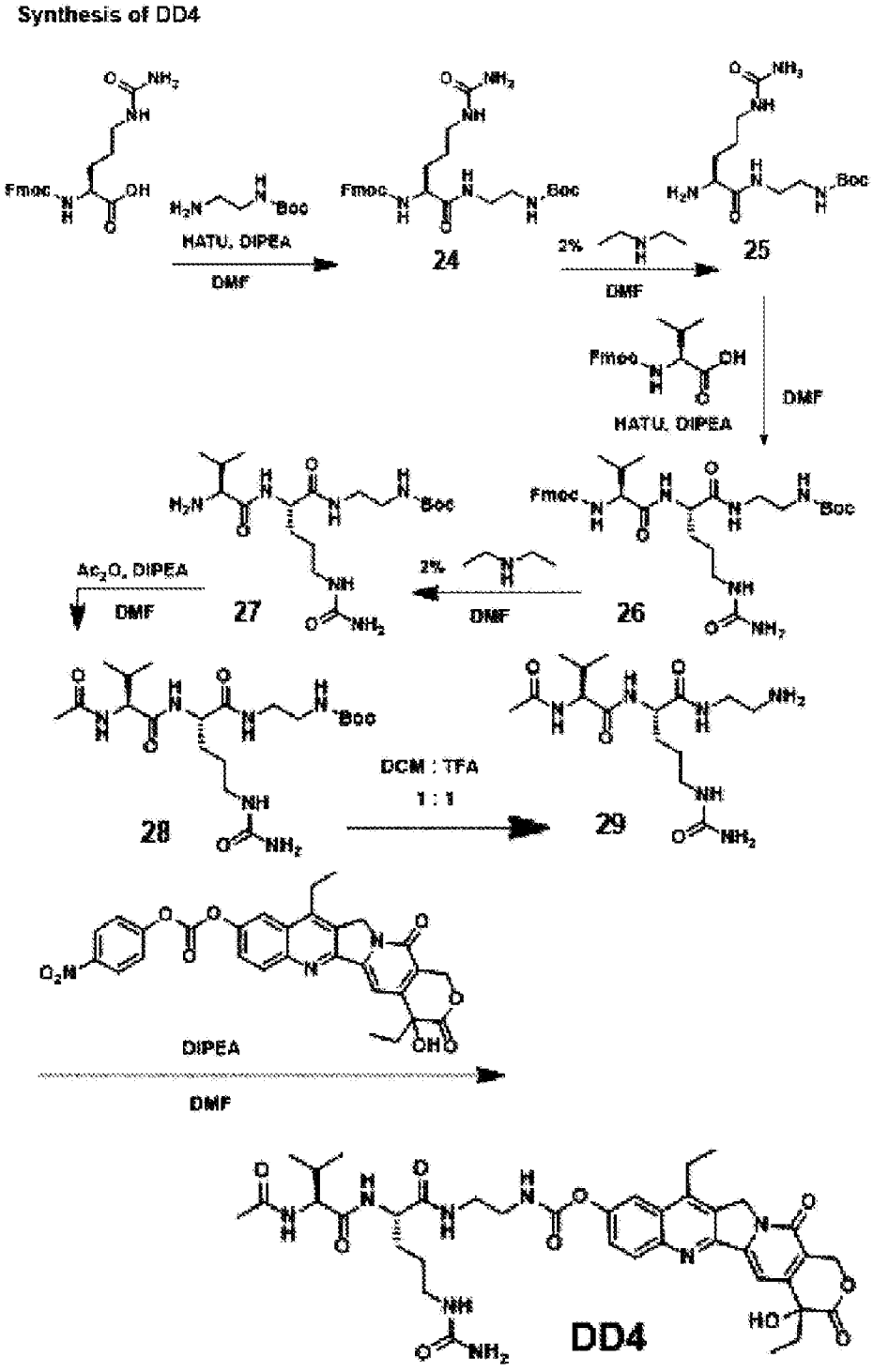
[1140] Confirmation of structure of VC linker (DD3)

[1141] LRMS (ESI): m/z 926.4 [$M+H^+$]

[1142] The results are shown in FIG. 29.

[1143] 1.3.3. Synthesis and confirmation of structure of VC linker (DD4)

[1144] [Scheme 12]



[1145] Synthesis of VC linker (DD4)

[1146] Synthesis of Compound 24

[1147] 5 g (14.7 mmol, 1.0 eq.) of Fmoc-Val-OH and 1.7 g (14.7 mmol, 1.0 eq.) of N-hydroxysuccinimide (NHS) were dissolved in 140 mL of dimethoxyethane (DME), and stirred. 2.5 mL (16.2 mmol, 1.1 eq.) of N,N'-diisopropylcarbodiimide (DIC) was added dropwise at 0°C, and stirred for 16 hours. The reaction solution was filtered under reduced pressure to remove floating matter, and the filtrate was concentrated under reduced pressure. The residue was dissolved in acetone, and stored at a low temperature for 4 hours in a refrigerator. Thereafter, the resulting solution was filtered under reduced pressure to remove the re-formed floating matter, and used in the next reaction without any purification (crude yield: 5.5 g, 86%). TLC (EA:Hex = 1:1); R_f = 0.5.

[1148] Synthesis of Compound 25

[1149] 2.0 g (11.5 mmol, 1.0 eq.) of L-citrulline was dissolved in 100 mL of a 1:1 mixed solution of tetrahydrofuran (THF) and water, and stirred. 988 mg of sodium hydrogen carbonate was added thereto, and stirred. Thereafter, 5.0 g of Compound 21 (11.5 mmol, 1.0 eq.) was dissolved in 80 mL of acetone, added dropwise to the reaction solution, and then stirred. The resulting mixture was stirred for 21 hours, and then concentrated under reduced pressure to remove the organic solvent. An aqueous layer was washed with ethyl acetate (EA), and then titrated to pH 3 by slow dropwise addition of 2 N HCl. EA was added thereto so that the precipitate in an organic layer was extracted. Then, the organic layer was dried over a saturated saline solution and sodium sulfate, and used in the next reaction without any purification (crude yield: 5.6 g, 98%). TLC (DCM:MeOH = 10:1, one drop of formic acid); R_f = 0.1.

[1150] Synthesis of Compound 26

[1151] 50 mL of 10% piperidine in N,N-dimethylformamide (DMF) was added dropwise to 2.84 g (5.72 mmol, 1.0 eq.) of Compound 25, and stirred. After 4 hours, the resulting mixture was concentrated under reduced pressure to remove a reaction solution, and the residue was dissolved in water, and filtered under reduced pressure to remove the formed floating matter. An aqueous layer was concentrated to obtain a target compound, which was used in the next reaction without any purification. TLC (DCM:MeOH = 10:1); R_f = 0.01.

[1152] Synthesis of Compound 27

[1153] 54 mg (0.22 mmol, 1.0 eq.) of Compound 26 was dissolved in 2 mL of DMF, and 0.05 mL (0.264 mmol, 1.2 eq.) of N,N-diisopropylethylamine (DIPEA) was added dropwise thereto. Compound 23 was completely dissolved by adding 2 mL of water, and 0.05 mL (0.528 mmol, 2.4 eq.) of an acetic anhydride was added dropwise thereto at room temperature. After 3 hours, the resulting mixture was concentrated under reduced pressure to remove a reaction solution, and the concentrate was purified by reversed-phase column chromatography to obtain a target

compound. TLC (DCM:MeOH = 10:1); R_f = 0.05.

[1154] Synthesis of Compound 28

[1155] 300 mg (0.95 mmol, 1.0 eq.) of Compound 27 was dissolved in 6 mL of DMF, and 550 mg (1.43 mmol, 1.5 eq.) of 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (HATU) was then added thereto. Thereafter, 0.25 mL (1.43 mmol, 1.5 eq.) of DIPEA was added dropwise, and 230 mg (1.43 mmol, 1.5 eq.) of 1-[(tert-butoxycarbonyl)amino]-2-aminoethane was dissolved in 3.5 mL of DMF, and added dropwise at room temperature. After 7 hours, the reaction solution was diluted with ethyl acetate (EA), and an organic layer was washed with a saturated sodium hydrogen carbonate solution. Then, the organic layer was dried over a saturated saline solution and sodium sulfate, and filtered to obtain a target compound. The obtained target compound was used in the next reaction without any purification. TLC (DCM:MeOH = 10:1); R_f = 0.5.

[1156] Synthesis of Compound 29

[1157] Compound 28 was dissolved in a 2:1 mixed solution of DCM and TFA, and a reaction was then carried out at 0°C. Thereafter, the reaction mixture was concentrated under reduced pressure to remove a reaction solution such as DCM, and this concentration was then repeated three times. The residue was dried under high vacuum, and purified by reversed-phase column chromatography to obtain 370 mg of a target compound. TLC (DCM:MeOH = 10:1); R_f = 0.01.

[1158] Synthesis of Compound DD4

[1159] 0.03 mL (0.17 mmol, 1.2 eq.) of DIPEA was added dropwise to a solution obtained by dissolving 50 mg (0.14 mmol, 1.0 eq.) of Compound 26 in 3 mL of DMF, and 107 mg (0.21 mmol, 1.5 eq.) of phenol-activated SN38 was added thereto. The resulting mixture was stirred for an hour, and then concentrated under reduced pressure to remove a reaction solution. The concentrate was purified by column chromatography (10% MeOH in DCM) to obtain a target compound. TLC (DCM:MeOH = 10:1); R_f = 0.1.

[1160] Confirmation of structure of VC linker (DD4)

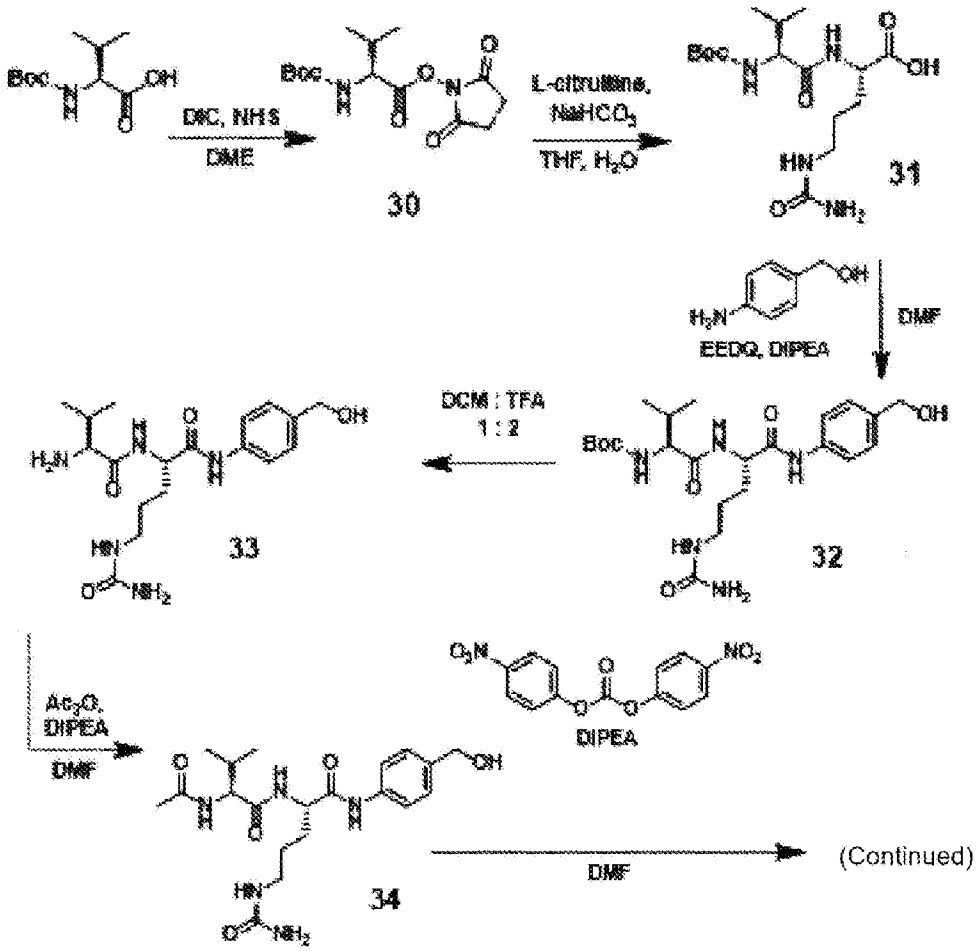
[1161] LRMS (ESI): m/z 777.3 [$M+H^+$]

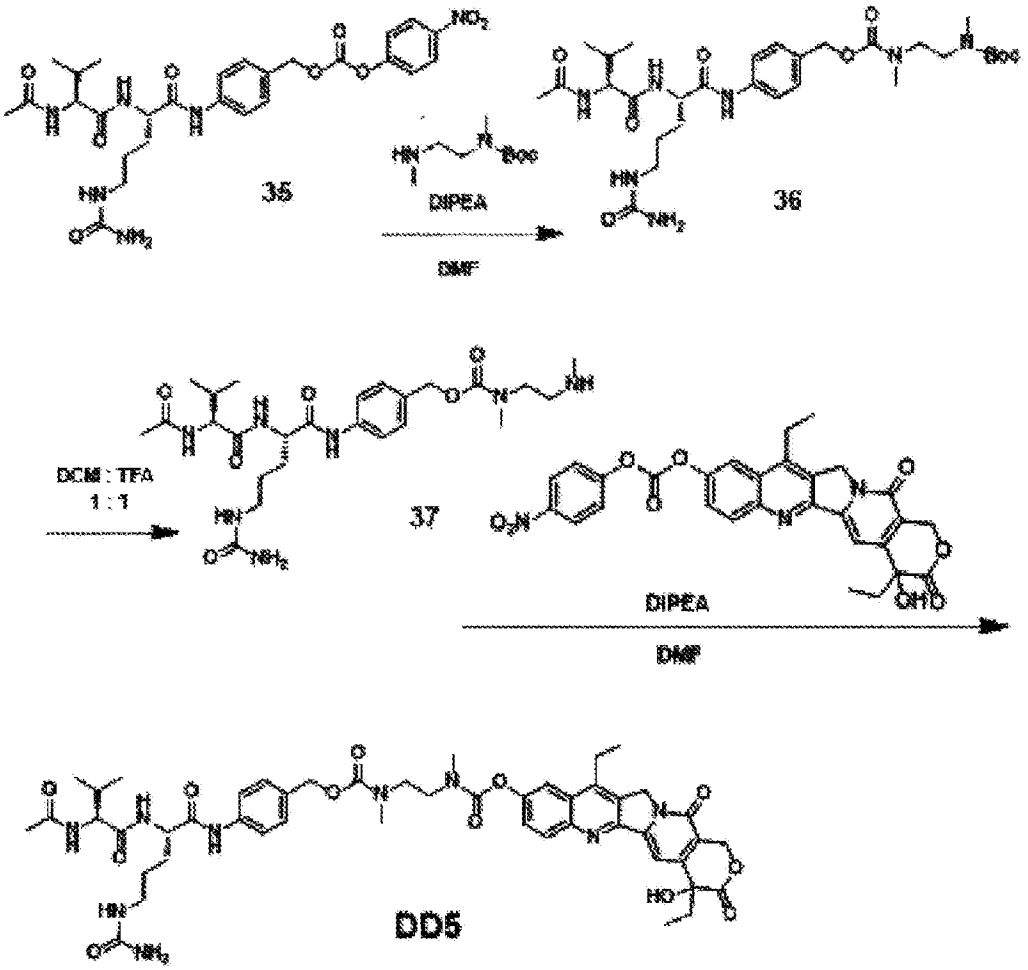
[1162] The results are shown in FIG. 30.

[1163] 1.3.4. Synthesis and confirmation of structure of VC linker (DD5)

[1164] [Scheme 13]

Synthesis of DD5





[1165] Synthesis of VC linker (DD5)

[1166] Synthesis of Compound 30

[1167] 5 g (14.7 mmol, 1.0 eq.) of Fmoc-Val-OH and 1.7 g (14.7 mmol, 1.0 eq.) of N-hydroxysuccinimide (NHS) were dissolved in 140 mL of dimethoxyethane (DME), and stirred. 2.5 mL (16.2 mmol, 1.1 eq.) of N,N'-diisopropylcarbodiimide (DIC) was added dropwise at 0°C, and stirred for 16 hours. The reaction solution was filtered under reduced pressure to remove floating matter, and the filtrate was concentrated under reduced pressure. The residue was dissolved in acetone, and stored at a low temperature for 4 hours in a refrigerator. Thereafter, the resulting solution was filtered under reduced pressure to remove the re-formed floating matter, and used in the next reaction without any purification (crude yield: 5.5 g, 86%). TLC (EA:Hex = 1:1); R_f = 0.5.

[1168] Synthesis of Compound 31

[1169] 2.0 g (11.5 mmol, 1.0 eq.) of L-citrulline was dissolved in 100 mL of a 1:1 mixed

solution of tetrahydrofuran (THF) and water, and stirred. 988 mg of sodium hydrogen carbonate was added thereto, and stirred. Thereafter, 5.0 g (11.5 mmol, 1.0 eq.) of Compound 30 was dissolved in 80 mL of acetone, added dropwise to the reaction solution, and then stirred. The resulting mixture was stirred for 21 hours, and then concentrated under reduced pressure to remove the organic solvent. An aqueous layer was washed with ethyl acetate (EA), and then titrated to pH 3 by slow dropwise addition of 2 N HCl. EA was added thereto so that the precipitate in an organic layer was extracted. Then, the organic layer was dried over a saturated saline solution and sodium sulfate, and used in the next reaction without any purification (crude yield: 5.6 g, 98%). TLC (DCM:MeOH = 10:1, one drop of formic acid); R_f = 0.1.

[1170] Synthesis of Compound 32

[1171] 50 mL of 10% piperidine in N,N-dimethylformamide (DMF) was added dropwise to 2.84 g (5.72 mmol, 1.0 eq.) of Compound 31, and stirred. After 4 hours, the resulting mixture was concentrated under reduced pressure to remove a reaction solution, and the residue was dissolved in water, and filtered under reduced pressure to remove the formed floating matter. An aqueous layer was concentrated to obtain a target compound, which was used in the next reaction without any purification. TLC (DCM:MeOH = 10:1); R_f = 0.01.

[1172] Synthesis of Compound 33

[1173] 54 mg (0.22 mmol, 1.0 eq.) of Compound 29 was dissolved in 2 mL of DMF, and 0.05 mL (0.264 mmol, 1.2 eq.) of N,N-diisopropylethylamine (DIPEA) was added dropwise thereto. Compound 32 was completely dissolved by adding 2 mL of water, and 0.05 mL (0.528 mmol, 2.4 eq.) of an acetic anhydride was added dropwise thereto at room temperature. After 3 hours, the resulting mixture was concentrated under reduced pressure to remove a reaction solution, and the concentrate was purified by reversed-phase column chromatography to obtain a target compound. TLC (DCM:MeOH = 10:1); R_f = 0.05.

[1174] Synthesis of Compound 34

[1175] 1 g (3.16 mmol, 1.0 eq.) of Compound 33 was dissolved in 30 mL of a 2:1 mixed solution of DCM and methanol, and 868 mg (3.48 mmol, 1.1 eq.) of N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) was added thereto, and stirred. 451 mg (3.66 mmol, 1.16 eq.) of 4-aminobenzyl alcohol was added thereto, and stirred for 5 hours. The resulting mixture was concentrated under reduced pressure to remove a reaction solution, and the concentrate was purified by column chromatography (10% MeOH in DCM) to obtain 419 mg of a target compound (yield: 32%). TLC (DCM:MeOH = 10:1); R_f = 0.1.

[1176] Synthesis of Compound 35

[1177] 240 mg (0.55 mmol, 1.0 eq.) of Compound 34 was dissolved in 10 mL of DMF, and

0.3 mL (1.65 mmol, 3.0 eq.) of DIPEA was added dropwise thereto, and stirred. Thereafter, a reaction was carried out by adding 509 mg (1.65 mmol, 3.0 eq.) of bis-(4-aminophenyl) carbonate. After 3 hours, the resulting mixture was concentrated under reduced pressure to remove a reaction solution, and diethyl ether was added thereto to precipitate a target compound, which was filtered under reduced pressure. The obtained target compound was used in the next reaction without any purification.

[1178] Synthesis of Compound 36

[1179] 33 mg (0.06 mmol, 1.0 eq.) of Compound 35 was dissolved in 5 mL of DMF, and 16 mg (0.08 mmol, 1.3 eq.) of N-[(tert-butoxy)carbonyl]-N,N'-dimethylethylenediamine and 0.02 mL (0.08 mmol, 1.3 eq.) of DIPEA were added dropwise thereto, and stirred. The resulting mixture was stirred for 7 hours, and then concentrated under reduced pressure to remove a reaction solution, and diethyl ether was added thereto to precipitate a target compound, which was filtered under reduced pressure. The obtained target compound was used in the next reaction without any purification. TLC (DCM:MeOH = 10:1); R_f = 0.1.

[1180] Synthesis of Compound 37

[1181] 17 mg (0.03 mmol, 1.0 eq.) of Compound 36 was dissolved in 1 mL of DCM, and 1 mL of trifluoroacetic acid (TFA) was then added dropwise thereto at 0°C. A reaction was carried out by stirring the resulting mixture at 0°C for 30 minutes, followed by stirring at room temperature for 30 minutes. Thereafter, the mixture was concentrated under reduced pressure to remove a reaction solution such as DCM, and this concentration was then repeated three times. The concentrated matter was dried under high vacuum, and purified by reversed-phase column chromatography to obtain 13 mg of a target compound. TLC (DCM:MeOH = 10:1); R_f = 0.01.

[1182] Synthesis of Compound DD5

[1183] 0.01 mL (0.04 mmol, 1.5 eq.) of DIPEA was added dropwise to a solution obtained by dissolving 13 mg (0.03 mmol, 1.0 eq.) of Compound 37 in 3 mL of DMF, and 22 mg (0.04 mmol, 1.5 eq.) of phenol-activated SN38 was added thereto. The resulting mixture was stirred for an hour, and then concentrated under reduced pressure to remove a reaction solution. The concentrate was purified by column chromatography (10% MeOH in DCM) to obtain a target compound. TLC (DCM:MeOH = 10:1); R_f = 0.2.

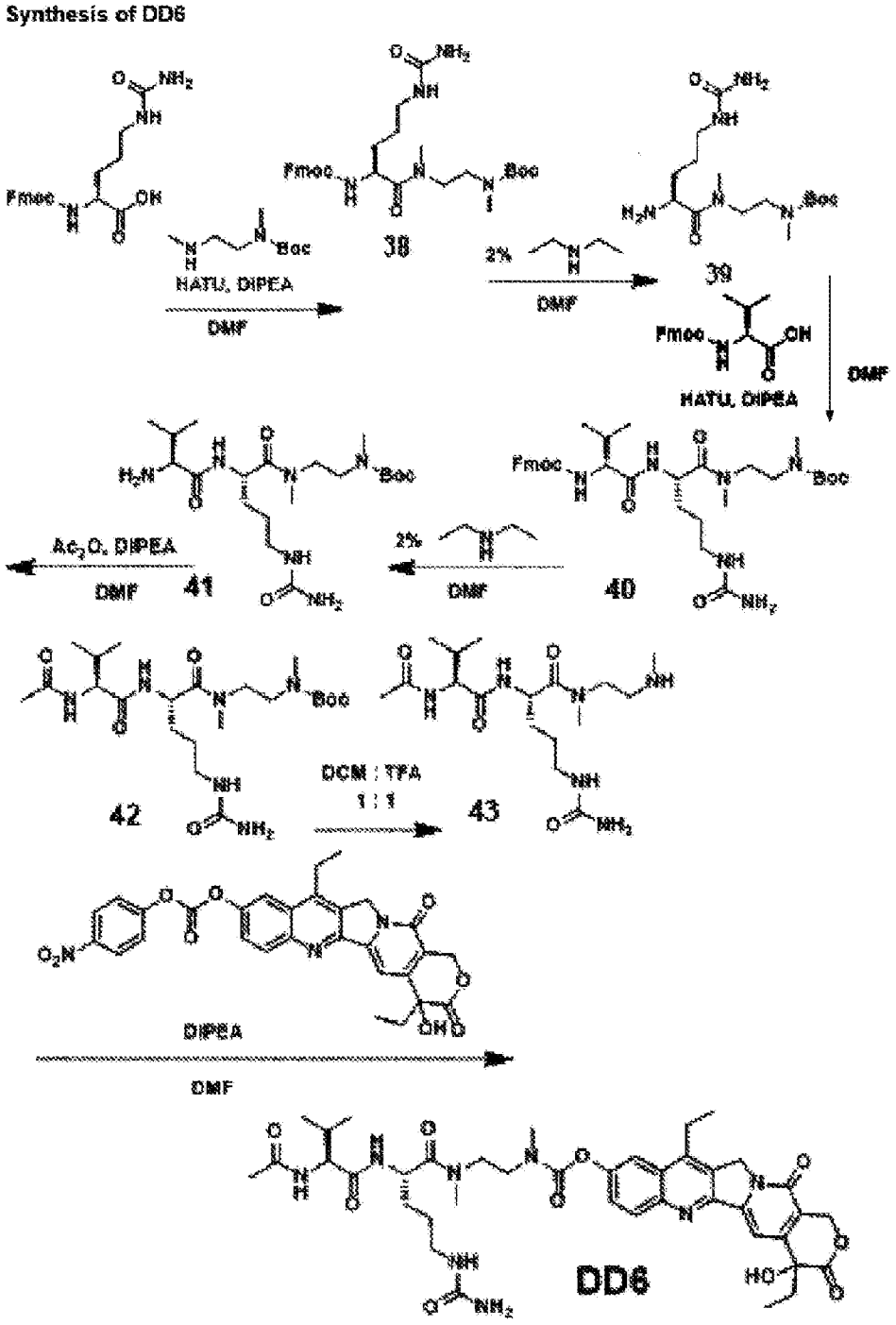
[1184] 3-4-2: Confirmation of structure of VC linker (DD5)

[1185] LRMS (ESI): m/z 954.4 [$M+H^+$]

[1186] The results are shown in FIG. 31.

[1187] 1.3.5. Synthesis and confirmation of structure of VC linker (DD6)

[1188] [Scheme 14]



[1189] Synthesis of VC linker (DD6)

[1190] Synthesis of Compound 38

[1191] 5 g (14.7 mmol, 1.0 eq.) of Fmoc-Val-OH and 1.7 g (14.7 mmol, 1.0 eq.) of N-hydroxysuccinimide (NHS) were dissolved in 140 mL of dimethoxyethane (DME), and stirred.

2.5 mL (16.2 mmol, 1.1 eq.) of N,N'-diisopropylcarbodiimide (DIC) was added dropwise at 0°C, and stirred for 16 hours. The reaction solution was filtered under reduced pressure to remove floating matter, and the filtrate was concentrated under reduced pressure. The residue was dissolved in acetone, and stored at a low temperature for 4 hours in a refrigerator. Thereafter, the resulting solution was filtered under reduced pressure to remove the re-formed floating matter, and used in the next reaction without any purification (crude yield: 5.5 g, 86%). TLC (EA:Hex = 1:1); R_f = 0.5.

[1192] Synthesis of Compound 39

[1193] 2.0 g (11.5 mmol, 1.0 eq.) of L-citrulline was dissolved in 100 mL of a 1:1 mixed solution of tetrahydrofuran (THF) and water, and stirred. 988 mg of sodium hydrogen carbonate was added thereto, and stirred. Thereafter, 5.0 g (11.5 mmol, 1.0 eq.) of Compound 38 was dissolved in 80 mL of acetone, added dropwise to the reaction solution, and then stirred. The resulting mixture was stirred for 21 hours, and then concentrated under reduced pressure to remove the organic solvent. An aqueous layer was washed with ethyl acetate (EA), and then titrated to pH 3 by slow dropwise addition of 2 N HCl. EA was added thereto so that the precipitate in an organic layer was extracted. Then, the organic layer was dried over a saturated saline solution and sodium sulfate, and used in the next reaction without any purification (crude yield: 5.6 g, 98%). TLC (DCM:MeOH = 10:1, one drop of formic acid); R_f = 0.1.

[1194] Synthesis of Compound 40

[1195] 50 mL of 10% piperidine in N,N-dimethylformamide (DMF) was added dropwise to 2.84 g (5.72 mmol, 1.0 eq.) of Compound 39, and stirred. After 4 hours, the resulting mixture was concentrated under reduced pressure to remove a reaction solution, and the residue was dissolved in water, and filtered under reduced pressure to remove the formed floating matter. An aqueous layer was concentrated to obtain a target compound, which was used in the next reaction without any purification. TLC (DCM:MeOH = 10:1); R_f = 0.01.

[1196] Synthesis of Compound 41

[1197] 54 mg (0.22 mmol, 1.0 eq.) of Compound 40 was dissolved in 2 mL of DMF, and 0.05 mL (0.264 mmol, 1.2 eq.) of N,N-diisopropylethylamine (DIPEA) was added dropwise thereto. Compound 37 was completely dissolved by adding 2 mL of water, and 0.05 mL (0.528 mmol, 2.4 eq.) of an acetic anhydride was added dropwise thereto at room temperature. After 3 hours, the resulting mixture was concentrated under reduced pressure to remove a reaction solution, and the concentrate was purified by reversed-phase column chromatography to obtain a target compound. TLC (DCM:MeOH = 10:1); R_f = 0.05.

[1198] Synthesis of Compound 42

[1199] 300 mg (0.95 mmol, 1.0 eq.) of Compound 41 was dissolved in 6 mL of DMF, and 550 mg (1.43 mmol, 1.5 eq.) of 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (HATU) was then added thereto. Thereafter, 0.25 mL (1.43 mmol, 1.5 eq.) of DIPEA was added dropwise, and 230 mg (1.43 mmol, 1.5 eq.) of N-[(tert-butoxy)carbonyl]-N,N'-dimethylethylenediamine was dissolved in 3.5 mL of DMF, and added dropwise at room temperature. After 7 hours, the reaction solution was diluted with ethyl acetate (EA), and an organic layer was washed with a saturated sodium hydrogen carbonate solution. Then, the organic layer was dried over a saturated saline solution and sodium sulfate, and filtered to obtain a target compound. The obtained target compound was used in the next reaction without any purification. TLC (DCM:MeOH = 10:1); R_f = 0.5.

[1200] Synthesis of Compound 43

[1201] Compound 42 was dissolved in a 2:1 mixed solution of DCM and TFA, and a reaction was then carried out at 0°C. Thereafter, the reaction mixture was concentrated under reduced pressure to remove a reaction solution such as DCM, and this concentration was then repeated three times. The residue was dried under high vacuum, and purified by reversed-phase column chromatography to obtain 370 mg of a target compound. TLC (DCM:MeOH = 10:1); R_f = 0.01.

[1202] Synthesis of Compound DD6

[1203] 0.03 mL (0.17 mmol, 1.2 eq.) of DIPEA was added dropwise to a solution obtained by dissolving 50 mg (0.14 mmol, 1.0 eq.) of Compound 43 in 3 mL of DMF, and 107 mg (0.21 mmol, 1.5 eq.) of phenol-activated SN38 was added thereto. The resulting mixture was stirred for an hour, and then concentrated under reduced pressure to remove a reaction solution. The concentrate was purified by column chromatography (10% MeOH in DCM) to obtain a target compound. TLC (DCM:MeOH = 10:1); R_f = 0.1.

[1204] Confirmation of structure of VC linker (DD6)

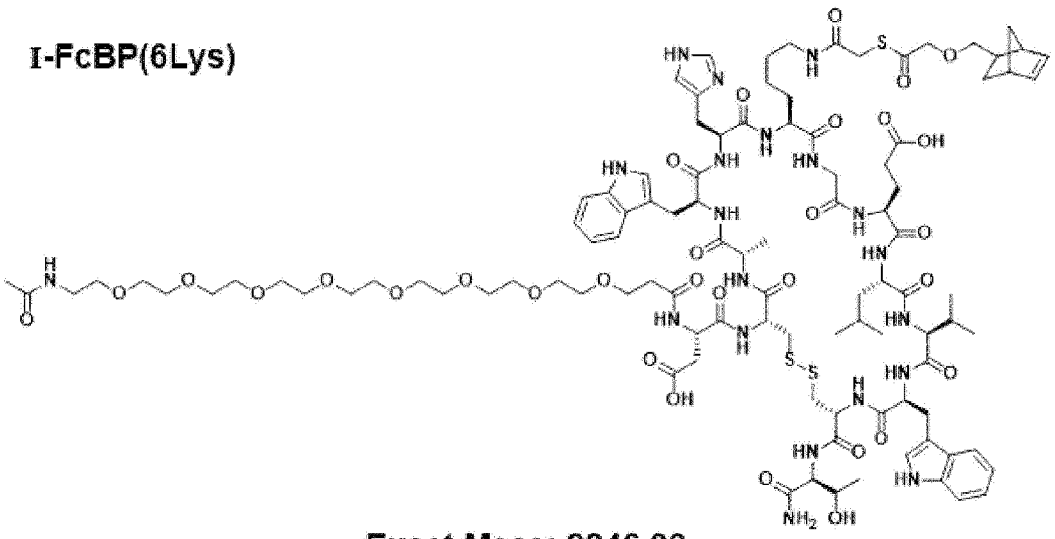
[1205] LRMS (ESI): m/z 805.5 [$M+H^+$]

[1206] The results are shown in FIG. 32.

[1207] 2. Preparation of agent for transferring site-specific first click-chemistry functional group to antibody (H_1-L_2-SSFI)

[1208] 2.1. Synthesis and confirmation of structure of Compound I-SSFI (where Xa_1 is lysine)

I-FcBP(6Lys)



Exact Mass: 2246.99

[1209] Synthesis method of Compound I-SSFI (Lys)

[1210] Synthesis of Compound I-SSFI was carried out in DMF, and 3 equivalents of DIPEA and 8.4 μmol of Compound I were dissolved in 7.3 μmol of SSFI, and stirred to introduce Compound I into SSFI.

[1211] After the termination of the reaction was confirmed, the reaction solution was concentrated, and purification of Compound I-SSFI by preparative-HPLC was attempted. After the purification, the Compound I-SSFI was freeze-dried to obtain a target compound, the amount of which was confirmed to be 12 mg (purity: >95% up (HPLC), and yield: 71%).

[1212] Confirmation of structure of Compound I-SSFI

[1213] Measuring equipment: Waters Quattro Premier Xe

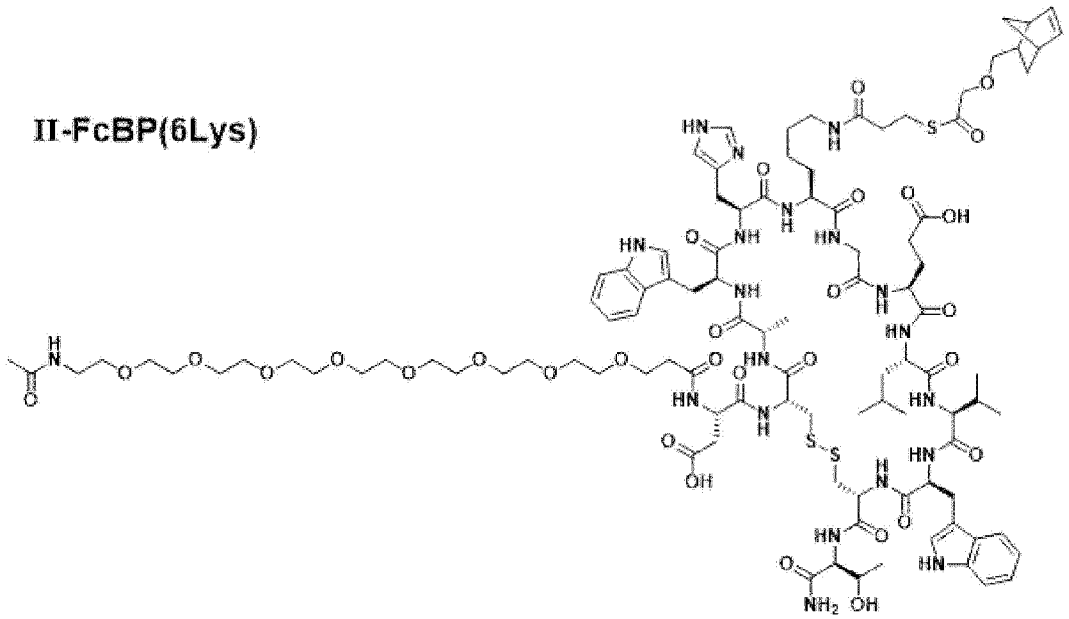
[1214] Calculated molecular weight: 2246.99 g/mol

[1215] Measured molecular weight $(M/2+H)^{2+}$: 1124.90 g/mol

[1216] The results are shown in FIG. 33.

[1217] 2.2. Synthesis and confirmation of structure of Compound II-SSFI (wherein Xa_1 is lysine)

II-FcBP(6Lys)



Exact Mass: 2261.01

[1218] Synthesis method of Compound II-SSFI (Lys)

[1219] Synthesis of Compound II-SSFI was carried out in DMF, and 3 equivalents of DIPEA and 8.4 μmol of Compound II were dissolved in 7.3 μmol of SSFI, and stirred to introduce Compound II into SSFI.

[1220] After the termination of the reaction was confirmed, the reaction solution was concentrated, and purification of Compound II-SSFI by preparative-HPLC was attempted. After the purification, the Compound II-SSFI was freeze-dried to obtain a target compound, the amount of which was confirmed to be 13 mg (purity: >95% up (HPLC), and yield: 78%).

[1221] Confirmation of structure of Compound II-SSFI (Lys)

[1222] Measuring equipment: Ultraflextreme (Bruker)

[1223] Measuring matrix: CHCA (α-Cyano-4-hydroxycinnamic acid) & DHB (2,5-Dihydroxybenzoic acid)

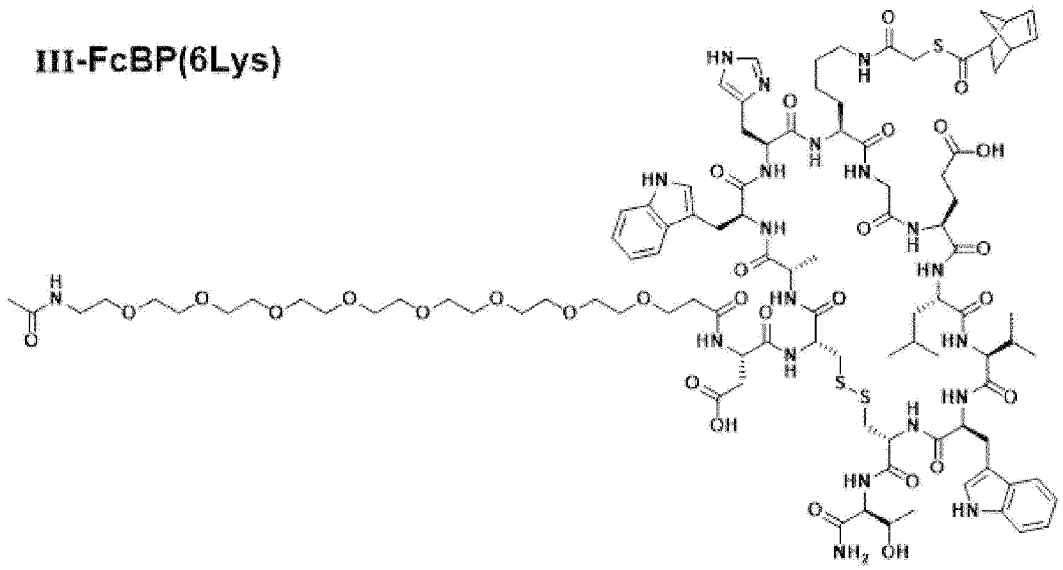
[1224] Calculated molecular weight: 2261.01 g/mol

[1225] Measured molecular weight $(M/2+H)^{2+}$: 2263.26 g/mol

[1226] The results are shown in FIG. 34.

[1227] 2.3. Synthesis and confirmation of structure of Compound III-SSFI (wherein Xa_1 is lysine)

III-FcBP(6Lys)



Exact Mass: 2202.97

[1228] Synthesis method of Compound III-SSFI (Lys)

[1229] Synthesis of Compound III-SSFI was carried out in DMF, and 3 equivalents of DIPEA and 8.4 μmol of Compound III were dissolved in 7.3 μmol of SSFI, and stirred to introduce Compound III into SSFI.

[1230] After the termination of the reaction was confirmed, the reaction solution was concentrated, and purification of Compound III-SSFI by preparative-HPLC was attempted. After the purification, the Compound III-SSFI was freeze-dried to obtain a target compound, the amount of which was confirmed to be 12 mg (purity: >95% up (HPLC), and yield: 75%).

[1231] Confirmation of structure of Compound III-SSFI (Lys)

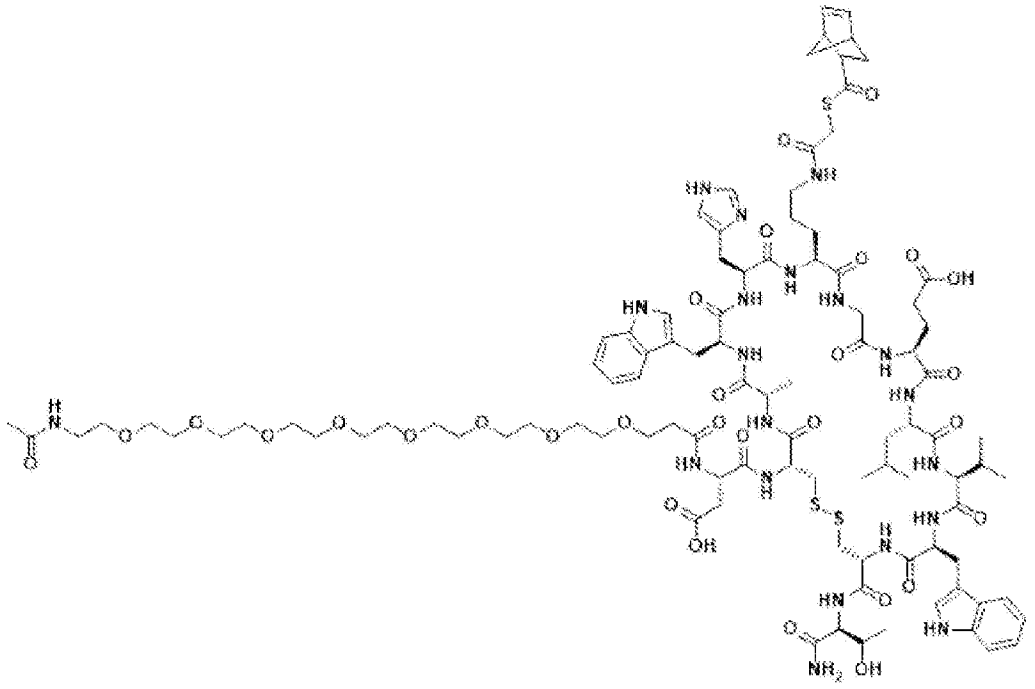
[1232] Measuring equipment: Waters Quattro Premier Xe

[1233] Calculated molecular weight: 2202.97 g/mol

[1234] Measured molecular weight $(M/2+H)^{2+}$: 1103.64 g/mol

[1235] The results are shown in FIG. 35.

[1236] 2.4. Synthesis and confirmation of structure of Compound III-SSFI (wherein Xa_1 is ornithine)



Exact Mass: 2188.95

[1237] Synthesis method of Compound III-SSFI (Orn)

[1238] Synthesis of Compound III-SSFI was carried out in DMF, and 3 equivalents of DIPEA and 8.4 μmol of Compound III were dissolved in 7.3 μmol of SSFI, and stirred to introduce Compound III into SSFI.

[1239] After the termination of the reaction was confirmed, the reaction solution was concentrated, and purification of Compound III-SSFI by preparative-HPLC was attempted. After the purification, the Compound III-SSFI was freeze-dried to obtain a target compound, the amount of which was confirmed to be 14 mg (purity: >95% up (HPLC), and yield: 87%).

[1240] Confirmation of structure of Compound III-SSFI (Orn)

[1241] Measuring equipment: Waters Quattro Premier Xe

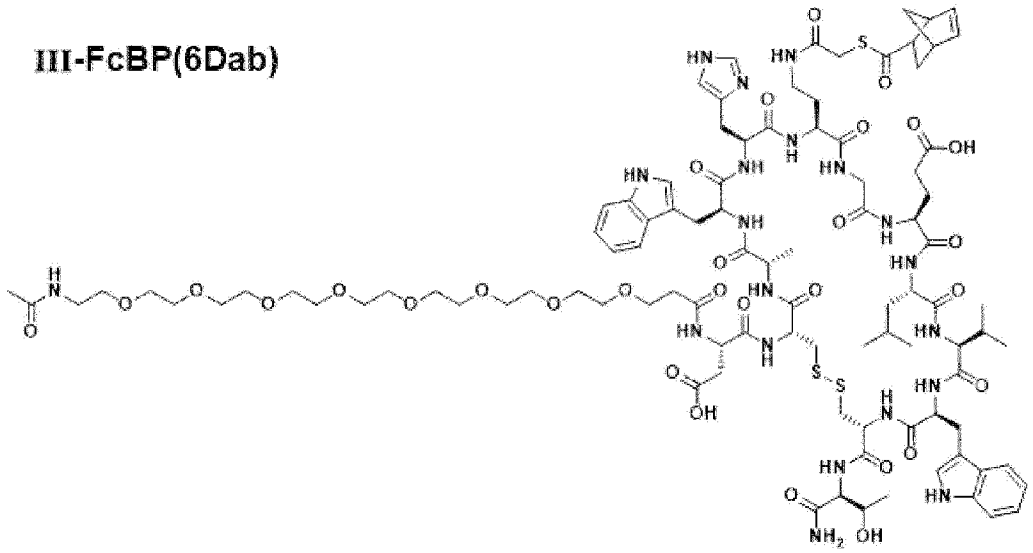
[1242] Calculated molecular weight: 2188.97 g/mol

[1243] Measured molecular weight $(M/2+H)^{2+}$: 1096.64 g/mol

[1244] The results are shown in FIG. 36.

[1245] 2.5. Synthesis and confirmation of structure of Compound III-SSFI (wherein Xa_1 is 2,4-diaminobutanoic acid (Dab))

III-FcBP(6Dab)



Exact Mass: 2174.94

[1246] Synthesis method of Compound III-SSFI (Dab)

[1247] Synthesis of Compound III-SSFI was carried out in DMF, and 3 equivalents of DIPEA and 8.4 μmol of Compound III were dissolved in 7.3 μmol of SSFI, and stirred to introduce Compound III into SSFI.

[1248] After the termination of the reaction was confirmed, the reaction solution was concentrated, and purification of Compound III-SSFI by preparative-HPLC was attempted. After the purification, the Compound III-SSFI was freeze-dried to obtain a target compound, the amount of which was confirmed to be 12 mg (purity: >95% up (HPLC), and yield: 75%).

[1249] Confirmation of structure of Compound III-SSFI (Dab)

[1250] Measuring equipment: Waters Quattro Premier Xe

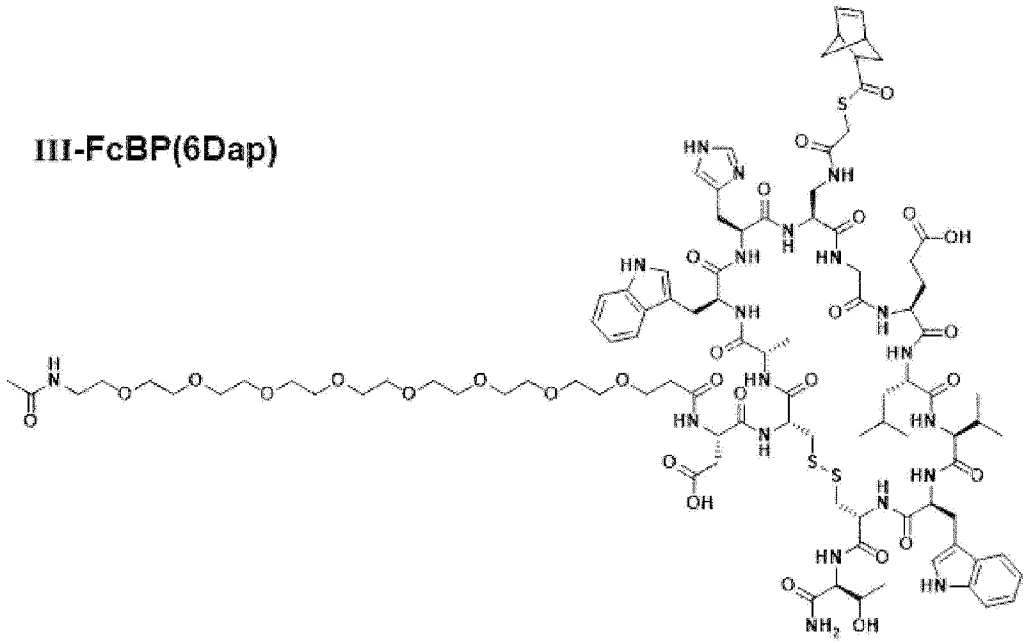
[1251] Calculated molecular weight: 2174.94 g/mol

[1252] Measured molecular weight $(M/2+H)^{2+}$: 1089.94 g/mol

[1253] The results are shown in FIG. 37.

[1254] 2.6. Synthesis and confirmation of structure of Compound III-SSFI (wherein Xa_1 is 2,3-diaminopropionic acid (Dap))

III-FcBP(6Dap)



Exact Mass: 2160.92

[1255] Synthesis method of Compound III-SSFI (Dap)

[1256] Synthesis of Compound III-SSFI was carried out in DMF, and 3 equivalents of DIPEA and 8.4 μmol of Compound III were dissolved in 7.3 μmol of SSFI, and stirred to introduce Compound III into SSFI.

[1257] After the termination of the reaction was confirmed, the reaction solution was concentrated, and purification of Compound III-SSFI by preparative-HPLC was attempted. After the purification, the Compound III-SSFI was freeze-dried to obtain a target compound, the amount of which was confirmed to be 12 mg (purity: >95% up (HPLC), and yield: 75%).

[1258] Confirmation of structure of Compound III-SSFI (Dap)

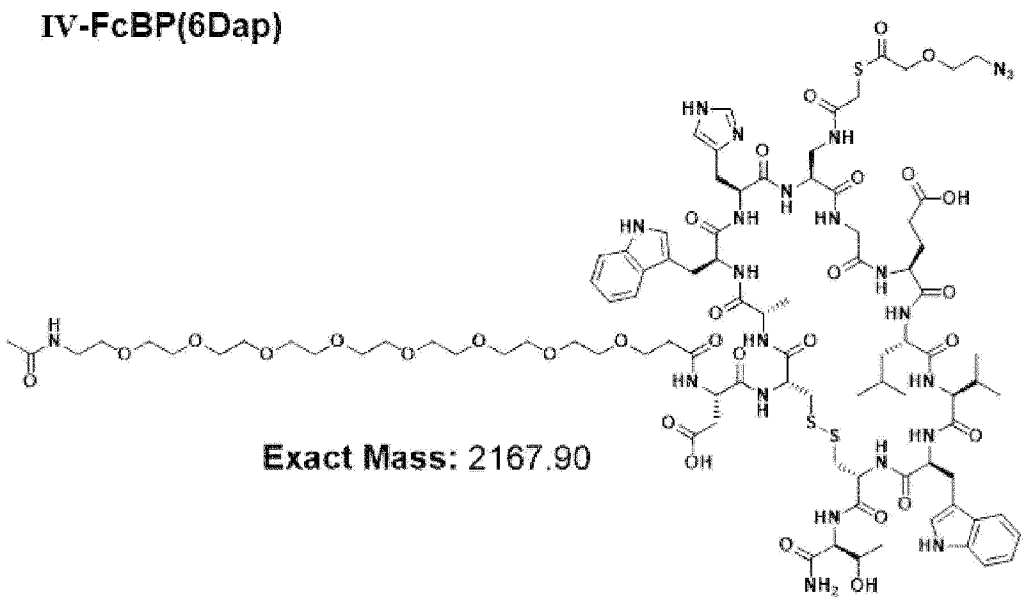
[1259] Measuring equipment: Waters Quattro Premier Xe

[1260] Calculated molecular weight: 2160.92 g/mol

[1261] Measured molecular weight $(M/2+H)^{2+}$: 1082.34 g/mol

[1262] The results are shown in FIG. 38.

[1263] 2.7. Synthesis and confirmation of structure of Compound IV-SSFI (wherein Xa_1 is Dap)



[1264] Synthesis method of Compound IV-SSFI (Dap)

[1265] Synthesis of Compound IV-SSFI was carried out in DMF, and 3 equivalents of DIPEA and 8.4 μmol of Compound IV were dissolved in 7.3 μmol of SSFI, and stirred to introduce Compound IV into SSFI.

[1266] After the termination of the reaction was confirmed, the reaction solution was concentrated, and purification of Compound IV-SSFI by preparative-HPLC was attempted. After the purification, the Compound IV-SSFI was freeze-dried to obtain a target compound, the amount of which was confirmed to be 12 mg (purity: >95% up (HPLC), and yield: 76%).

[1267] Confirmation of structure of Compound IV-SSFI (Dap)

[1268] Measuring equipment: Waters Quattro Premier Xe

[1269] Calculated molecular weight: 2167.90 g/mol

[1270] Measured molecular weight $(M/2+H)^{2+}$: 1085.60 g/mol

[1271] The results are shown in FIG. 39.

[1272] 3. Synthesis and confirmation of structure of antibody containing first click-chemistry functional group in site-specific manner

[1273] 3.1. Synthesis of Antibody-Norbornene

[1274] Synthesis method of Trastuzumab-Norbornene (1)

[1275] Introduction reaction using Compound I-SSFI (Lys)

[1276] Synthesis of Ab (246/248 Lys)-norbornene was carried out in a buffer using Compound I-SSFI (Lys). A 1× PBS buffer (0.01% Tween 20, pH 7.4) was used as the reaction buffer. To introduce norbornene at a certain site of an antibody (trastzumab, 4 mg/mL), 8

equivalents of Compound I-SSFI (Lys) was added per one antibody, and reacted at room temperature for 72 hours, and the reaction monitoring and termination were confirmed by HIC-HPLC. As the binding of norbornene to the antibody proceeds, the peaks on the chromatogram were observed to shift from 4 minutes to 4.5 minutes and 5 minutes, thereby confirming a degree of progression of the reaction. The Ab-norbornene conjugate was dialyzed three times using a 1× PBS buffer (pH 7.4), and purified using a size exclusion chromatography technique.

[1277] Observation of the binding reaction between trastuzumab and Compound I-SSFI (Lys) according to reaction time is shown in FIG. 40.

[1278] Synthesis method of Trastuzumab-Norbornene (2)

[1279] Introduction reaction using Compound II-SSFI (Lys)

[1280] Synthesis of Ab (246/248 Lys)-norbornene was carried out in a buffer using Compound II-SSFI (Lys). A 1× PBS buffer (0.01% Tween 20, pH 7.4) was used as the reaction buffer. To introduce norbornene at a certain site of an antibody (trastzumab, 4 mg/mL), 8 equivalents of Compound II-SSFI (Lys) was added per one antibody, and reacted at room temperature for 72 hours, and the reaction monitoring and termination were confirmed by HIC-HPLC. As the binding of norbornene to the antibody proceeds, the peaks on the chromatogram were observed to shift from 4 minutes to 4.5 minutes, thereby confirming a degree of progression of the reaction. The Ab-norbornene conjugate was dialyzed three times using a 1× PBS buffer (pH 7.4), and purified using a size exclusion chromatography technique.

[1281] Observation of the binding reaction between trastuzumab and Compound II-SSFI (Lys) according to reaction time is shown in FIG. 41.

[1282] Synthesis method of Trastuzumab-Norbornene (3)

[1283] Introduction reaction using Compound III-SSFI (Dap, Dab, Orn, or Lys)

[1284] Synthesis of Ab (246/248 Lys)-norbornene was carried out in a buffer using Compound III-SSFI (Lys). A 1× PBS buffer (0.01% Tween 20, pH 7.4) was used as the reaction buffer. To introduce norbornene at a certain site of an antibody (Herceptin or trastzumab, 4 mg/mL), 10 equivalents of Compound III-SSFI (Dap, Dab, Orn, or Lys) was added per one antibody, and reacted at room temperature for 28 hours, and the reaction monitoring and termination were confirmed by HIC-HPLC. As the binding of norbornene to the antibody proceeds, the peaks on the chromatogram were observed to shift from 6.2 minutes to 6.4 to 6.7 minutes, thereby confirming a degree of progression of the reaction. The Ab-norbornene conjugate was dialyzed using a 1× PBS buffer (pH 7.4), and purified using a size exclusion chromatography technique.

[1285] The reaction between Compound III-SSFI (Dap, Dab, Orn, or Lys) and the antibody is shown in FIG. 42, and the structure of the final product (i.e., Ab(246/248)-Norbornene) is shown in FIG. 43.

[1286] Observation of the binding reaction between trastuzumab and Compound III-SSFI (Dap) according to reaction time is shown in FIG. 44.

[1287] Observation of the binding reaction between trastuzumab and Compound III-SSFI (Dab) according to reaction time is shown in FIG. 45.

[1288] Observation of the binding reaction between trastuzumab and Compound III-SSFI (Orn) according to reaction time is shown in FIG. 46.

[1289] Observation of the binding reaction between trastuzumab and Compound III-SSFI (Lys) according to reaction time is shown in FIG. 47.

[1290] 3.2. Synthesis of Antibody-Azide

[1291] Synthesis method of Trastuzumab-Azide (4)

[1292] Introduction reaction using Compound IV-SSFI (Dap)

[1293] Synthesis of Ab (246/248 Lys)-azide was carried out in a buffer using Compound IV-SSFI (Dap). A 1× PBS buffer (0.01% Tween 20, pH 7.4) was used as the reaction buffer. To introduce an azide at a certain site of an antibody (trastzumab, 4 mg/mL), 10 equivalents of Compound IV-SSFI (Dap) was added per one antibody, and reacted at room temperature for 24 hours, and the reaction monitoring and termination were confirmed by HIC-HPLC. As the binding of norbornene to the antibody proceeds, the peaks on the chromatogram were observed to shift from 6 minutes to 6.3 minutes, thereby confirming a degree of progression of the reaction. The Ab-norbornene conjugate was dialyzed three times using a 1× PBS buffer (pH 7.4), and purified using a size exclusion chromatography technique.

[1294] Observation of the binding reaction between trastuzumab and Compound IV-SSFI (Dap) according to reaction time is shown in FIG. 48.

[1295] 3.3. Confirmation of structure and site-specific binding of trastuzumab-norbornene conjugate

[1296] An antibody-norbornene complex in which a norbornene linker was bound to an antibody via Compound III-SSFI (Dap) was confirmed by mass spectrometry. It was determined to which site of the antibody the norbornene was bound via F(ab')2 and Fc/2 after the antibody-norbornene complex was treated with an IdeS enzyme. It was confirmed that the Ides Fc/2-norbornene complex was found to have an "Obs-Theo" value of 2991.68, a value which is an exact match in consideration of the combined value of the mass value of N-glycan, the value of a

loss of one lysine residue, and the value of one norbornene molecule, which corresponded to the molecular weight of 120 Da, (a value of -0.10 was considered to be a system error in the high-mass molecular weight analysis). The observed mass spectrum of the Ides_F(ab')2 was detected, but the mass value of one charge was not shifted due to the complexity of the respective mass spectra, thereby making impossible to obtain the corresponding mass value.

[1297] The spectra of the complex having an increased molecular weight due to the antibody-norbornene binding are shown in FIGS. 49 and 50, and changes in molecular weights due to the norbornene conjugation are as listed in Table 12.

<Table 12> Changes in molecular weights of antibody-norbornene conjugates

Sample		Theoretical mass (peptide full seq.)	Observed mass	Δ mass (Obs-Theo) (continued-)
Trastuzumab	Ides Fc/2	23916.08	25231.10	1315.02
Trastuzumab-norbornene conjugate	Ides Fc/2	23916.08	25353.53	1437.45

(-continued) N-glcan (G0F/G0F)	C-term (-2K)	Norbornene conjugate	Δ mass	
1445.00	128	ND	-1.98	(G0F/-K)
1445.00	128	122	-1.55	(G0F/-K)

[1298] Also, the norbornene binding site of the antibody-norbornene complex was confirmed through comparison of the MS/MS spectrum with trastuzumab. As a result, it was

confirmed that the norbornene molecule was bound to a K248 site of the sequence LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF. The MS/MS chromatograms are shown FIGS. 51 to 54, and the sequence matching results based on the MS/MS spectra are shown in FIG. 55.

[1299] The conditions for the analysis are as follows:

[1300] UPLC conditions

[1301] Measuring equipment: Acquity UPLC I-Class system

[1302] 1. Column: Thermo MAbPac™ RP (2.1 mm × 5 mm)

[1303] 2. Column Temp.: 60 °C

[1304] 3. Mobile phase

[1305] A. 0.1% Formic acid in water

[1306] B. 0.1% Formic acid in acetonitrile

[1307] Mass spectroscopy conditions

[1308] Measuring equipment: LTQ Elite (Thermo)

[1309] Source type: HESI

[1310] Capillary Temp.: 320 °C

[1311] Source heater temp.: 300 °C

[1312] Sheath gas flow: 40.00

[1313] Aux gas flow: 20.00

[1314] Sweep gas flow: 5.00

[1315] Source voltage (KV): 4.00

[1316] FTMS resolution: 120,000

[1317] FTMS mass range: 400 to 4,000

[1318] 3.4. Confirmation of structure of Trastuzumab-Azide

[1319] The antibody-azide complex in which an azide linker was bound to an antibody via Compound IV-SSFI (Dap) was confirmed using mass spectrometry. A molecular weight increase of 249 Da was confirmed when two molecules of an azide compound were bound to trastuzumab. The measured mass spectrum of trastuzumab is shown in FIG. 56, and the mass spectrum of the antibody-azide complex to which two azide molecules were bound is shown in FIG. 57.

[1320] Measuring equipment: Ultraflex III (TOF/TOF)

[1321] Analysis mode: Linear mode

[1322] Polarity: Positive

[1323] Detection: m/z 2,000 to 300,000

[1324] Laser repetition rate: 100 Hz

[1325] Number of shots: 1,000 shots

[1326] Deflection: On, 5,000 Da

[1327] Voltage: Ion Source kV, Ion Source II 23.00 kV, Lens 9.00 kV

[1328] Calculated molecular weight: 148,271 g/mol

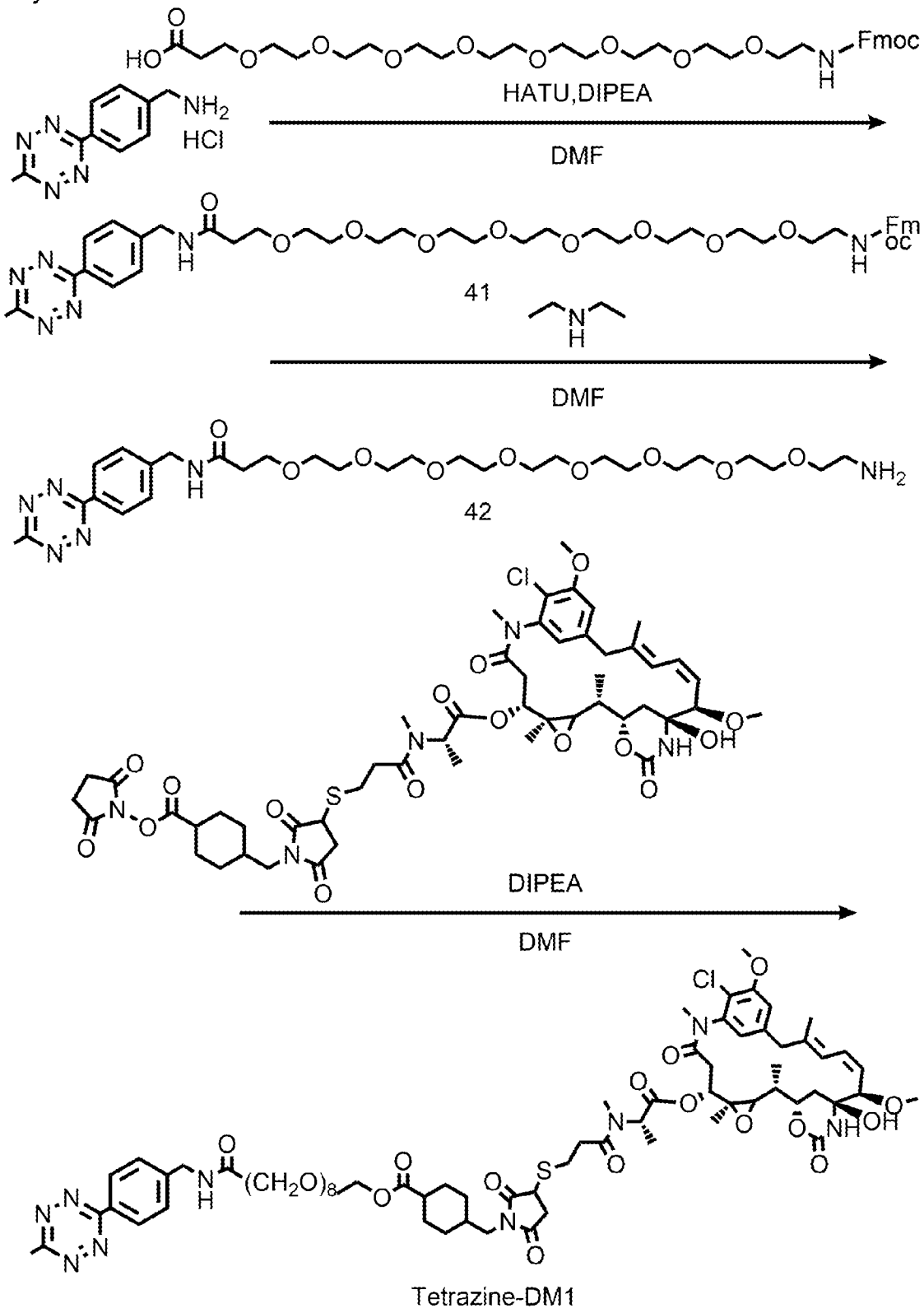
[1329] Measured molecular weight: 148,262 g/mol

[1330] 4. Synthesis and confirmation of structure of antibody-payload conjugate

[1331] 4.1. Synthesis method and confirmation of structure of payload

[1332] [Scheme 15]

Synthesis of Tetrazine-DM1



Tetrazine-DM1

[1333] Synthesis method of Tetrazine-DM1

[1334] Synthesis of Compound 41

[1335] 0.53 g (0.8 mmol, 1.0 eq.) of Fmoc-PEG_8-OH was dissolved in 7 mL of DMF, and then stirred. Thereafter, 0.38 g (1.0 mmol, 1.25 eq.) of HATU and 0.3 mL (1.7 mmol, 2.1 eq.) of DIPEA were added dropwise thereto. 0.205 g (0.86 mmol, 1.1 eq.) of methyltetrazine-amine·HCl was added dropwise, and 0.3 mL (1.7 mmol, 2.1 eq.) of DIPEA was added dropwise thereto. The resulting mixture was stirred for 4 hours, and then concentrated under reduced pressure to remove the solvent. The mixture was purified by column chromatography (5% MeOH in DCM) to obtain a target compound. TLC (DCM:MeOH = 20:1); R_f = 0.3.

[1336] Synthesis of Compound 42

[1337] 0.527 g (0.62 mmol, 1.0 eq.) of Compound 41 was dissolved in 6 mL of DMF, and 0.3 mL of diethylamine was added dropwise thereto. The resulting mixture was stirred for 2.5 hours, and then concentrated under reduced pressure to remove the solvent. The mixture was purified by column chromatography (DCM:MeOH:NH_4OH= 80:25:2.5) to obtain a target compound, the amount of which was confirmed to be 0.273 g (yield: 70%). TLC (DCM:MeOH:NH_4OH = 80:25:2.5); R_f = 0.1.

[1338] Synthesis of Tetrazine-DM1

[1339] 0.355 g (0.57 mmol, 1.5 eq.) of Compound 42 was dissolved in 25 mL of DMF, and then stirred. 0.405 g (0.38 mmol, 1.0 eq.) of DM1-SMCC-NHS was added thereto, and DIPEA was added dropwise until the pH of the mixture reached pH 9.0. Thereafter, the mixture was stirred for 2 hours. The reaction solution was concentrated under reduced pressure, and then purified by column chromatography (10% MeOH in DCM) to obtain a target compound, the amount of which was confirmed to be 0.434 g (yield: 73%). TLC (DCM:MeOH = 10:1); R_f = 0.5.

[1340] Confirmation of structure of Tetrazine-DM1

[1341] LRMS (ESI): m/z 782.6 [$M+2H^+$]

[1342] The results are shown in FIG. 58.

[1343] 4.2. Synthesis and confirmation of structure of antibody-payload conjugate

[1344] 4.2.1. Antibody-Norbornene-Tetrazine-DM1

[1345] Synthesis method of Trastuzumab-Norbornene-Tetrazine-DM1 (1)

[1346] Introduction reaction of Tetrazine-DM1 into Trastuzumab-Norbornene using Compound III-SSFI (Dap)

[1347] An antibody-payload conjugate was constructed using trastuzumab into which two norbornene molecules were introduced via Compound III-SSFI (Dap). A reaction was carried

out using 25 mL of the antibody-payload conjugate at a concentration of 4.5 mg/mL, and conjugation of a tetrazine-PEG8-DM1 drug was attempted for a biorthogonal reaction (i.e., biorthogonal chemistry) with norbornene conjugated to the antibody. 40 Equivalents of the drug was used per one antibody, and the conjugation reaction was performed at room temperature for 24 hours in a 20 mM histidine acetate solution (pH 5.5). The conjugation reaction was monitored by HIC-HPLC, and it was observed that the peaks appearing at 7.7 minutes after only a norbornene molecule was bound to trastuzumab were shifted to 8.7 minutes (DAR 1) and 10.5 minutes (DAR 2) as the tetrazine-PEG8-DM1 drug reacted with the antibody-norbornene linker. As a result, formation of the antibody-payload conjugate was observed.

[1348] The structure of the product 'antibody-payload conjugate' is shown in FIG. 59, and the reaction monitoring by HIC-HPLC is shown in FIG. 60 (Table 13).

<Table 13>

Reaction monitoring by HIC-HPLC for formation of antibody-payload conjugate

Retention time	Composition
6.6 min	Trastuzumab
7.7 min	Trastuzumab-norbornene conjugate
8.7 min	Trastuzumab-DM1 conjugate (1 site, DAR1)
10.5 min	Trastuzumab-DM1 conjugate (2 site, DAR2)

[1349] Confirmation of Trastuzumab-Norbornene-Tetrazine-DM1 structure (2)

[1350] An antibody-drug conjugate in which tetrazine-DM1 was bound to Trastuzumab-Norbornene was confirmed by mass spectrometry. It was determined to which site of the antibody the tetrazine-DM1 was bound via F(ab')2 and Fc/2 after the antibody-drug conjugate was treated with an IdeS enzyme. It was confirmed that the Ides Fc/2-DM1 complex was found to have an "Obs-Theo" value of 1673.5, indicating that the drug was bound to the conjugate in consideration of the combined value of one tetrazine-DM1 molecule, which corresponded to the molecular weight of 1,688.80 Da (a dehydration reaction was observed in the molecular weight analysis of the DM1-series drug). The observed mass spectrum of the Ides_F(ab')2 was detected, but the mass value of one charge was not shifted due to the complexity of the respective mass spectra, thereby making impossible to obtain the corresponding mass value.

[1351] The spectra of the complex having an increased molecular weight due to the trastuzumab-norbornene-tetrazine-DM1 binding are shown in FIGS. 61 and 62.

[1352] UPLC conditions

[1353] Measuring equipment: Acquity UPLC I-Class system

[1354] 1. Column: Thermo MAbPac™ RP (2.1 mm × 5 mm)

[1355] 2. Column Temp: 60 °C

[1356] 3. Mobile phase

[1357] A. 0.1% Formic acid in water

[1358] B. 0.1% Formic acid in acetonitrile

[1359] Mass spectroscopy conditions

[1360] Measuring equipment: LTQ Elite (Thermo)

[1361] Source type: HESI

[1362] Capillary Temp.: 320 °C

[1363] Source heater temp.: 300 °C

[1364] Sheath gas flow: 40.00

[1365] Aux gas flow: 20.00

[1366] Sweep gas flow: 5.00

[1367] Source voltage (KV): 4.00

[1368] FTMS resolution: 120,000

[1369] FTMS mass range: 400 to 4,000

[1370] 5. FcRn binding analysis of antibody-drug conjugate

[1371] The antibody-drug conjugate constructed in this patent was named 'AbClick®Pro.'

[1372] To check whether or not the antibody-drug conjugate (see FIG. 59; AbClick®Pro (NC, DM1)) had an antibody recycling function, the binding affinity of the conjugate for a neonatal Fc receptor (FcRn), which is closely associated with the *in vivo* antibody recycling, was analyzed. As one of the biomolecular interaction systems, Octet (model: Octet QK384) was used to determine the reaction affinity of FcRn for the constructed ADC. As a difference in reflection of light caused by a surface change, which occurs according to a degree of reaction between molecules attached to a surface of a sensor and molecules in a sample, was recognized as being free of a label, kinetic analysis was performed. Trastuzumab, Kadcyla, and AbClick®Pro (NC, DM1) were used as the samples to measure binding affinities for FcRn under a condition of pH 6.0. As a result, it was confirmed that the measured KD values were 1.12×10^{-7} M, 1.12×10^{-7} M, and 8.84×10^{-8} M, respectively, indicating that AbClick®Pro had a similar or superior binding affinity, compared to Kadcyla (Table 14).

<Table 14> FcRn binding analysis of antibody-drug conjugate

pH 6.0	FcRn binding		
	KD_Kinetics (M)	Ka (1/Ms)	Kd (1/S)
Trastuzumab	1.12E-7	3.31E+05	3.69E-02
Kadcyla	1.12E-7	2.10E+05	2.37E-02
*AbClick® Pro	8.84E-8	2.26E+05	1.99E-02

[1373] 6. Antigen binding analysis of antibody-drug conjugate

[1374] It was determined through enzyme-linked immunosorbent assay (ELISA) analysis whether or not a Her2 protein was bound to the antibody-drug conjugate. The antibody-norbornene complex constructed using trastuzumab and Compound III-SSFI (Dap) was used as the antibody-drug conjugate for measurement of antigen binding affinity. Structures of three drugs to be bound to the antibody-norbornene complex are shown in FIG. 63.

[1375] The dissociation constants (Kd) of Herceptin (trastuzumab), Kadcyla, AbClick®Pro (NC, DM1), AbClick®Pro (VC, DM1), and AbClick®Pro (VC, MMAE) from the antigen were confirmed to be approximately 98.6 pM, approximately 94.8 pM, approximately 121.4 pM, approximately 137.0 pM, and approximately 117.3 pM, respectively. Based on the results, it was confirmed that the binding site of the drug did not affect the binding affinity of the constructed ADC for the antigen.

[1376] A graph of measuring the antigen binding affinities of the antibody-drug conjugates is shown in FIG. 64 (Table 15).

<Table 15> Antigen binding analysis of antibody-drug conjugate

Antibody	K_D Value (binding affinity, pM)
Herceptin	98.6
Kadcyla	94.7
AbClick®Pro (NC,DM1)	121.4
AbClick®Pro (VC,DM1)	137.0
AbClick®Pro (VC,MMAE)	117.3

[1377] 7. Confirmation of serum stability of antibody-drug conjugate

[1378] It was determined through enzyme-linked immunosorbent assay (ELISA) analysis whether or not the antibody-drug conjugate was stable in serum. The stabilities of the antibody-drug conjugates in rat, rabbit and human sera were tested at room temperature. It was confirmed that a pattern of an intact ADC (i.e., drug-bound ADC) residual level of the self-constructed ADC "AbClick®Pro (NC, DM1)" over time was consistent with that of the Kadcyla® group.

[1379] The results of serum stability analysis of the antibody-drug conjugate are shown in FIGS. 65 to 67.

[1380] 8. Evaluation of medicinal effect of antibody-drug conjugate (AbClick®Pro) at cellular level (*in vitro* cytotoxicity test)

[1381] Medicinal effects of the three antibody-drug conjugates (ADC, AbClick®Pro series) using trastuzumab were evaluated according to a level of a target marker in cancer cells, and a cytotoxicity test was performed using NCI-N87 and MDA-MB-468 as a positive cell line expressing the target antigen (Her2) 'cell line' and a negative cell line expressing BT474, respectively. The three antibody-drug conjugates (AbClick®Pro (NC, DM1), AbClick®Pro (VC, DM1), and AbClick®Pro (VC, MMAE)) constructed in the Her2-overexpressing cells NCI-N87 were observed after the cells were treated with different concentrations of the antibody-drug conjugates. As a result, it was confirmed that the antibody-drug conjugates had IC50 values of 207.7 ng/mL, 93.9 ng/mL, and 57.7 ng/mL, indicating that the antibody-drug conjugates had an excellent anti-cancer effect. The three antibody-drug conjugates (AbClick®Pro (NC, DM1), AbClick®Pro (VC, DM1), and AbClick®Pro (VC, MMAE)) constructed in another Her2-overexpressing cell line BT474 were

observed after the cells were treated with different concentrations of the antibody-drug conjugates. As a result, it was confirmed that the antibody-drug conjugates had IC50 values of 47.7 ng/mL, 44.6 ng/mL, and 1.09 ng/mL, indicating that the antibody-drug conjugates had an excellent anti-cancer effect. The utility of the AbClick®Pro (VC, MMAE) as the novel ADC was confirmed because the AbClick®Pro (VC, MMAE) was observed to have an equal or superior apoptotic effect, compared to Kadcyla as the commercially available Herceptin ADC.

[1382] The test results are shown in FIGS. 68 to 70.

[1383] 9. Evaluation of medicinal effect of antibody-drug conjugate (AbClick®Pro) at animal level (*in vitro* cytotoxicity test)

[1384] A target antigen-overexpressing NCI-N87 gastric cancer cell line was subcutaneously transplanted into mice to establish a xenograft model, and the mice were divided into 4 groups to perform an anti-cancer efficacy test on the materials administered. Because the BALB/c nude mice used in this test were deficient in T cells, cancer cells were easily transplanted into the BALB/c nude mice. Therefore, the BALB/c nude mice were used as a model suitable for an anti-cancer efficacy test using a rodent.

[1385] (a) Preparation of cell line

[1386] One vial of a human tumor cell line (NCI-N87 cell line) was put into a cell culture flask containing an RPMI1640 medium (Gibco, 22400-089) supplemented with heat-inactivated 10% fetal bovine serum (FBS; Gibco, 10082-742), and cultured at 37°C in a 5% CO_2 incubator. The cell culture medium was washed with PBS, and diluted 10-fold with 2.5% trypsin-EDTA (Gibco, 15090). Thereafter, the diluted cell culture medium was added to separate the cells, and the cells were centrifuged (at 1,000 rpm for 5 minutes) to discard a supernatant. A fresh medium was added to the cell pellets to obtain a cell suspension. The viability of the cells was confirmed using a microscope, and the cells were diluted to a concentration of 1.25×10^7 cells/mL with a solution obtained by mixing a medium and Matrigel at a mixing ratio of 1:1 to prepare a cell line.

[1387] (b) Transplantation of cell line

[1388] A cell line was prepared using a method described in the section "4. 3) (4) Preparation of cell line." The cells were re-suspended and homogenized to prepare a cell line, and the prepared cell line was immediately administered to animals. For transplantation of the cell line, the back region of the animal was sterilized with 70% alcohol, the dorsal skin was pulled with the thumb and forefinger to form a space between the skin and muscles. Thereafter, a syringe with a 26 gauge needle was stuck in a subcutaneous pocket between the thumb and forefinger, and the cell line was then subcutaneously administered at a dose of 2.5×10^6 cells/0.2 mL/head from the front of the animal. During an acclimation period, healthy animals were

selected, and subjected to cell line inoculation. When the size of a tumor in the cell line-transplanted site reached approximately 100 to 150 mm^3, the mice were divided according to the size of a ranked tumor so that the tumor sizes in each group were distributed as uniformly as possible.

[1389] (c) Configuration of experimental groups and determination of dose and administration method

[1390] Cell line = NCI-N87

[1391] Mouse type = BALB/c nude (CAnN.Cg-Foxn1nu/CrljOri)

[1392] Number per group = 5

[1393] Administration method = Intravenous injection (using a 26-gauge needle syringe)

[1394] Dose = 5 mg/kg

[1395] Number of administration = Once/ 3 day, administered three times

[1396] Observation period = 5 weeks

[1397] Group 1: PBS; Group 2: Herceptin (trastuzumab); Group 3: Kadcyla; Group 4 : AbClick®Pro(NC, DM1); Group 5 : AbClick®Pro(VC, DM1); and Group 6: AbClick®Pro(VC, MMAE)

[1398] (d) Observation and inspection items

[1399] General symptoms

[1400] During the administration and observation periods, the type of general symptoms (including death), the symptom onset date, and the severity of symptoms were observed once a day, and recorded for each individual. The individuals whose general symptoms worsened were quarantined.

[1401] Body weight

[1402] The body weight was measured on the grouping date or the start date for administration of the test substance, and then measured twice a week.

[1403] Measurement of tumor size

[1404] The tumor size was measured twice a week for 5 weeks from the start date for administration of the test substance. The major- and minor-axis lengths of a tumor were measured using calipers, and the tumor size was calculated according to the following equation.

[1405] Tumor size = $ab^2/2$ (a: a major-axis length; and b: a minor-axis length)

[1406] (e) Results

[1407] The mice in the groups into which phosphate buffered saline (PBS) and Herceptin (trastuzumab) were injected did not show a tendency to suppress the growth of tumor for an observation period of 5 weeks. It was confirmed that the AbClick®Pro-series antibody-drug

conjugates constructed according to the present invention has a superior ability to suppress the growth of tumor compared to Herceptin, indicating that the antibody-drug conjugates according to the present invention successfully function as the ADC.

[1408] The related results are shown in FIGS. 71 and 72.

[1409] 10. Pharmacokinetics (PK) test of antibody-drug conjugate

[1410] To analyze the pharmacokinetic characteristics of the antibody-drug conjugate (ADC) manufactured using the AbClick®Pro, an enzyme-linked immunosorbent assay (ELISA) was performed to confirm the pharmacokinetics of the antibody-drug conjugate. The manufactured ADC had an AbClick®Pro (NC, DM1) structure as shown in FIG. 59, and comparative validation with Kadcyla was carried out. Rats (n = 3) were used for an animal model. In this case, the rats were intravenously injected with 5 mg/kg of ADC, and then monitored over time. It was confirmed that the intact ADC form (an intact ADC to which a drug was bound) of the administered AbClick®Pro (NC, DM1) was reduced over time. It was judged that the intact ADC had similar or improved drug characteristics, compared to Kadcyla.

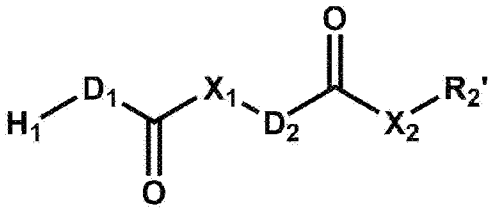
[1411] The results are shown in FIG. 73.

[1412] This application contains references to amino acid sequences and/or nucleic acid sequences which have been submitted concurrently herewith as the sequence listing entitled "000072uscoa_SequenceListing.XML", file size 62 kilobytes (KB), created on 16 January 2023. The aforementioned sequence listing is hereby incorporated by reference in its entirety pursuant to 37 C.F.R. §1.52(e)(5).

WHAT IS CLAIMED IS:

1. A compound of Formula 2:

[formula 2]



wherein,

H_1 is a first click chemistry functional group,

D_1 is selected from a covalent bond, $C_{1\sim4}$alkylene, $C_{2\sim4}$alkenylene, $C_{2\sim4}$alkynlene, and $C_{3\sim8}$cycloalkylene,

X_1 is S,

D_2 is selected from $C_{1\sim7}$alkylene, $C_{2\sim7}$alkenylene, $C_{2\sim7}$alkynylene, and $C_{3\sim8}$cycloalkynlene,

X_2 is O, R_2' is N-succinimide, p-nitrophenyl, or pentafluorophenyl.

2. A compound according to claim 1,

wherein H_1 is selected from terminal alkyne, azide, strained alkyne, diene, dienophile, alkene, thiol, and tetrazine.

3. A compound according to claim 2,

wherein H_1 is selected from norbornene, tetrazine, azide and dibenzocyclooctyne-amine.

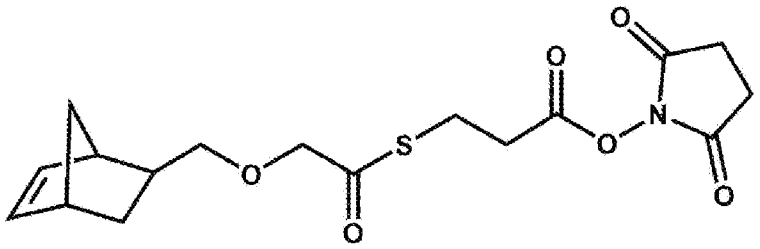
4. A compound according to claim 1,

wherein R_2' is N-succinimide.

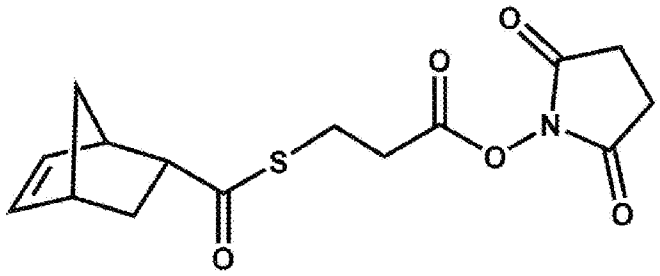
5. A compound according to claim 1,

wherein the formula 2 is formula 2-1, 2-2, or 2-3:

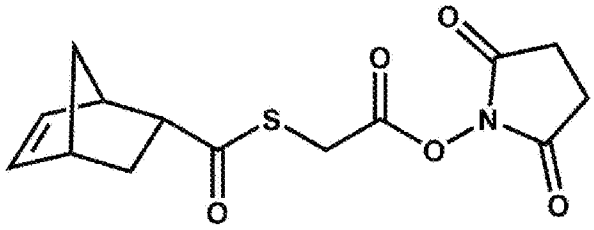
[formula 2-1]



[formula 2-2]



[formula 2-3]



6. A peptide of formula 4-2:

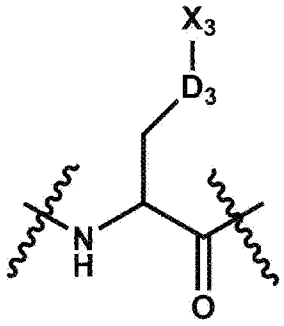
[formula 4-2]

$$(Xaa)_{1\text{-}3}\text{-}C\text{-}(Xaa)_2\text{-}H\text{-}Xa_1\text{-}G\text{-}Xa_2\text{-}L\text{-}V\text{-}Xa_3\text{-}C\text{-}(Xaa)_{1\text{-}3}$$

wherein,

each Xaa is independently any amino acid residue that is not a cysteine residue,

C is a cysteine residue, H is a histidine residue, G is a glycine residue, Xa_2 is a glutamic acid residue or an asparagine residue, L is a leucine residue, V is a valine residue, Xa_3 is selected from a tryptophan residue, a naphthylalanine residue, and a phenylalanine residue, and

Xa_1 is  , wherein D_3 is a covalent bond or $C_{1\sim3}$alkylene and X_3 is NH_2,

wherein the peptide consists of 13 to 17 amino acid residues,

wherein the peptide exhibits the activity of binding to human immunoglobulin G (IgG),

and wherein the cysteine residue that is between two to four amino acids from the N-terminus of formula 4-2 and the cysteine residue that is between two to four amino acids from the C-terminus of formula 4-2 are optionally linked.

7. A peptide according to claim 6,

wherein D_3 is a covalent bond, methylene, or ethylene.

8. A peptide according to claim 6,

wherein the formula 4-2 is formula 4-6:

[formula 4-6]

D-C-A-W-H-Xa_1-G-E-L-V-W-C-T

wherein,

D is an aspartic acid residue, A is an alanine residue, E is a glutamic acid residue, W is a tryptophan residue, and T is a threonine residue.

9. A peptide-compound conjugate of formula 6-2:

[formula 6-2]

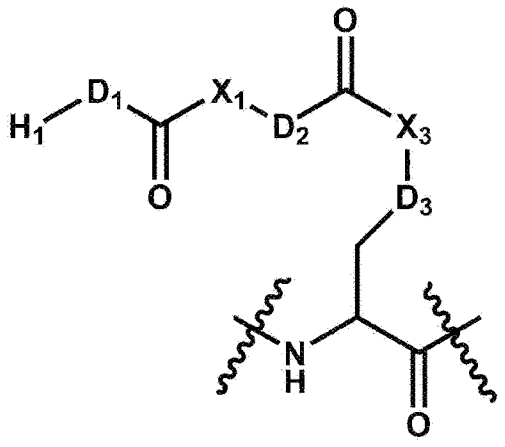
$(Xaa)_{1-3}$-C-$(Xaa)_2$-H-$(Xa_1)'$-G-Xa_2-L-V-Xa_3-C-$(Xaa)_{1-3}$

wherein,

each Xaa is independently any amino acid residue that is not a cysteine residue,

C is a cysteine residue, H is a histidine residue, G is a glycine residue, Xa_2 is a glutamic acid residue or an asparagine residue, L is a leucine residue, V is a valine residue, Xa_3 is selected from a tryptophan residue, a naphthylalanine residue, and a phenylalanine residue,

and

$(Xa_1)'$ is  , wherein

H_1 is a first click chemistry functional group,

D_1 is selected from a covalent bond, $C_{1\sim4}$alkylene, $C_{2\sim4}$alkenylene, $C_{2\sim4}$alkynylene, and $C_{3\sim8}$cycloalkynlene,

X_1 is S, D_2 is selected from $C_{1\sim7}$alkylene, $C_{2\sim7}$alkenylene, $C_{2\sim7}$alkynylene, and $C_{3\sim8}$cycloalkynlene, D_3 is a covalent bond, or $C_{1\sim3}$alkylene, and X_3 is NH,

wherein the peptide consists of 13 to 17 amino acid residues,

wherein the peptide exhibits the activity of binding to human immunoglobulin G (IgG),

and wherein the cysteine residue that is between two to four amino acids from the N-terminus of formula 6-2 and the cysteine residue that is between two to four amino acids from the C-terminus of formula 6-2 are optionally linked.

10. A peptide-compound conjugate according to claim 9,

wherein a distance from a beta carbon of the $(Xa_1)'$ to a first carbonyl carbon of the $(Xa_1)'$ is less than approx. 11.668Å.

11. A peptide-compound conjugate according to claim 9,

wherein D_2 is $C_y$alkylene, $C_y$alkenylene, or $C_y$alkynylene,

D_3 is $C_x$alkylene,

wherein y is an integer greater than or equal to 1, and $1 \leq x+y \leq 5$.

12. A peptide-compound conjugate according to claim 9,

wherein a distance from a beta carbon of the $(Xa_1)'$ to a first carbonyl carbon of the $(Xa_1)'$ is greater than approx. 16.208Å.

13. A peptide-compound conjugate according to claim 9,
wherein D_2 is $C_y$alkylene, $C_y$alkenylene, or $C_y$alkynylene,
and D_3 is $C_x$alkylene,
wherein y is an integer greater than or equal to 1, and $9 \leq x+y \leq 12$.

14. A peptide-compound conjugate according to claim 13,
wherein D_2 is $C_y$alkenylene, or $C_y$alkynylene.

15. A peptide-compound conjugate according to claim 9,
a distance from a beta carbon of the $(Xa_1)'$ to a first carbonyl carbon of the $(Xa_1)'$ is approx. 11.668Å to approx. 16.208Å.

16. A peptide-compound conjugate according to claim 9,
wherein D_2 is $C_y$alkylene, $C_y$alkenylene, or $C_y$alkynylene,
and D_3 is $C_x$alkylene,
wherein y is an integer greater than or equal to 1, and $6 \leq x+y \leq 8$.

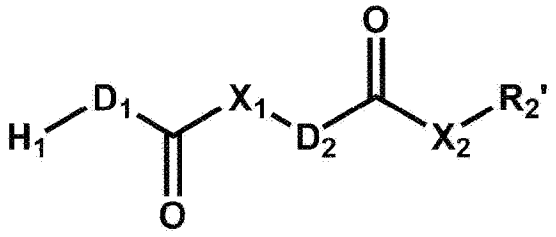
17. A peptide-compound conjugate according to claim 9,
wherein the formula 6-2 is formula 6-3:
[formula 6-3]

D-C-A-W-H-$(Xa_1)'$-G-E-L-V-W-C-T

wherein,
D is an aspartic acid residue, A is an alanine residue, E is a glutamic acid residue, W is a tryptophan residue, T is a threonine residue.

18. A peptide-compound conjugate according to claim 9,
wherein D_1 is a covalent bond and D_2 is methylene.

19. A method for preparing an agent for transferring a first click chemical functional group to an antibodycomprising
reacting a compound of formula 2:
[formula 2]



wherein,

H_1 is a first click chemistry functional group,

D_1 is selected from a covalent bond, $C_{1\sim4}$alkylene, $C_{2\sim4}$alkenylene, $C_{2\sim4}$alkynylene, and $C_{3\sim8}$cycloalkynlene,

X_1 is S, D_2 is selected from $C_{1\sim7}$alkylene, $C_{2\sim7}$alkenylene, $C_{2\sim7}$alkynylene, and $C_{3\sim8}$cycloalkynlene,

X_2 is O, R_2' is N-succinimide, p-nitrophenyl, or pentafluorophenyl, with a peptide of formula 4-2:

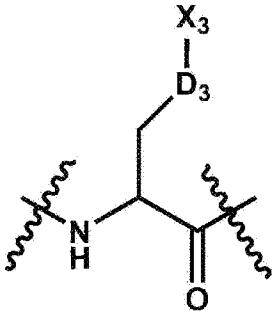
[formula 4-2]

$$\mathbf{(Xaa)_{1-3}\text{-}C\text{-}(Xaa)_2\text{-}H\text{-}Xa_1\text{-}G\text{-}Xa_2\text{-}L\text{-}V\text{-}Xa_3\text{-}C\text{-}(Xaa)_{1-3}}$$

wherein,

each Xaa is independently any amino acid residue that is not a cysteine residue,

C is a cysteine residue, H is a histidine residue, G is a glycine residue, Xa_2 is a glutamic acid residue or an asparagine residue, L is a leucine residue, V is a valine residue, Xa_3 is selected from a tryptophan residue, a naphthylalanine residue, and a phenylalanine residue, and

Xa_1 is  , wherein D_3 is a covalent bond or $C_{1\sim3}$alkylene and X_3 is NH_2,

wherein the peptide consists of 13 to 17 amino acid residues,

wherein the peptide exhibits the activity of binding to human immunoglobulin G (IgG),

and wherein the cysteine residue that is between two to four amino acids from the N-terminus of formula 4-2 and the cysteine residue that is between two to four amino acids from the C-terminus of formula 4-2 are optionally linked.

20. A method for preparing an agent for transferring a first click chemical functional group to an antibodyaccording to claim 19,

wherein D_2 is $C_y$alkylene, $C_y$alkenylene, or $C_y$alkynylene,

and D_3 is $C_x$alkylene,

wherein y is an integer greater than or equal to 1, and $1 \leq x+y \leq 5$,

characterized in that the agent for transferring a first click chemical functional group to an antibodyprepared by the method can react with an antibody to deliver the first click chemistry functional group specifically to a 248 a lysine residue of an Fc domain of the antibody.

21. A method for preparing an agent for transferring a first click chemical functional group to an antibodyaccording to claim 19,

wherein D_2 is $C_y$alkylene, $C_y$alkenylene, or $C_y$alkynylene,

and D_3 is $C_x$alkylene,

wherein y is an integer greater than or equal to 1, and $9 \leq x+y \leq 12$,

characterized in that the agent for transferring a first click chemical functional group to an antibodyprepared by the method can react with an antibody to deliver the first click chemistry functional group specifically to a 246 a lysine residue of an Fc domain of the antibody.

22. A method for preparing an agent for transferring a first click chemical functional group to an antibodyaccording to claim 19,

wherein D_2 is $C_y$alkylene, $C_y$alkenylene, or $C_y$alkynylene,

and D_3 is $C_x$alkylene,

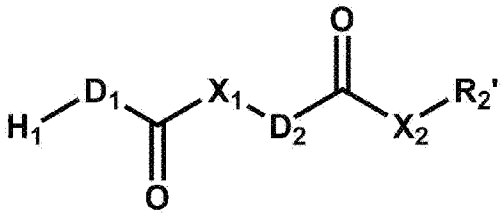
wherein y is an integer greater than or equal to 1, and $6 \leq x+y \leq 8$,

characterized in that the agent for transferring a first click chemical functional group to an antibodyprepared by the method can react with an antibody to deliver the first click chemistry functional group selectively to a 246 a lysine residue or a 248 a lysine residue of an Fc domain of the antibody.

23. A kit for preparing a first click chemistry functional group transferring to an antibody comprising

a compound of formula 2:

[formula 2]



wherein,

H_1 is a first click chemistry functional group,

D_1 is selected from a covalent bond, $C_{1\sim4}$alkylene, $C_{2\sim4}$alkenylene, $C_{2\sim4}$alkynylene, and $C_{3\sim8}$cycloalkynlene,

X_1 is S,

D_2 is selected from $C_{1\sim7}$alkylene, $C_{2\sim7}$alkenylene, $C_{2\sim7}$alkynylene, and $C_{3\sim8}$cycloalkynlene,

X_2 is O, R_2' is N-succinimide, p-nitrophenyl, or pentafluorophenyl; and

a peptide of formula 4-2:

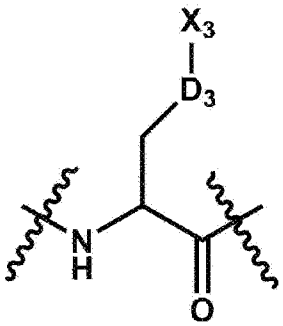
[formula 4-2]

$$\mathbf{(Xaa)_{1-3}\text{-}C\text{-}(Xaa)_2\text{-}H\text{-}Xa_1\text{-}G\text{-}Xa_2\text{-}L\text{-}V\text{-}Xa_3\text{-}C\text{-}(Xaa)_{1-3}}$$

wherein,

each Xaa is independently any amino acid residue that is not a cysteine residue,

C is a cysteine residue, H is a histidine residue, G is a glycine residue, Xa_2 is a glutamic acid residue or an asparagine residue, L is a leucine residue, V is a valine residue, Xa_3 is selected from a tryptophan residue, a naphthylalanine residue, and a phenylalanine residue, and

Xa_1 is  , wherein D_3 is a covalent bond or $C_{1\sim3}$alkylene and X_3 is NH_2,

wherein the peptide consists of 13 to 17 amino acid residues,

wherein the peptide exhibits the activity of binding to human immunoglobulin G (IgG),

and wherein the cysteine residue that is between two to four amino acids from the N-terminus of formula 4-2 and the cysteine residue that is between two to four amino acids from

the C-terminus of formula 4-2 are optionally linked.

24. An antibody or a fragment thereof comprising one or more amino acid sequence selected from formula 8-1, formula 8-2, and formula 8-3:

[formula 8-1]

G—P—S—V—F—L—F—P—P-(K)'-P—K—D—T—L—M—I

[formula 8-2]

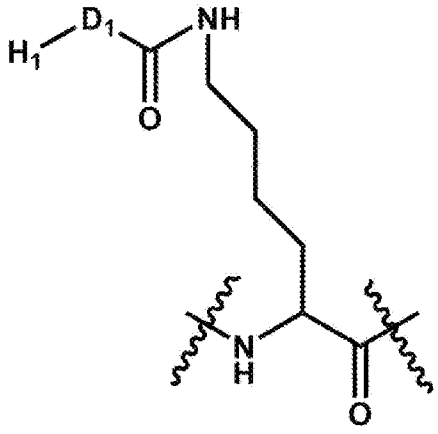
G—P—S—V—F—L—F—P—P—K—P-(K)'-D—T—L—M—I

[formula 8-3]

G—P—S—V—F—L—F—P—P-(K)'-P-(K)'-D—T—L—M—I

wherein,

G is a glycine residue, P is a proline residue, S is a serine residue, V is a valine residue, F is a phenylalanine residue, L is a leucine residue, K is a lysine residue, D is an aspartic acid residue, T is a threonine residue, M is a methionine residue, I is an isoleucine residue, and

(K)' is 

, wherein

H_1 is a first click chemistry functional group,

D_1 is selected from a covalent bond, $C_{1\sim4}$alkylene, $C_{2\sim4}$alkenylene, $C_{2\sim4}$alkynylene, and $C_{3\sim8}$cycloalkynlene.

25. An antibody or a fragment thereof according to claim 24, wherein D_1 is a covalent bond.

26. An antibody or a fragment thereof according to claim 24, comprising the amino acid sequence of formula 8-1 and not comprising the amino acid sequence of formula 8-2 and 8-3.

27. An antibody or a fragment thereof according to claim 26, comprising the amino acid sequence of formula 8-1 in both two Fc domains.

28. An antibody or a fragment thereof according to claim 24, comprising the amino acid sequence of formula 8-2 and not comprising the amino acid sequence of formula 8-1 and 8-3.

29. An antibody or a fragment thereof according to claim 28, comprising the amino acid sequence of formula 8-2 in both two Fc domains.

30. An antibody or a fragment thereof according to claim 24, comprising the amino acid sequence of formula 8-3 and not comprising the amino acid sequence of formula 8-1 and 8-2.

31. An antibody or a fragment thereof according to claim 30, comprising the amino acid sequence of formula 8-3 in both two Fc domains.

32. A method for preparing an antibody or a fragment thereof comprising first click chemistry functional group comprising

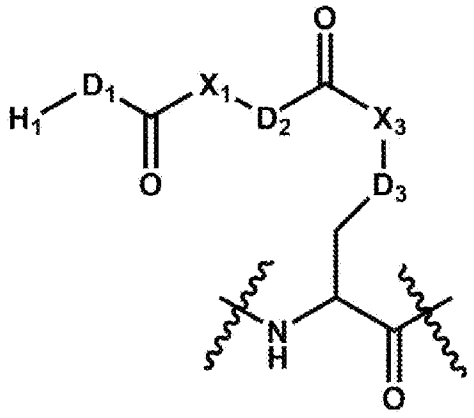
reacting a peptide-compound conjugate of formula 6-2:

[formula 6-2]

$$(Xaa)_{1-3}\text{-}C\text{-}(Xaa)_2\text{-}H\text{-}(Xa_1)'\text{-}G\text{-}Xa_2\text{-}L\text{-}V\text{-}Xa_3\text{-}C\text{-}(Xaa)_{1-3}$$

wherein,

each Xaa is independently any amino acid residue that is not a cysteine residue, C is a cysteine residue, H is a histidine residue, G is a glycine residue, Xa_2 is a glutamic acid residue or an asparagine residue, L is a leucine residue, V is a valine residue, Xa_3 is selected from a tryptophan residue, a naphthylalanine residue, and a phenylalanine residue, and

$(Xa_1)'$ is  , wherein

H_1 is a first click chemistry functional group,

D_1 is selected from a covalent bond, $C_{1\sim4}$alkylene, $C_{2\sim4}$alkenylene, $C_{2\sim4}$alkynylene, and $C_{3\sim8}$cycloalkynlene,

X_1 is S,

D_2 is selected from $C_{1\sim7}$alkylene, $C_{2\sim7}$alkenylene, $C_{2\sim7}$alkynylene, and $C_{3\sim8}$cycloalkynlene,

D_3 is a covalent bond or $C_{1\sim3}$alkylene and X_3 is NH,

wherein the peptide consists of 13 to 17 amino acid residues,

wherein the peptide exhibits the activity of binding to human immunoglobulin G (IgG),

and wherein the cysteine residue that is between two to four amino acids from the N-terminus of formula 6-2 and the cysteine residue that is between two to four amino acids from the C-terminus of formula 6-2 are optionally linked, with

an antibody or a fragment thereof.

33. A kit for preparing an antibody or a fragment thereof comprising first click chemistry functional group comprising

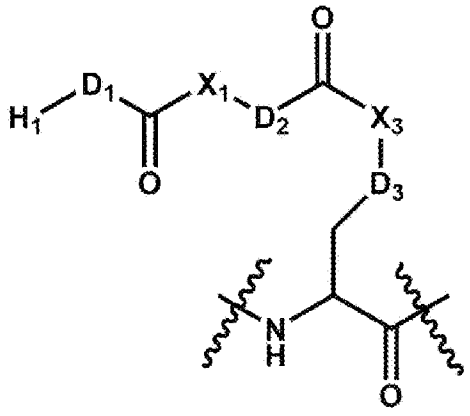
a peptide-compound conjugate of formula 6-2:

[formula 6-2]

$$(Xaa)_{1\text{-}3}\text{-}C\text{-}(Xaa)_2\text{-}H\text{-}(Xa_1)'\text{-}G\text{-}Xa_2\text{-}L\text{-}V\text{-}Xa_3\text{-}C\text{-}(Xaa)_{1\text{-}3}$$

wherein,

each Xaa is independently any amino acid residue that is not a cysteine residue, C is a cysteine residue, H is a histidine residue, G is a glycine residue, Xa_2 is a glutamic acid residue or an asparagine residue, L is a leucine residue, V is a valine residue, W is a tryptophan residue,

$(Xa_1)'$ is  , wherein

H_1 is a first click chemistry functional group,

D_1 is selected from a covalent bond, $C_{1\sim4}$alkylene, $C_{2\sim4}$alkenylene, $C_{2\sim4}$alkynylene, and $C_{3\sim8}$cycloalkynlene,

X_1 is S,

D_2 is selected from $C_{1\sim7}$alkylene, $C_{2\sim7}$alkenylene, $C_{2\sim7}$alkynylene, and $C_{3\sim8}$cycloalkynlene,

D_3 is a covalent bond or $C_{1\sim3}$alkylene and X_3 is NH,

wherein the peptide consists of 13 to 17 amino acid residues,

wherein the peptide exhibits the activity of binding to human immunoglobulin G (IgG),

and wherein the cysteine residue that is between two to four amino acids from the N-terminus of formula 6-2 and the cysteine residue that is between two to four amino acids from the C-terminus of formula 6-2 are optionally linked; and

an antibody or a fragment thereof.

34. A compound of formula 9:

[formula 9]



wherein,

C_m is a cargo moiety,

H_2 is a second click chemistry functional group.

35. A method for preparing an antibody-drug conjugate comprising

reacting an antibody or a fragment thereof comprising one or more amino acid sequence selected from formula 8-1, formula 8-2, and formula 8-3:

[formula 8-1]

G—P—S—V—F—L—F—P—P-(K)'-P—K—D—T—L—M—I

[formula 8-2]

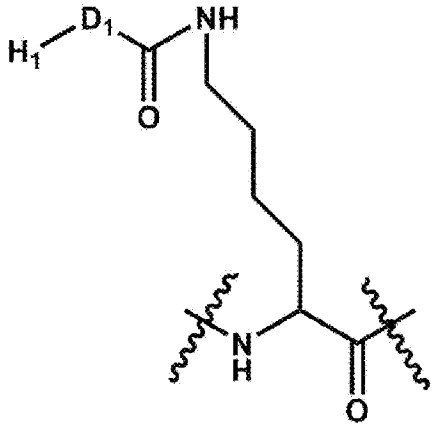
G—P—S—V—F—L—F—P—P—K—P-(K)'-D—T—L—M—I

[formula 8-3]

G—P—S—V—F—L—F—P—P-(K)'-P-(K)'-D—T—L—M—I

wherein,

G is a glycine residue, P is a proline residue, S is a serine residue, V is a valine residue, F is a phenylalanine residue, L is a leucine residue, K is a lysine residue, D is an aspartic acid residue, T is a threonine residue, M is a methionine residue, I is an isoleucine residue, and

(K)' is 

, wherein

H_1 is a first click chemistry functional group,

D_1 is selected from a covalent bond, $C_{1\sim4}$alkylene, $C_{2\sim4}$alkenylene, $C_{2\sim4}$alkynylene, and $C_{3\sim8}$cycloalkynlene, with

a compound of formula 9:

[formula 9]

C_m———H_2

wherein,

C_m is a cargo moiety,

H_2 is a second click chemistry functional group which is complementary with the first click chemistry functional group.

36. A kit for preparing an antibody-drug conjugate comprising

an antibody or a fragment thereof comprising one or more amino acid sequence selected from formula 8-1, formula 8-2, and formula 8-3:

[formula 8-1]

G—P—S—V—F—L—F—P—P-(K)'-P—K—D—T—L—M—I

[formula 8-2]

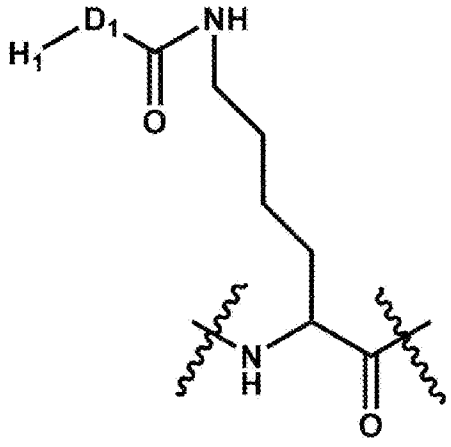
G—P—S—V—F—L—F—P—P—K—P-(K)'-D—T—L—M—I

[formula 8-3]

G—P—S—V—F—L—F—P—P-(K)'-P-(K)'-D—T—L—M—I

wherein,

G is a glycine residue, P is a proline residue, S is a serine residue, V is a valine residue, F is a phenylalanine residue, L is a leucine residue, K is a lysine residue, D is an aspartic acid residue, T is a threonine residue, M is a methionine residue, I is an isoleucine residue, and

(K)' is 

, wherein

H_1 is a first click chemistry functional group,

D_1 is selected from a covalent bond, $C_{1\sim4}$alkylene, $C_{2\sim4}$alkenylene, $C_{2\sim4}$alkynylene, and $C_{3\sim8}$cycloalkynlene; and

a compound of formula 9:

[formula 9]

C_m———H_2

wherein,

C_m is a cargo moiety,

H_2 is a second click chemistry functional group which is complementary with the first click chemistry functional group.

37. An antibody or a fragment thereof comprising one or more amino acid sequence selected from formula 10-1, formula 10-2, and formula 10-3:

[formula 10-1]

G—P—S—V—F—L—F—P—P–(K)"-P—K—D—T—L—M—I

[formula 10-2]

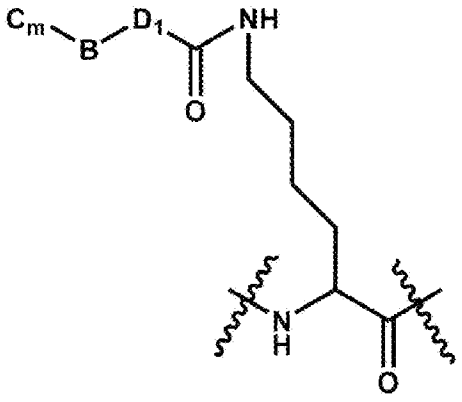
G—P—S—V—F—L—F—P—P—K—P–(K)"-D—T—L—M—I

[formula 10-3]

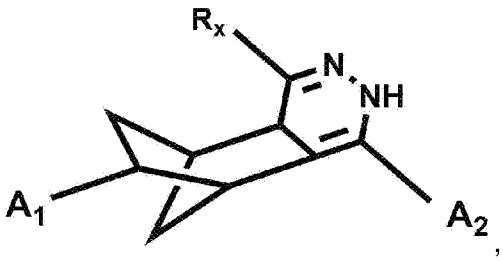
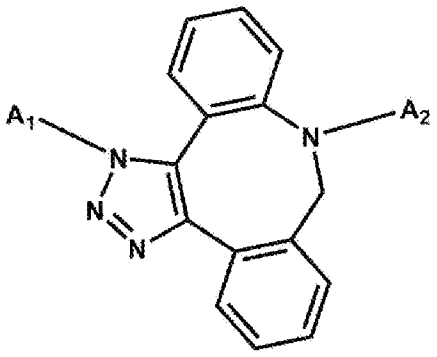
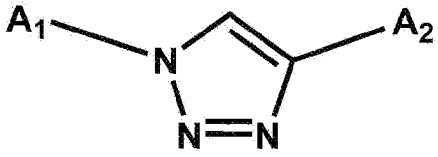
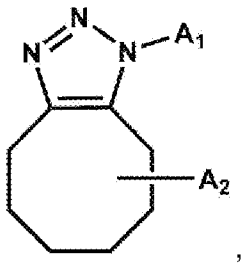
G—P—S—V—F—L—F—P—P–(K)"-P–(K)"-D—T—L—M—I

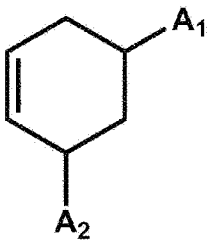
wherein,

G is a glycine residue, P is a proline residue, S is a serine residue, V is a valine residue, F is a phenylalanine residue, L is a leucine residue, K is a lysine residue, D is an aspartic acid residue, T is a threonine residue, M is a methionine residue, I is an isoleucine residue, and

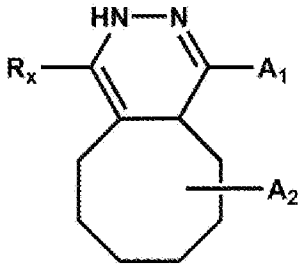
(K)" is  , wherein

C_m is a cargo moiety,

B is selected from  ,  ,  ,  ,



,


, and


, wherein A_1 and A_2 is connected to the cargo moiety or D_1 and they are not both connected to same, R_x is selected from H, halogen, and $C_{1\sim3}$alkyl,

D_1 is selected from a covalent bond, $C_{1\sim4}$alkylene, $C_{2\sim4}$alkenylene, $C_{2\sim4}$alkynylene, and $C_{3\sim8}$cycloalkynlene.

38. An antibody or a fragment thereof according to claim 37,

comprising the amino acid sequence of formula 10-1 and not comprising the amino acid sequence of formula 10-2 and 10-3.

39. An antibody or a fragment thereof according to claim 38,

comprising the amino acid sequence of formula 10-1 in both two Fc domains.

40. An antibody or a fragment thereof according to claim 37,

comprising the amino acid sequence of formula 10-2 and not comprising the amino acid sequence of formula 10-1 and 10-3.

41. An antibody or a fragment thereof according to claim 40,

comprising the amino acid sequence of formula 10-2 in both two Fc domains.

42. An antibody or a fragment thereof according to claim 37,

comprising the amino acid sequence of formula 10-3 and not comprising the amino acid sequence of formula 10-1 and 10-2.

43. An antibody or a fragment thereof according to claim 42,

comprising the amino acid sequence of formula 10-3 in both two Fc domains.

44. An antibody or a fragment thereof according to claim 37,

wherein the cargo moiety comprises a drug moiety.

45. An antibody or a fragment thereof according to claim 44,
wherein the cargo moiety comprises more than one drug moiety.

46. An antibody or a fragment thereof according to claim 44,
wherein the drug moiety is an anticancer agent.

47. An antibody or a fragment thereof according to claim 46,
wherein the anticancer agent is at least one selected from DM1, DM3, DM4, abrin, ricin A, pseudomonas exotoxin, cholera toxin, diphtheria toxin, tumor necrosis factor, α-interferon, β-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, a cytokine, an apoptotic agen, an anti-angiogenic agent, a lymphokine, taxane, a DNA-alkylating agent, anthracycline, tubulysin analogs, duocarmycin analogs, auristatin E, auristatin F, maytansinoid, a cytotoxic agent comprising a reactive polyethylene glycol moiety, taxon, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, t. colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoid, procaine, tetracaine, lidocaine, propranolol, puromycin, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine, mechlorethamine, thiotepachlorambucil, meiphalan, carmustine, lomustine, cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, cisplatin, dactinomycin, bleomycin, anthramycin, calicheamicin, Gemcitabine, bendamustine, bortezomib, carboplatin, cabazitaxel, dasatinib, docetaxel, epirubicin, erlotinib, everolimus, gemcitabine, gefitinib, idarubicin, imatinib, hydroxyurea, lapatinib, leuprorelin, melphalan, nedaplatin, nilotinib, oxaliplatin, pazopanib, pemetrexed, picoplatin, romidepsin, satraplatin, sorafenib, vemurafenib, sunitinib, teniposide, triplatin, and vinorelbine.

48. A pharmaceutical composition for treating cancer comprising an antibody-drug conjugate according to claim 46.

49. A pharmaceutical composition for treating cancer according to claim 48,
wherein the cancer is selected from bladder cancer, bone cancer, brain tumor, breast cancer, heart cancer, cervical cancer, colorectal cancer, rectal cancer, esophageal cancer, fibrosarcoma, gastric cancer, gastrointestinal cancer, head and neck cancer, Kaposi sarcoma, renal cancer, leukemia, liver cancer, lung cancer, lymphoma, melanoma, myeloma, ovarian

cancer, pancreatic cancer, penile cancer, prostate cancer, testicular germ cell cancer, thymoma and thymic carcinoma.

50. A pharmaceutical composition for treating cancer according to claim 49, wherein the cancer is breast cancer.

51. A method for treating cancer comprising administering a pharmaceutical composition comprising an antibody-drug conjugate according to claim 46 to a subject.

52. A method for treating cancer according to claim 51, wherein the cancer is selected from bladder cancer, bone cancer, brain tumor, breast cancer, heart cancer, cervical cancer, colorectal cancer, rectal cancer, esophageal cancer, fibrosarcoma, gastric cancer, gastrointestinal cancer, head and neck cancer, Kaposi sarcoma, renal cancer, leukemia, liver cancer, lung cancer, lymphoma, melanoma, myeloma, ovarian cancer, pancreatic cancer, penile cancer, prostate cancer, testicular germ cell cancer, thymoma and thymic carcinoma.

53. A method for treating cancer according to claim 52, wherein the cancer is breast cancer.

ABSTRACT

The present invention relates to technology capable of labeling a certain site of an antibody with a certain number of chemical functional groups or cargo moieties. The present invention may provide an antibody product having high uniformity. The present invention may provide an antibody product whose antibody functions are not degraded. That is, the present invention may provide an antibody product whose antibody binding affinity and half-life are not degraded. The present invention is of great significance as being the first technology allowing site-specific labeling of an antibody without any complicated processes.

PTO/AIA/82A (07-13)
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Application Number	To Be Assigned
Filing Date	16 January 2023
First Named Inventor	Sang Jeon CHUNG
Title	SITE-SPECIFIC ANTIBODY CONJUGATION AND ANTIBODY-DRUG CONJUGATE AS SPECIFIC EMBODIMENT THEREOF
Art Unit	To Be Assigned
Examiner Name	To Be Assigned
Attorney Docket Number	18896-000072-US-COA

SIGNATURE of Applicant or Patent Practitioner

Signature	/Kisuk Lee/	Date (Optional)	16 January 2023
Name	Kisuk Lee	Registration Number	66,861
Title (if Applicant is a juristic entity)	Attorney for Applicant		
Applicant Name (if Applicant is a juristic entity)	AbTis Co., Ltd.		

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

☑ *Total of 1 forms are submitted.

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

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

Application Number	Filing Date

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

28997

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

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Address					
City		State		Zip	
Country					
Telephone		Email			

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

AbTis Co., Ltd.

☐ Inventor or Joint Inventor (title not required below)

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

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

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

SIGNATURE of Applicant for Patent

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

Signature		Date (Optional)	
Name	SANG JEON CHUNG		
Title	, AbTis Co., Ltd.		

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

☐ Total of forms are submitted.

This collection of information is required by 37 CFR 1.131, 1.32, and 1.33. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 3 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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Docket No.: 18896-000072-US-COA
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Sang Jeon CHUNG et al.

Application No.: Not Yet Assigned	Confirmation No.: Not Yet Assigned
Filed: Concurrently Herewith	Art Unit: Not Yet Assigned
For: SITE-SPECIFIC ANTIBODY CONJUGATION AND ANTIBODY-DRUG CONJUGATE AS SPECIFIC EMBODIMENT THEREOF	Examiner: Not Yet Assigned

INFORMATION DISCLOSURE STATEMENT UNDER 37 C.F.R. §1.97(B)(3)

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

Dear Commissioner:

Applicant hereby submits an Information Disclosure Statement (IDS) under 37 C.F.R. §1.97(b)(3): before the mailing of a first Office Action on the merits. Applicant does not believe any fee is owed to submit the present IDS. However, authorization is provided herewith to charge any such fee that may be required under 37 C.F.R. §1.17(p) to Deposit Account No. 08-0750.

In accordance with 37 C.F.R. §1.97(g) and (h), this submission shall not be construed as a representation that a search has been made or as admission that information presented or cited herein is, or is considered to be, material to patentability as defined in 37 C.F.R. §1.56(b).

KL/ei

In accordance with 37 C.F.R. §1.98, Applicant submits herewith a listing on Form HDP-1449 of documents requested to be considered by the Examiner. Consideration by the Examiner of the present submission is requested.

Applicant believes no fee is due with this response. However, if a fee is due, please charge our Deposit Account No. 08-0750, under Order No. 18896-000072-US-COA from which the undersigned is authorized to draw.

Dated: January 16, 2023

Respectfully submitted,

Electronic signature: /Kisuk Lee/
Kisuk Lee
Registration No.: 66,861
HARNESS, DICKEY & PIERCE, P.L.C.
7700 Bonhomme
Suite 400
Clayton, Missouri 63105
314.446.7670
Attorney for Applicant

Enclosures:
HDP Form 1449
Cited documents