

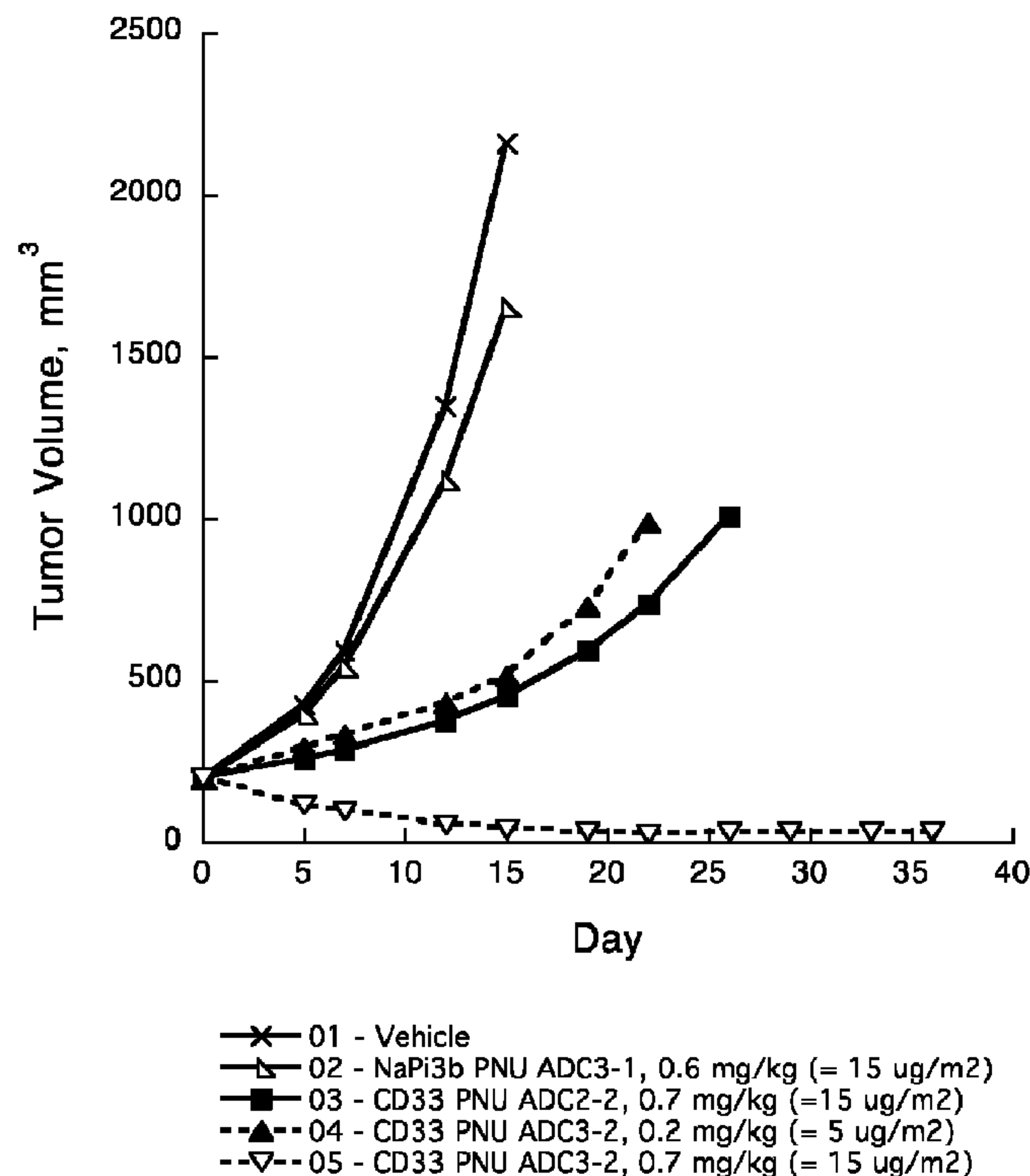


(86) **Date de dépôt PCT/PCT Filing Date:** 2014/12/16
 (87) **Date publication PCT/PCT Publication Date:** 2015/06/25
 (85) **Entrée phase nationale/National Entry:** 2016/04/27
 (86) **N° demande PCT/PCT Application No.:** US 2014/070654
 (87) **N° publication PCT/PCT Publication No.:** 2015/095223
 (30) **Priorité/Priority:** 2013/12/16 (US61/916,680)

(51) **Cl.Int./Int.Cl. A61K 47/48** (2006.01),
A61P 35/00 (2006.01)
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(54) **Titre : COMPOSES PEPTIDOMIMETIQUES ET CONJUGUES ANTICORPS-MEDICAMENT DE CEUX-CI**
 (54) **Title: PEPTIDOMIMETIC COMPOUNDS AND ANTIBODY-DRUG CONJUGATES THEREOF**

Figure 1 (HL-60)



(57) **Abrégé/Abstract:**

This invention relates to novel peptidomimetic compounds which are useful as linkers of antibody-drug conjugates (ADC). This invention also relates to ADCs containing peptidomimetic linkers and anthracycline derivatives. It is contemplated that the ADC of



(57) Abrégé(suite)/Abstract(continued):

the present invention may be used to treat various diseases or disorders, e.g. characterised by the overexpression of a tumour antigen.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau(10) International Publication Number
WO 2015/095223 A3(43) International Publication Date
25 June 2015 (25.06.2015)

(51) International Patent Classification:

A61K 47/48 (2006.01) *A61P 35/00* (2006.01)

(21) International Application Number:

PCT/US2014/070654

(22) International Filing Date:

16 December 2014 (16.12.2014)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

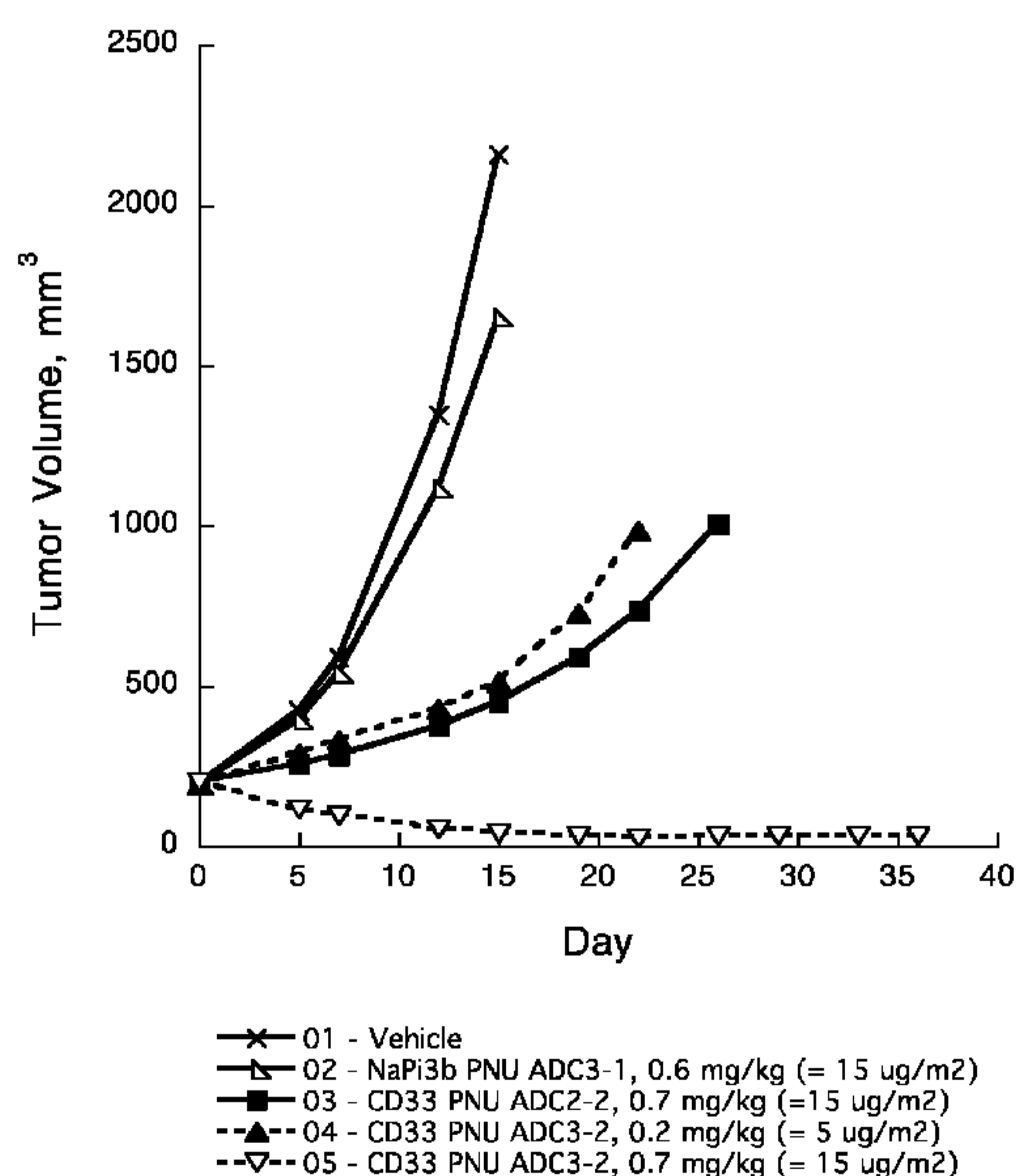
61/916,680 16 December 2013 (16.12.2013) US

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Stop 49, South San Francisco, CA 94080 (US).(81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,
AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY,
BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM,
DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT,
HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR,
KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG,
MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM,
PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC,
SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN,
TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.(84) Designated States (unless otherwise indicated, for every
kind of regional protection available): ARIPO (BW, GH,
GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ,
TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU,

[Continued on next page]

(54) Title: PEPTIDOMIMETIC COMPOUNDS AND ANTIBODY-DRUG CONJUGATES THEREOF

Figure 1 (HL-60)

(57) Abstract: This invention relates to novel peptidomimet-
ic compounds which are useful as linkers of antibody-drug
conjugates (ADC). This invention also relates to ADCs con-
taining peptidomimetic linkers and anthracycline derivatives.
It is contemplated that the ADC of the present invention may
be used to treat various diseases or disorders, e.g. character-
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TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

(88) Date of publication of the international search report:
3 September 2015

Published:

— *with international search report (Art. 21(3))*

PEPTIDOMIMETIC COMPOUNDS AND ANTIBODY-DRUG CONJUGATES THEREOF**CROSS REFERENCE TO RELATED APPLICATIONS**

5

This application claims benefit under 35 U.S.C. §119 to provisional U.S. Application No. 61/916680, filed December 16, 2013, the contents of which are hereby incorporated by reference in their entirety.

10

FIELD OF INVENTION

This invention relates to novel peptidomimetic compounds which are useful as linkers of antibody-drug conjugates(ADC). This invention also relates to ADCs containing peptidomimetic linkers and anthracycline derivatives. This invention also relates to methods of treating diseases in humans.

15

BACKGROUND OF THE INVENTION

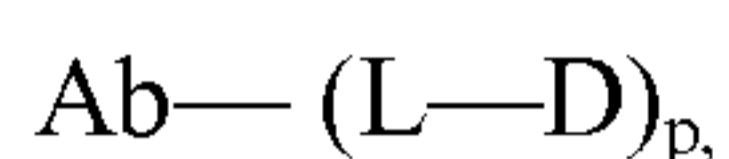
The use of monoclonal antibodies (mABs) to deliver anticancer drugs directly to tumor cells has attracted a great deal of focus in recent years. Two new antibody-drug conjugates have been
20 approved by the FDA for the treatment of cancer. Adcetris® (brentuximab vedotin) is a CD30-directed antibody-drug conjugate (ADC) indicated for the treatment of relapsed or refractory Hodgkin lymphoma and systemic anaplastic large cell lymphoma (ALCL). Kadcyla® (ado-trastuzumab emtansine), is a new therapy approved for patients with HER2-positive, late-stage (metastatic) breast cancer. To obtain a therapeutic both potent anti-tumor activity and acceptable
25 therapeutic index in an ADC, several aspects of design may be optimized. Particularly, it is well known that the chemical structure of the linker can have significant impact on both the efficacy and the safety of ADC (Ducry & Stump, Bioconjugate Chem, 2010, 21, 5-13). Choosing the right linker influences proper drug delivery to the intended cellular compartment of cancer cells. Linkers can be generally divided into two categories: cleavable (such as peptide, hydrzone, or
30 disulfide) or non-cleavable (such as thioether). Peptide linkers, such as Valine-Citrulline (Val-Cit), that can be hydrolyzed by lysosomal enzymes (such as Cathepsin B) have been used to connect the drug with the antibody (US6214345). They have been particularly useful, due in part to their relative stability in systemic circulation and the ability to efficiently release the drug in tumor. ADCs containing the Val-Cit linker have been shown to be relatively stable in vivo (t1/2
35 for drug release ~7 days (Doronina et al (2008), Bioconjugate Chem., 19, 1960-1963). However, the chemical space represented by natural peptides is limited; therefore, it is desirable to have a

variety of non-peptide linkers which act like peptides and can be effectively cleaved by lysosomal proteases. The greater diversity of non-peptide structures may yield novel, beneficial properties that are not afforded by the peptide linkers. Provided herein are different types of non-peptide linkers for ADC that can be cleaved by lysosomal enzymes.

5

SUMMARY OF THE INVENTION

This invention relates to antibody-drug conjugates represented by Formula (I)



10 Ab is an antibody;

L is a peptidomimetic linker represented by the following formula

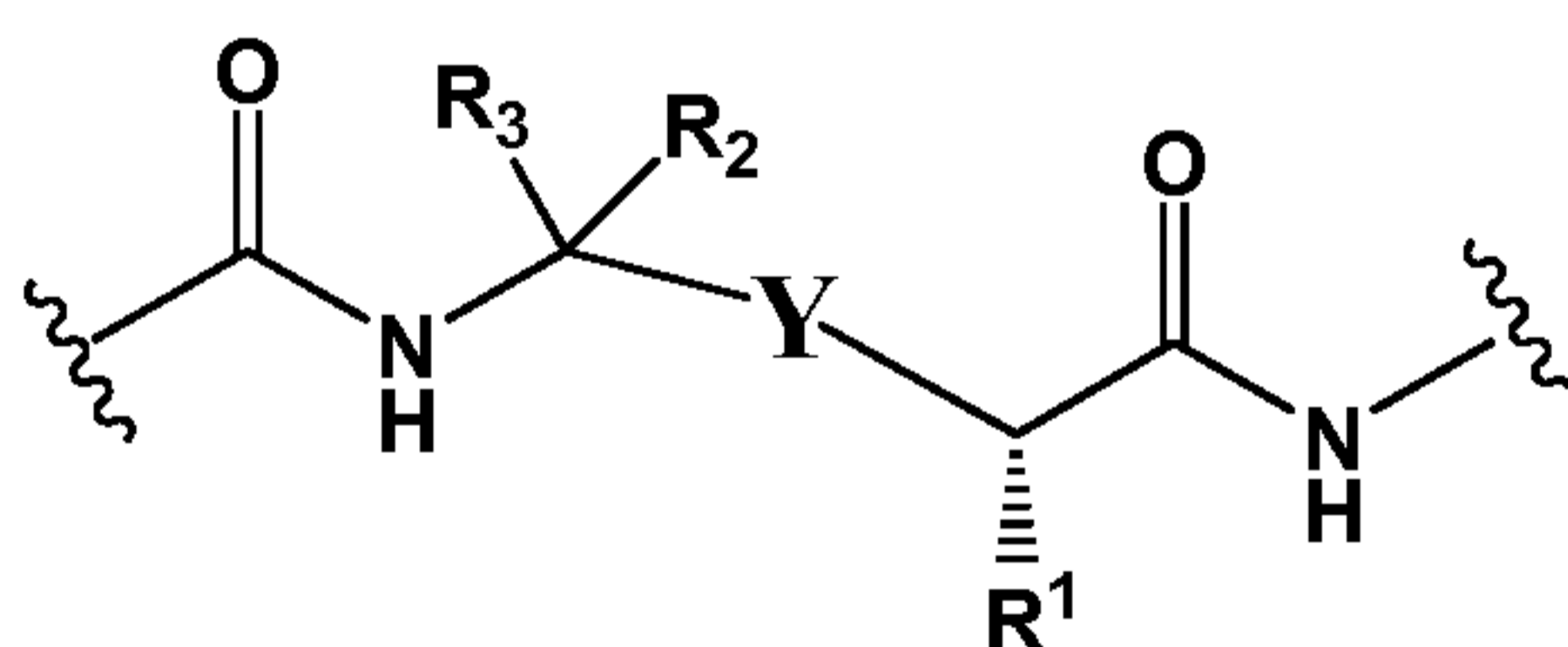
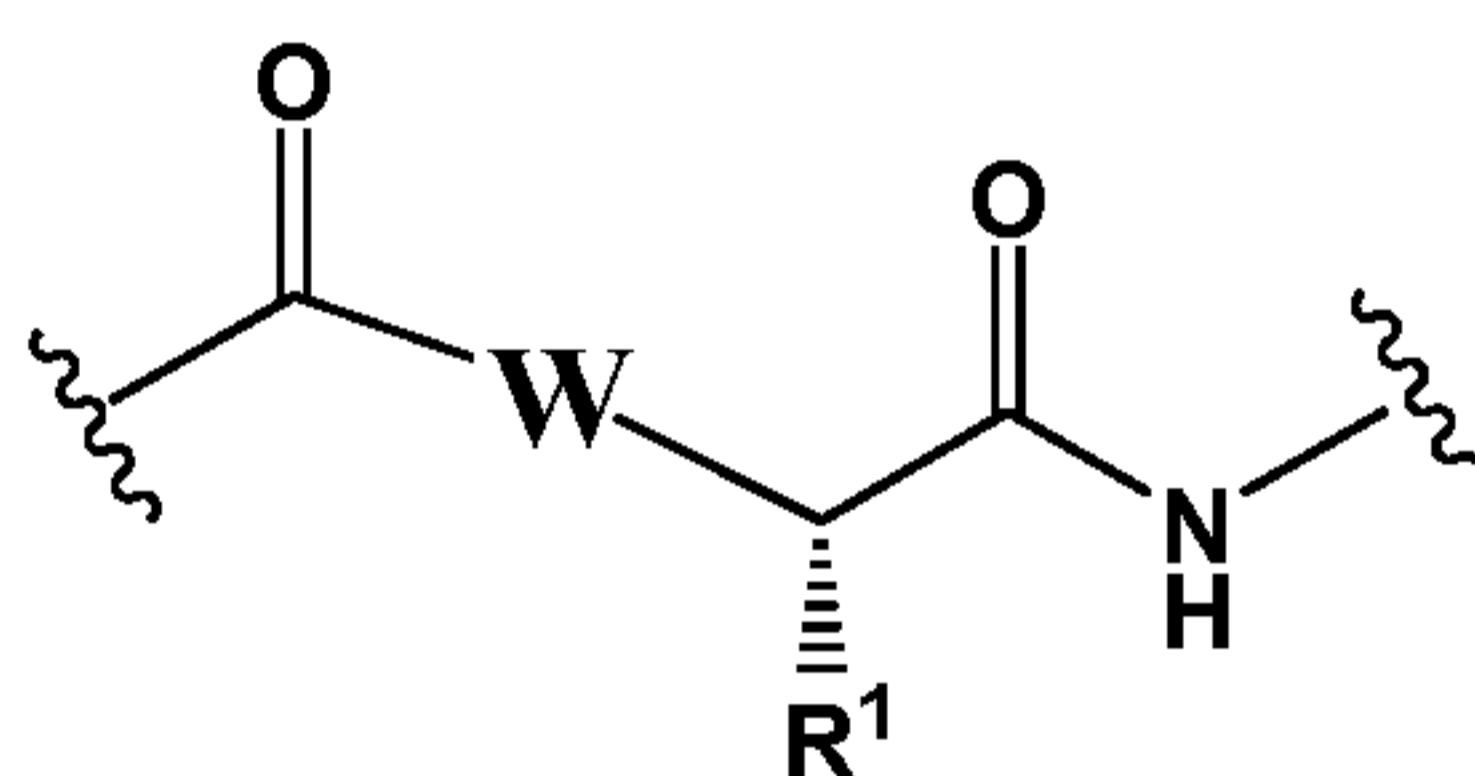


wherein

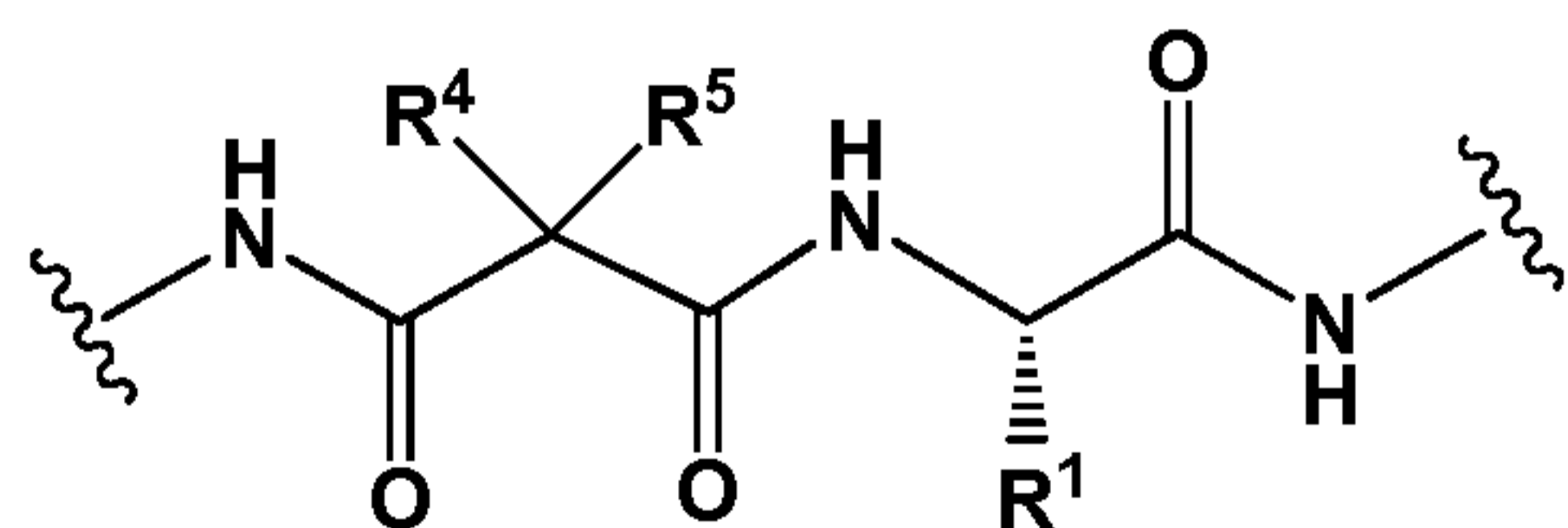
15 Str is a stretcher unit covalently attached to Ab;

Sp is a bond or spacer unit covalently attached to a drug moiety;

PM is a non-peptide chemical moiety selected from the group consisting of:



and



20

W is -NH-heterocycloalkyl- or heterocycloalkyl;

Y is heteroaryl, aryl, $-C(O)C_1-C_6$ alkylene, C_1-C_6 alkylene-NH₂, C_1-C_6 alkylene-NH-CH₃, C_1-C_6 alkylene-N-(CH₃)₂, C_1-C_6 alkenyl or C_1-C_6 alkylenyl;

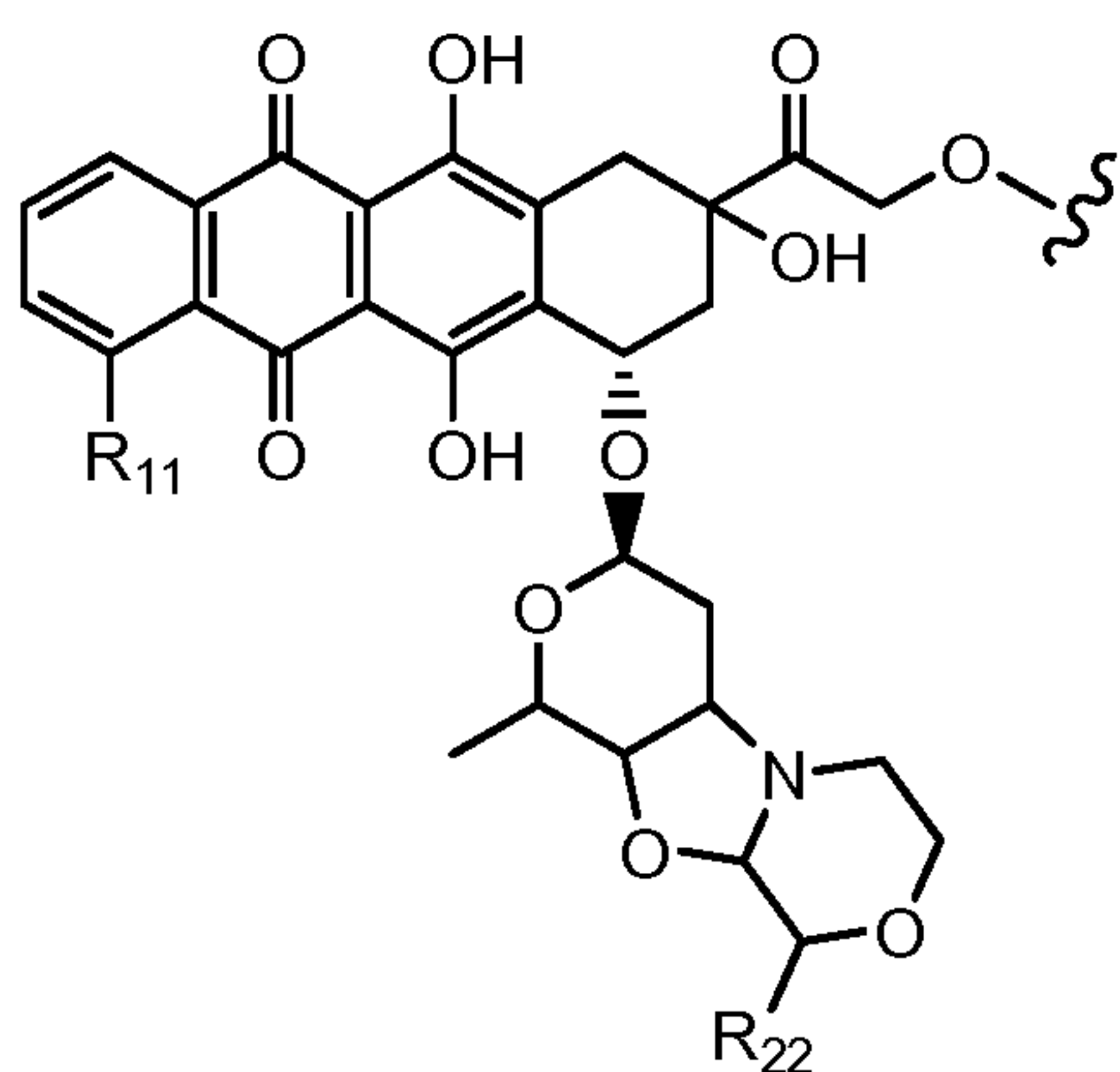
each R¹ is independently C_1-C_{10} alkyl, C_1-C_{10} alkenyl, $(C_1-C_{10}alkyl)NHC(NH)NH_2$ or $(C_1-C_{10}alkyl)NHC(O)NH_2$;

5 R³ and R² are each independently H, C_1-C_{10} alkyl, C_1-C_{10} alkenyl, arylalkyl or heteroarylalkyl, or R³ and R² together may form a C_3-C_7 cycloalkyl;

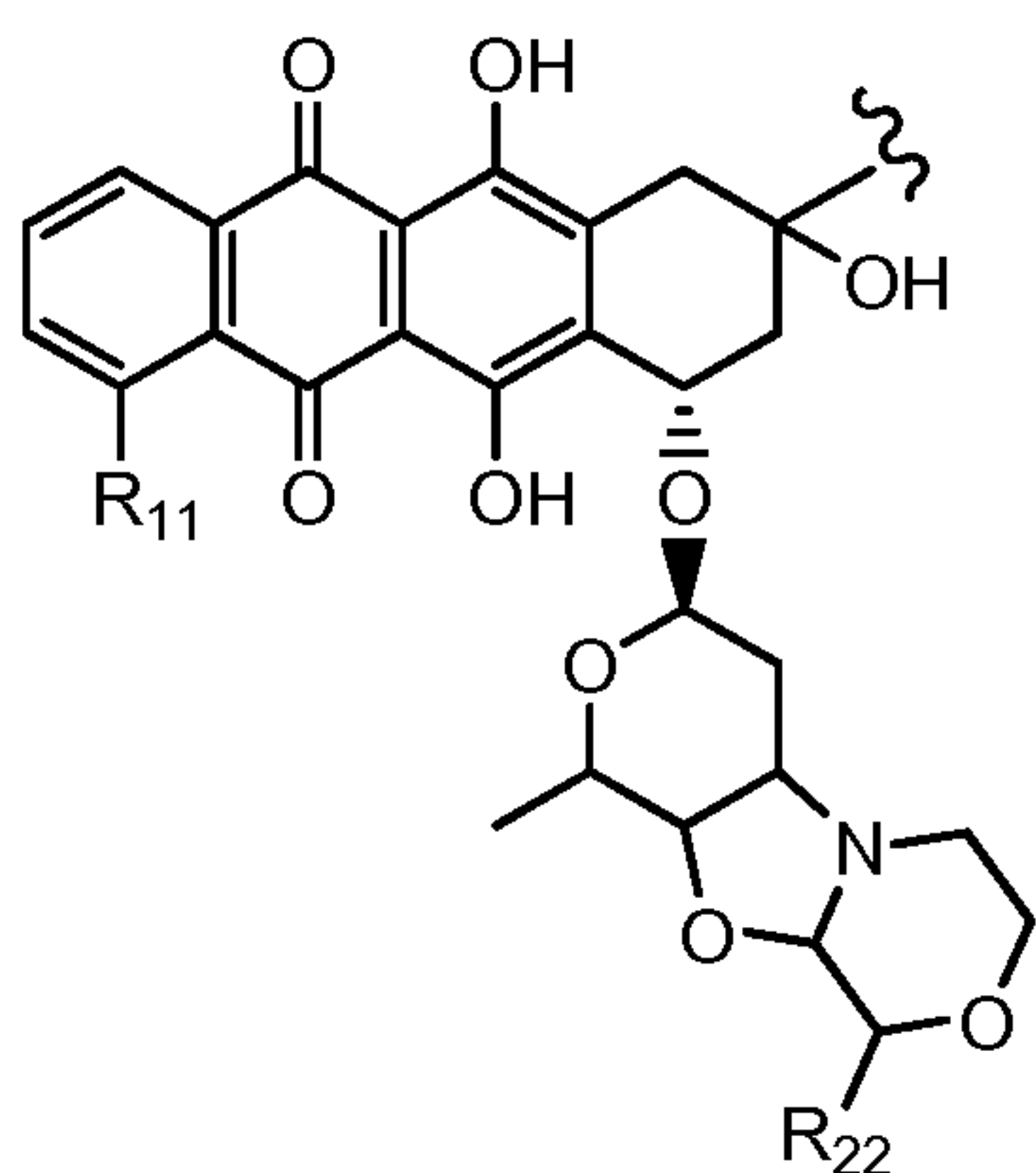
R⁴ and R⁵ are each independently C_1-C_{10} alkyl, C_1-C_{10} alkenyl, arylalkyl, heteroarylalkyl, $(C_1-C_{10}alkyl)OCH_2-$, or R⁴ and R⁵ may form a C_3-C_7 cycloalkyl ring;

p is an integer from 1 to 8;

10 D is a drug moiety of Formula (IA) or (IB)



(IA)



(IB)

15 wherein R¹¹ is hydrogen atom, hydroxy or methoxy group and R²² is a C_1-C_5 alkoxy group.

This invention also relates to pharmaceutical compositions of antibody-drug conjugates of Formula (I).

This invention also relates to a method of treating cancer, use of antibody-drug conjugates of Formula (I) in therapy, and use of antibody-drug conjugates of Formula (I) in manufacturing a medicament for treating cancer.

20

This invention also relates to method of preparing antibody-drug conjugates of Formula (I).

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows efficacy comparison of CD33 ADCs (CD33 PNU ADC3-2 and ADC2-2) in SCID mice with HL-60 human acute myeloid leukemia tumors.

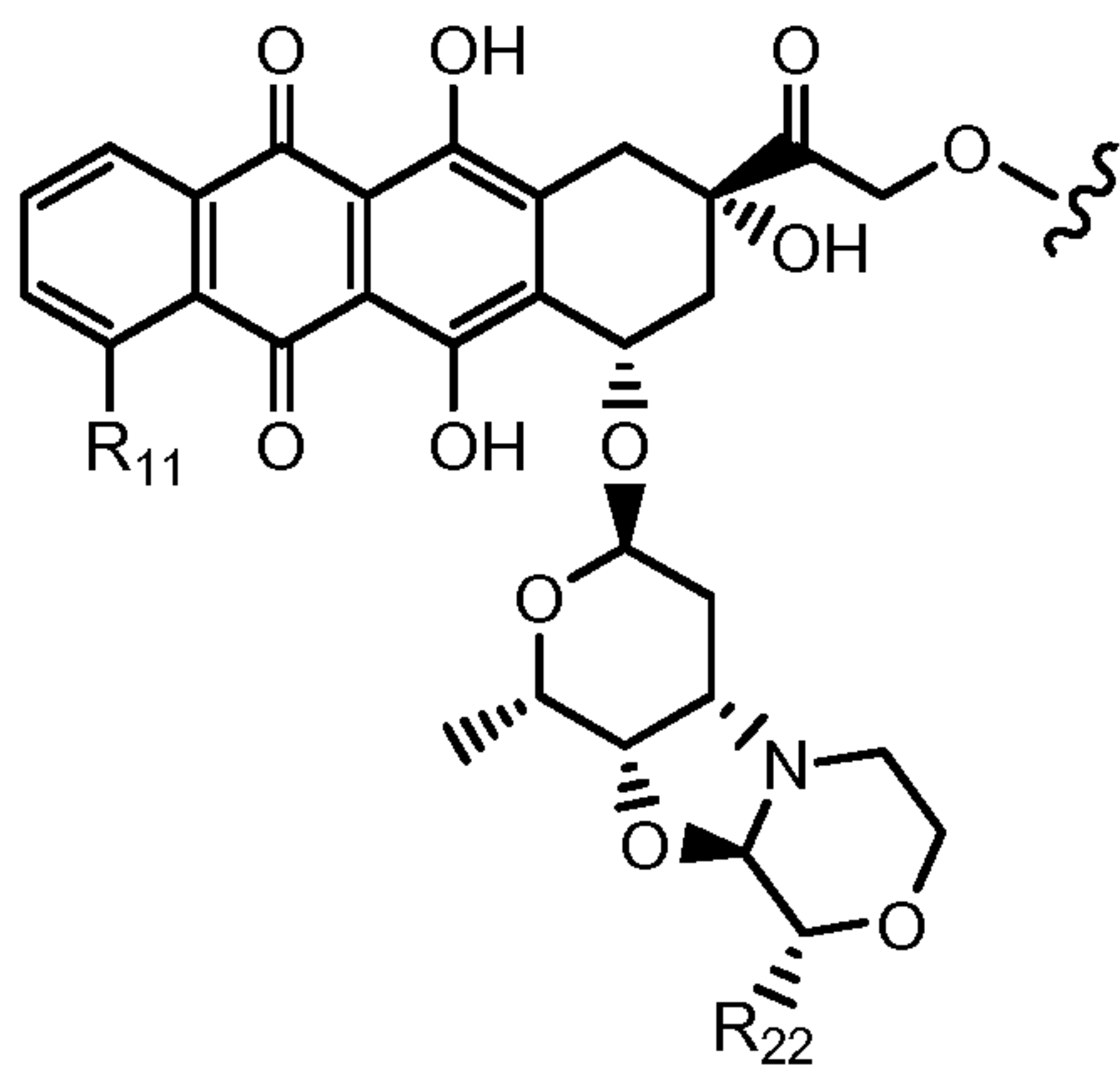
- 5 Figure 2 shows efficacy comparison of CD33 ADCs (CD33 PNU ADC4-2 and ADC2-2) in SCID mice with HL-60 human acute myeloid leukemia tumors.

DETAILED DESCRIPTION OF THE INVENTION

- 10 Provided herein are different types of non-peptide linkers for ADC that are cleavable by lysosomal enzymes. For example, the amide bond in the middle of a dipeptide (e.g. Val-Cit) was replaced with an amide mimic; and/or entire amino acid (e.g., valine amino acid in Val-Cit dipeptide) was replaced with a non-amino acid moiety (e.g., cycloalkyl dicarbonyl structures (for example, ring size = 4 or 5)).

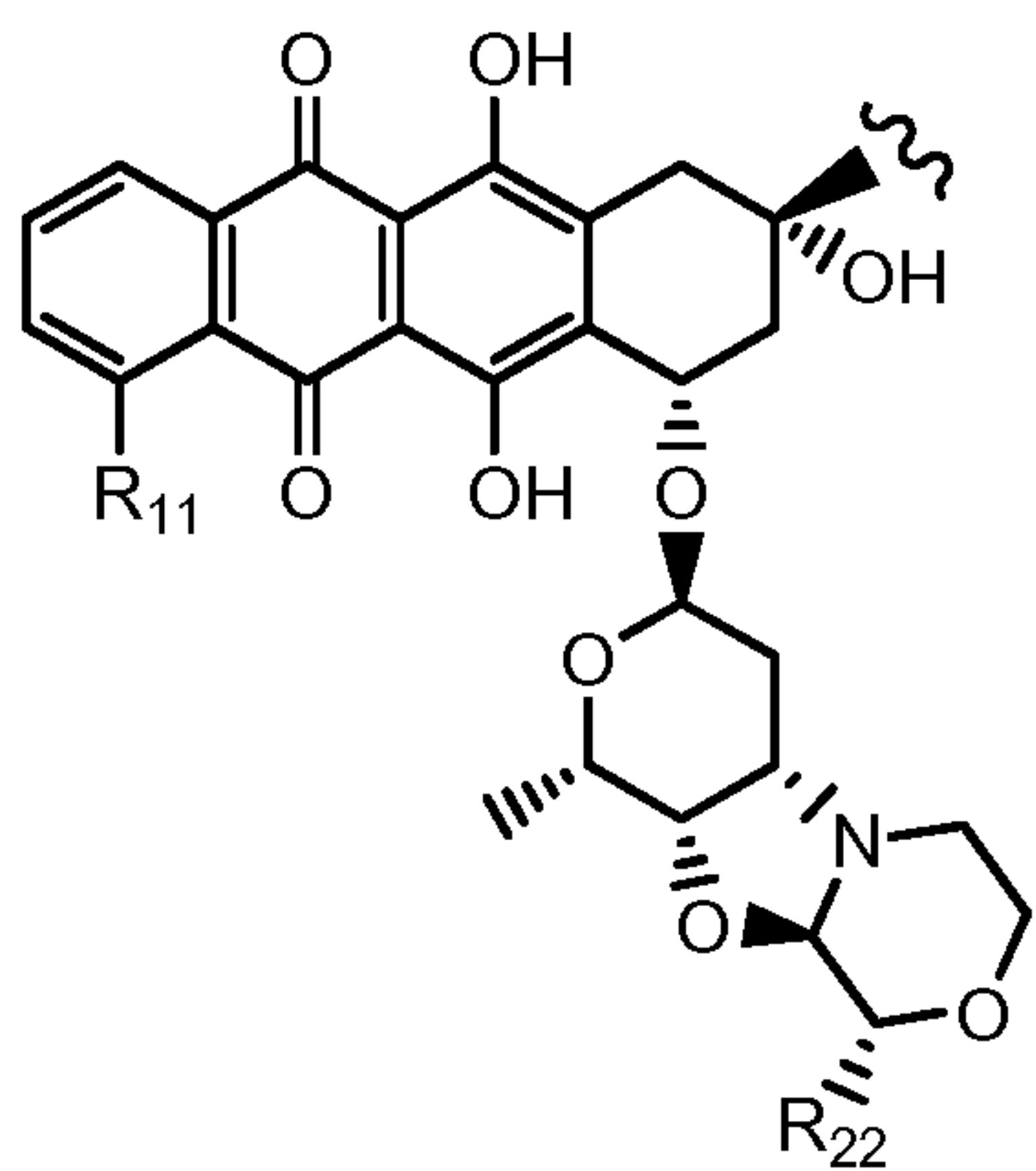
- 15 This invention relates to antibody-conjugates of Formula (I).

This invention also relates to antibody-conjugates of Formula (I), wherein (IA) is:



(Ia)

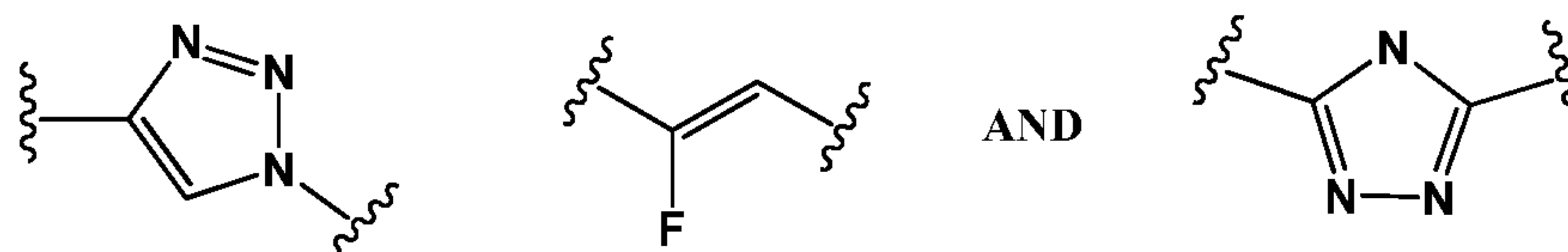
and (IB) is



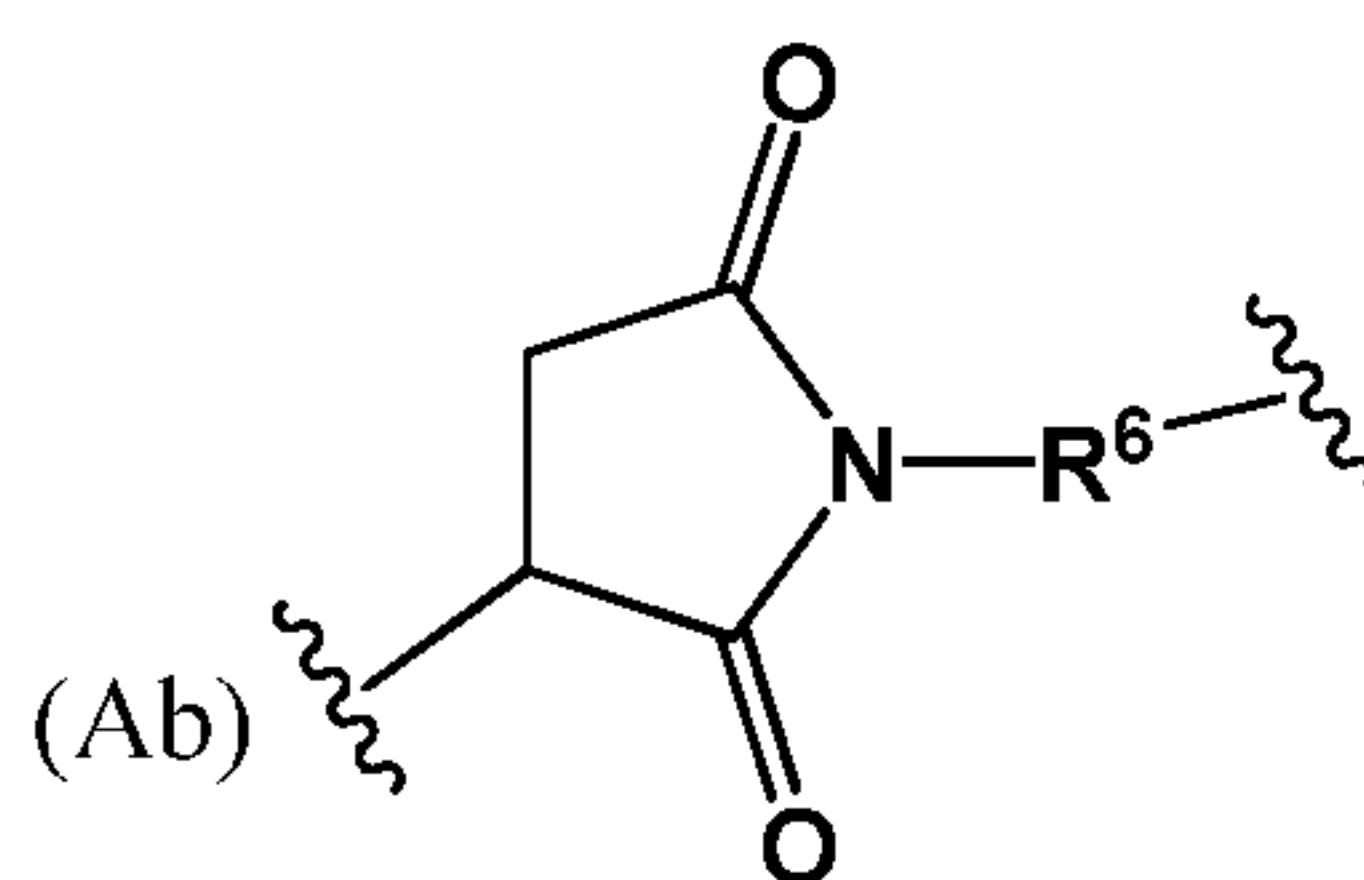
20

This invention also relates to antibody-conjugates of Formula (I), wherein Y is heteroaryl; R⁴ and R⁵ together form a cyclobutyl ring.

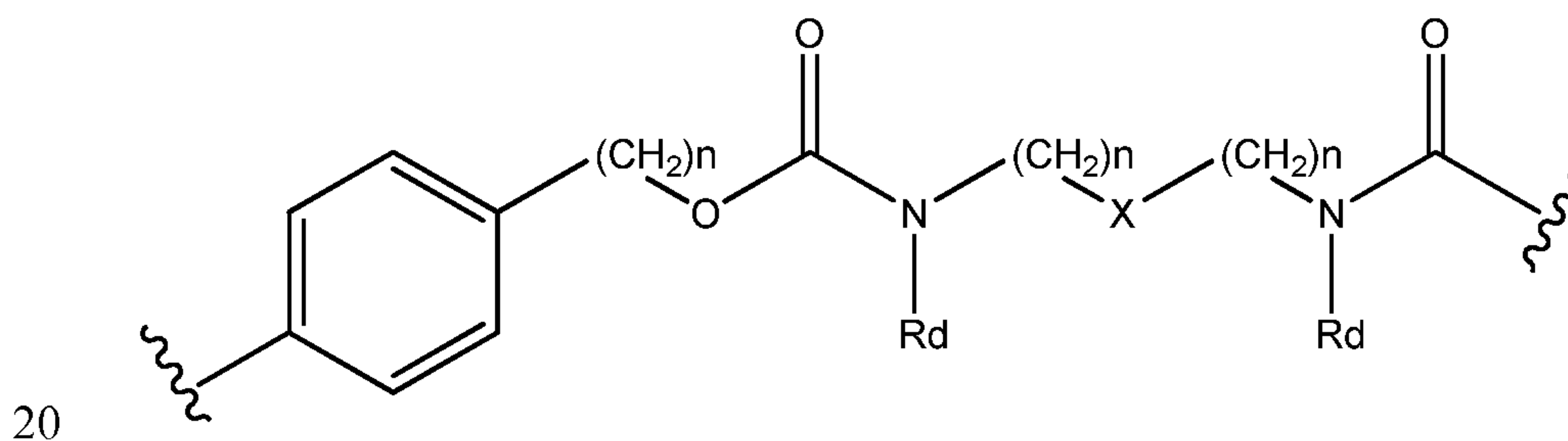
This invention also relates to antibody-conjugates of Formula (I), wherein Y is a moiety selected
5 from the group consisting of



This invention also relates to antibody-conjugates of Formula (I), wherein
10 Str is a chemical moiety represented by the following formula:



wherein R⁶ is selected from the group consisting of C₁-C₁₀alkylene, C₁-C₁₀alkenyl, C₃-C₈cycloalkyl, (C₁-C₈alkylene)O-, and C₁-C₁₀alkylene-C(O)N(R^a)-C₂-C₆alkylene, where each
15 alkylene may be substituted by one to five substituents selected from the group consisting of halo, trifluoromethyl, difluoromethyl, amino, alkylamino, cyano, sulfonyl, sulfonamide, sulfoxide, hydroxy, alkoxy, ester, carboxylic acid, alkylthio, aryl, arylalkyl, C₃-C₈cycloalkyl, C₄-C₇heterocycloalkyl, heteroarylalkyl and heteroaryl each R^a is independently H or C₁-C₆alkyl; Sp is —Ar—R^b—, wherein Ar is aryl or heteroaryl, R^b is (C₁-C₁₀alkylene)O-, or Sp is the
20 following formula



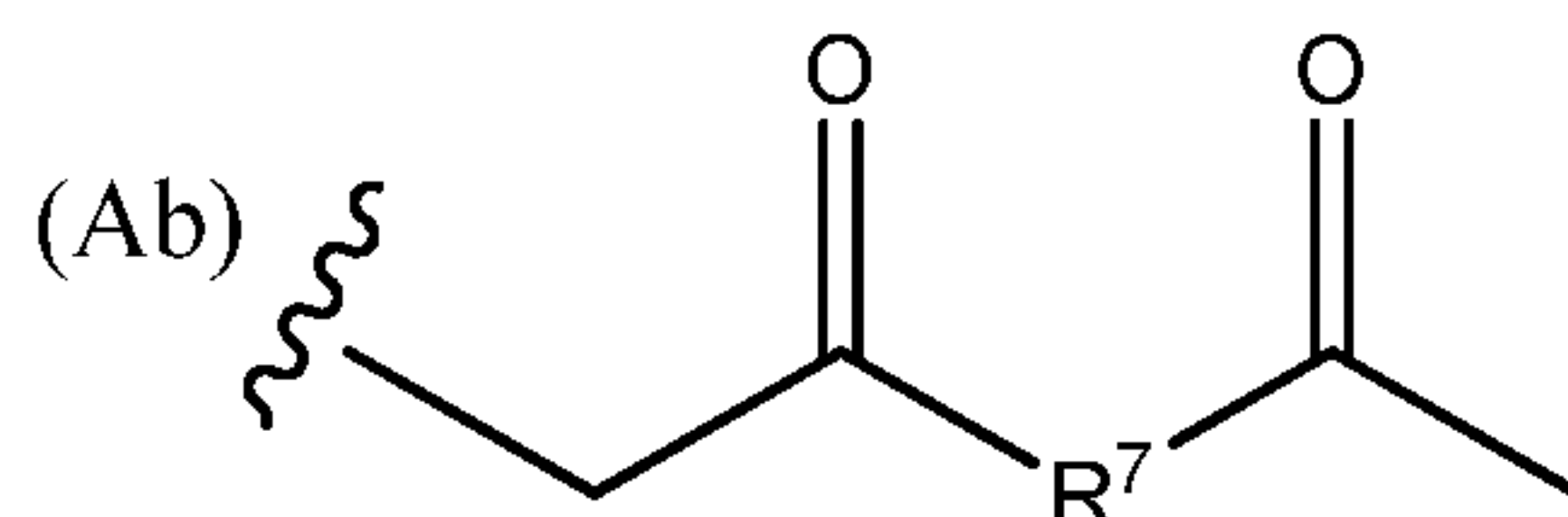
wherein

each n is independently 1-6;

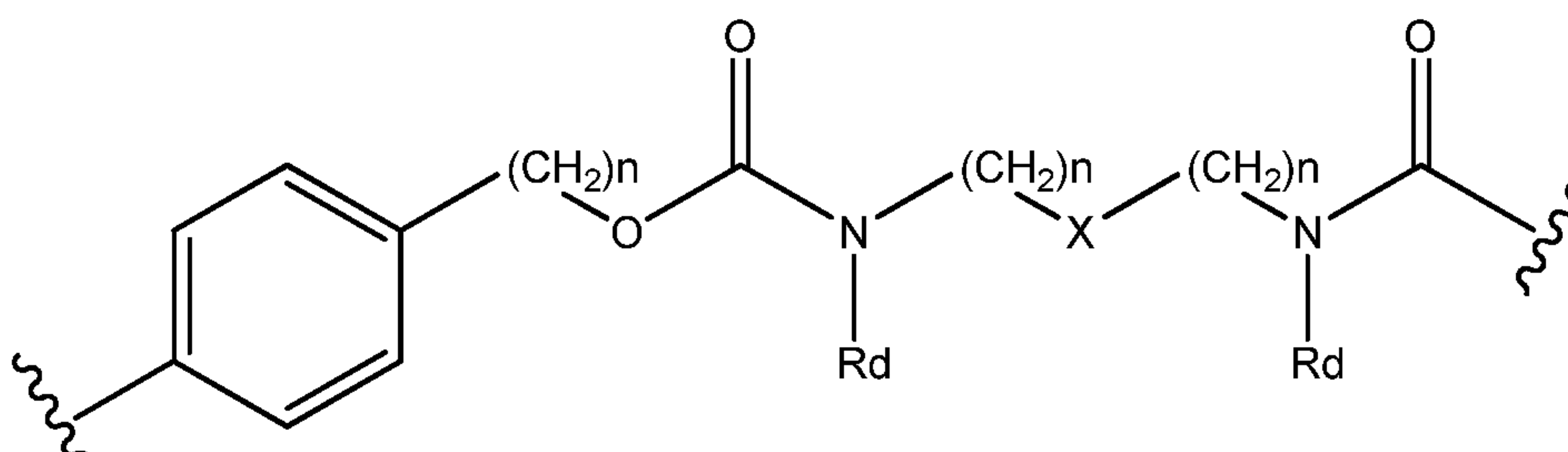
X is N, CH₂ or a bond; and

25 each R^d is independently H or C₁-C₃alkyl.

This invention also relates to antibody-conjugates of Formula (I), wherein Str has the formula:

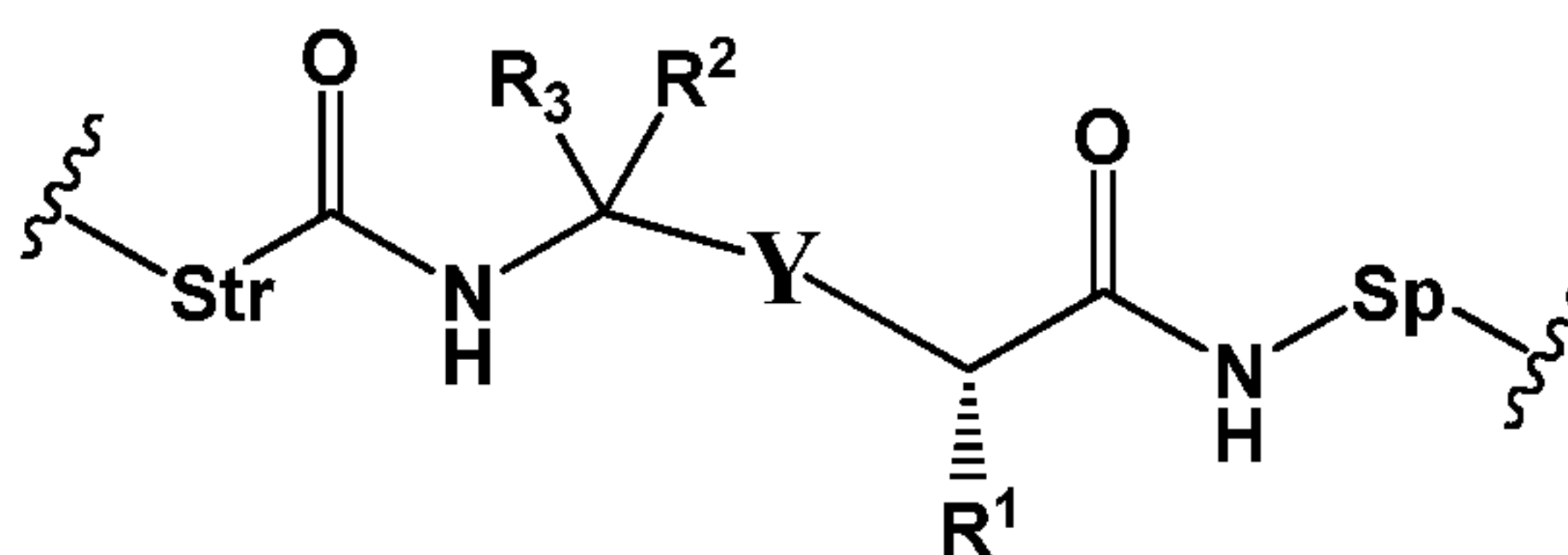


wherein R^7 is selected from C_1 - C_{10} alkylene, C_1 - C_{10} alkenyl, $(C_1$ - C_{10} alkylene)O-, $N(R^c)$ -(C_2 - C_6 alkylene)- $N(R^c)$ and $N(R^c)$ -(C_2 - C_6 alkylene); where each R^c is independently H or C_1 - C_6 alkyl; Sp is —Ar— R^b —, wherein Ar is aryl or heteroaryl, R^b is $(C_1$ - C_{10} alkylene)O- or Sp is the following formula



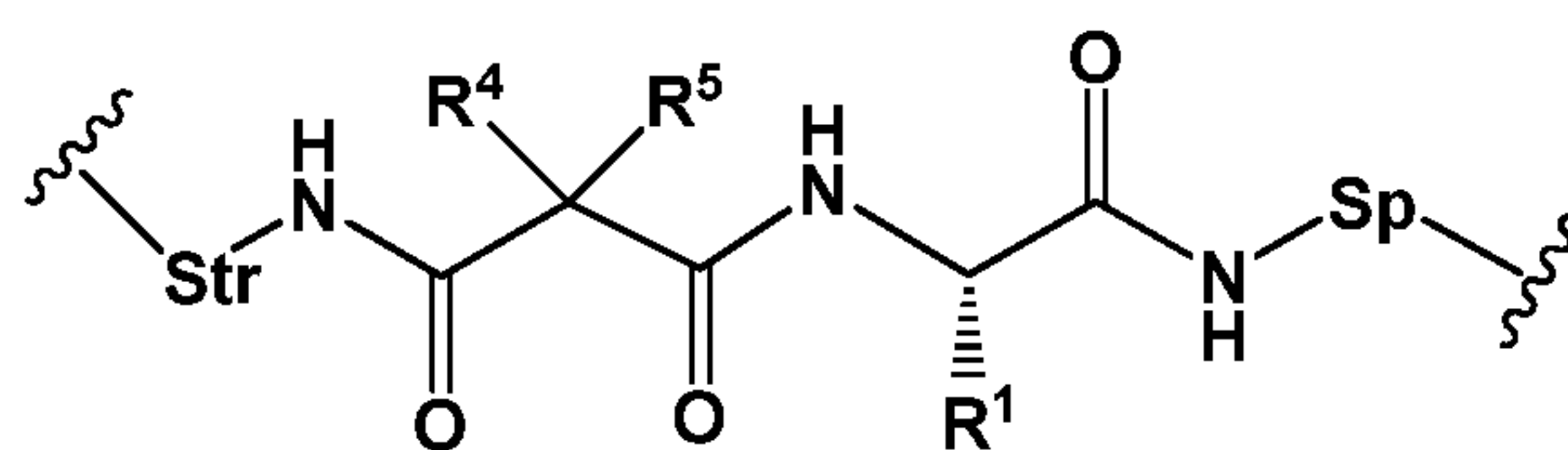
wherein
 10 each n is independently 1-6;
 X is N, CH_2 or a bond; and
 each R^d is independently H or C_1 - C_3 alkyl.

This invention also relates to antibody-conjugates of Formula (I),
 15 wherein
 L is non-peptide chemical moiety represented by the following formula



R^1 is C_1 - C_6 alkyl, C_1 - C_6 alkenyl, $(C_1$ - C_6 alkyl)NHC(NH)NH₂ or $(C_1$ - C_6 alkyl)NHC(O)NH₂;
 20 R^3 and R^2 are each independently H, C_1 - C_{10} alkyl.

This invention also relates to antibody-conjugates of Formula (I),
 wherein
 L is non-peptide chemical moiety represented by the following formula

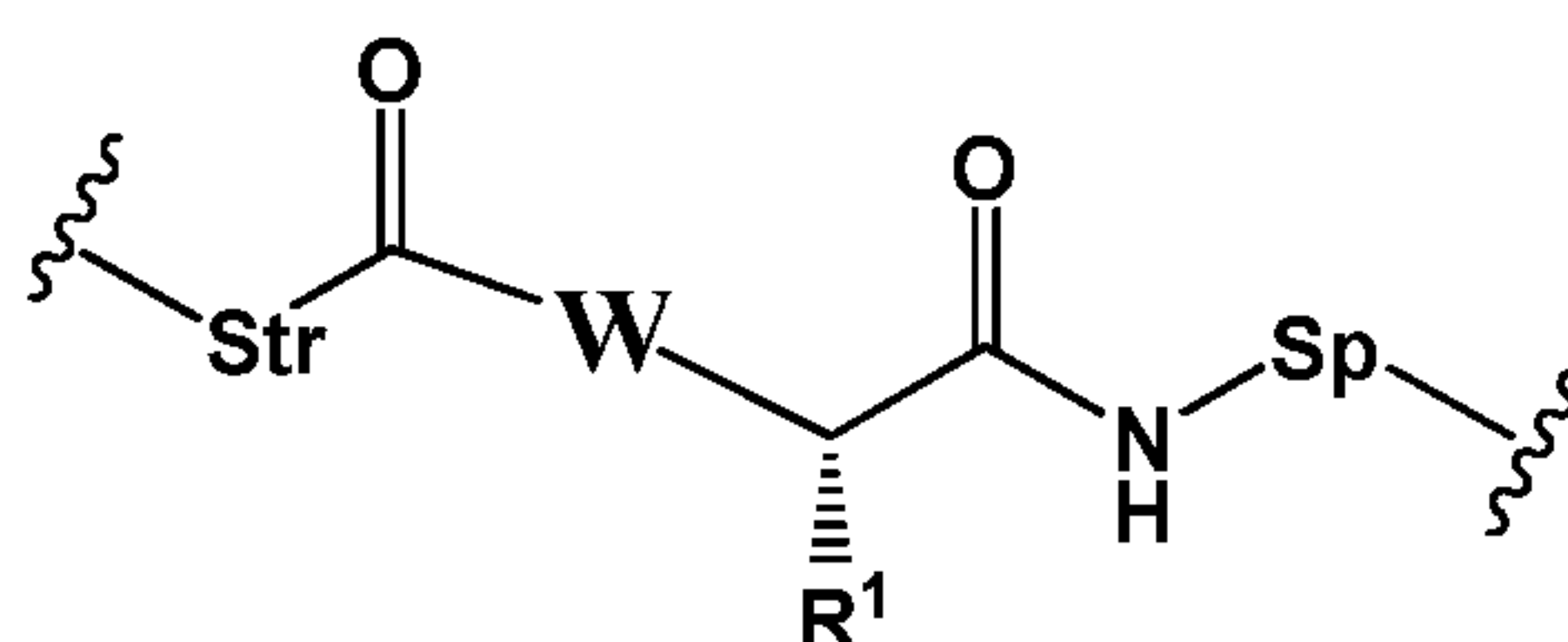


R^1 is C_1 - C_6 alkyl, $(C_1$ - C_6 alkyl) $NHC(NH)NH_2$ or $(C_1$ - C_6 alkyl) $NHC(O)NH_2$;

R^4 and R^5 together form a C_3 - C_7 cycloalkyl ring.

- 5 This invention also relates to antibody-conjugates of Formula (I),
wherein

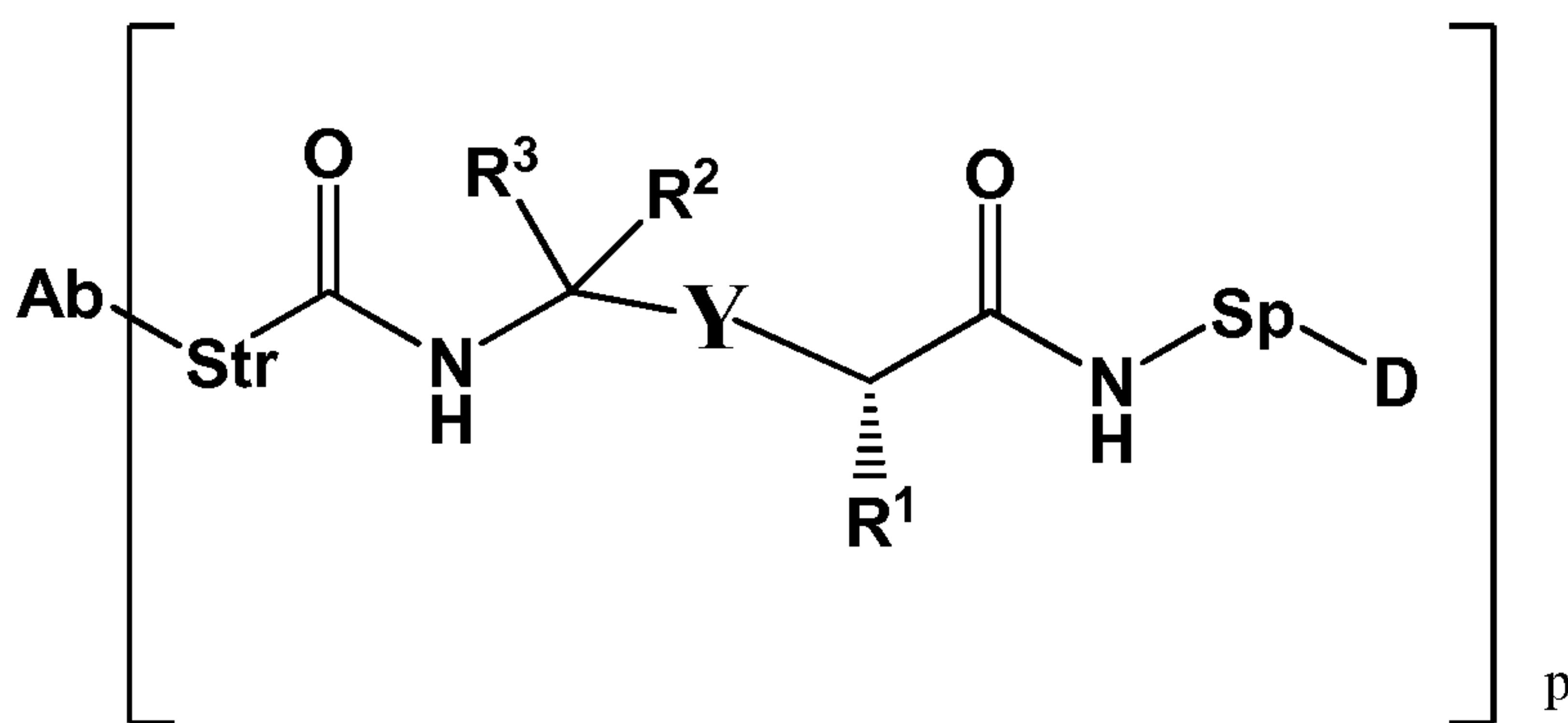
L is non-peptide chemical moiety represented by the following formula



R^1 is C_1 - C_6 alkyl, $(C_1$ - C_6 alkyl) $NHC(NH)NH_2$ or $(C_1$ - C_6 alkyl) $NHC(O)NH_2$.

10

This invention also relates to antibody-conjugates of Formula (I) represented by the following formula:

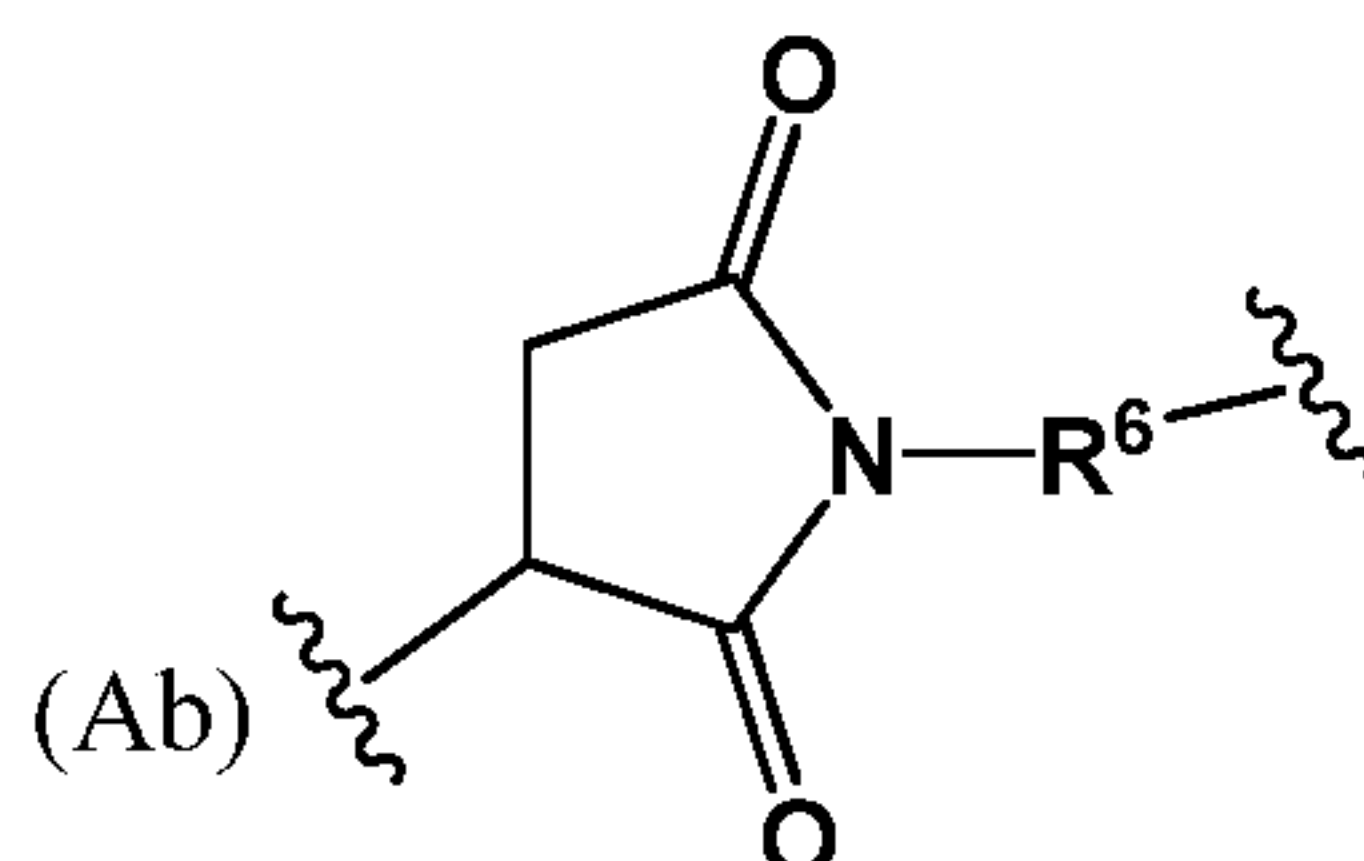


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(I)(A1)

wherein

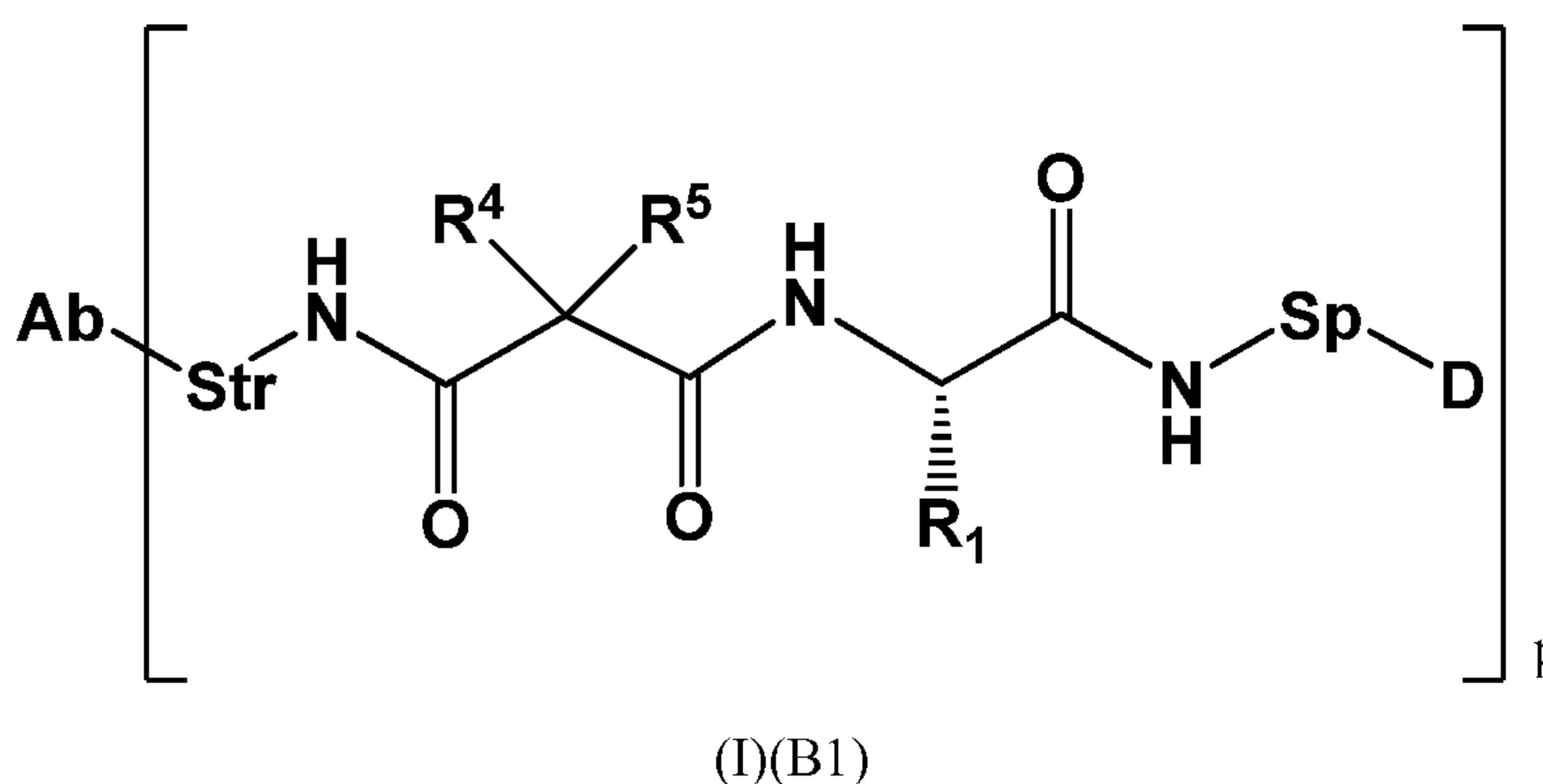
Str is a chemical moiety represented by the following formula:



wherein R^6 is selected from the group consisting of C_1 - C_{10} alkylene, and C_1 - C_{10} alkylene- $C(O)N(R^a)$ - C_2 - C_6 alkylene, where each alkylene may be substituted by one to five substituents selected from the group consisting of halo, trifluoromethyl, difluoromethyl, amino, alkylamino, cyano, sulfonyl, sulfonamide, sulfoxide, hydroxy, alkoxy, ester, carboxylic acid, alkylthio, aryl, arylalkyl, C_3 - C_8 cycloalkyl, C_4 - C_7 heterocycloalkyl, heteroarylalkyl and heteroaryl each R^a is independently H or C_1 - C_6 alkyl;

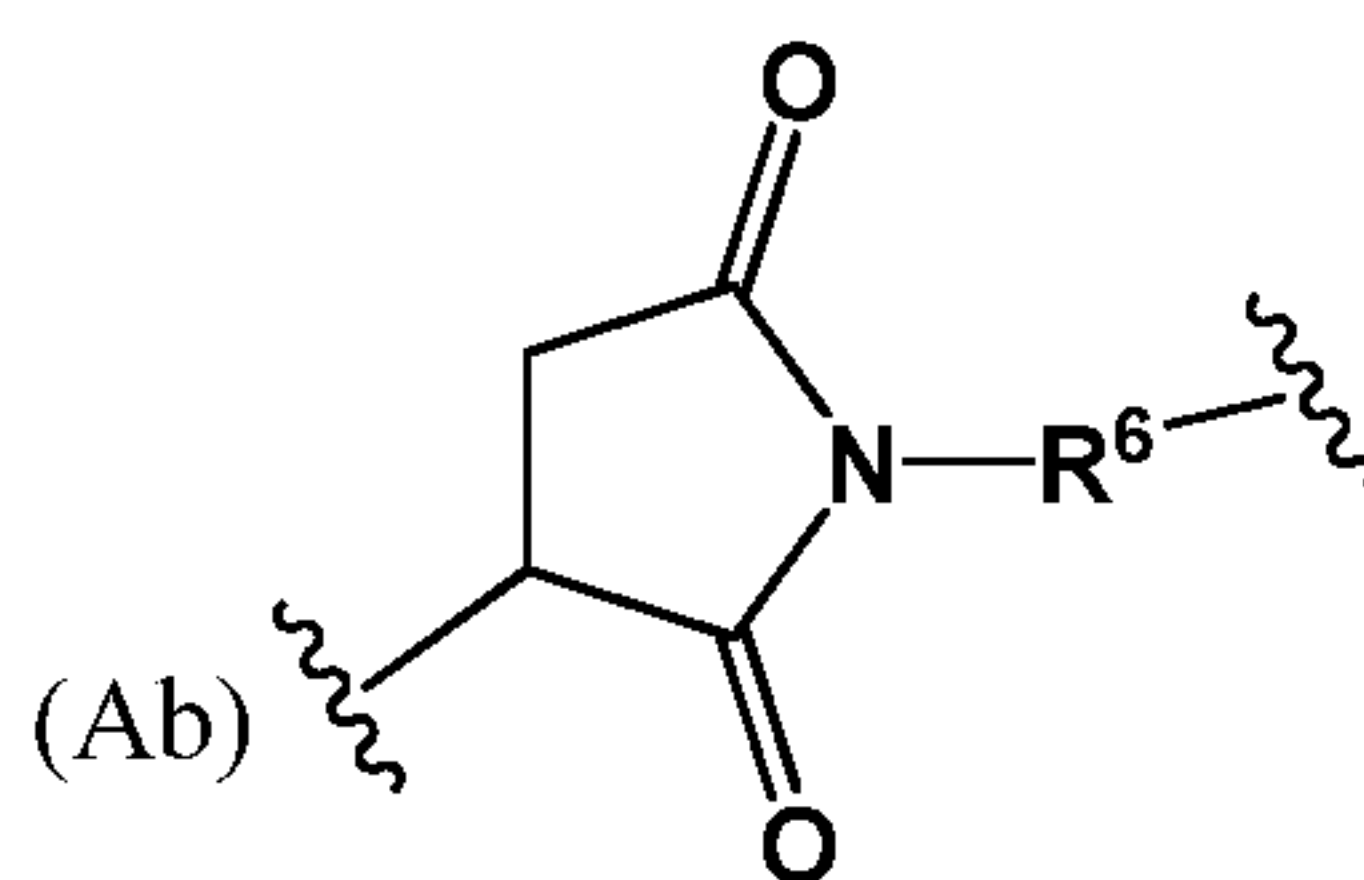
5 p is 1, 2, 3 or 4.

This invention also relates to antibody-conjugates of Formula (I) represented by the following
10 formula:



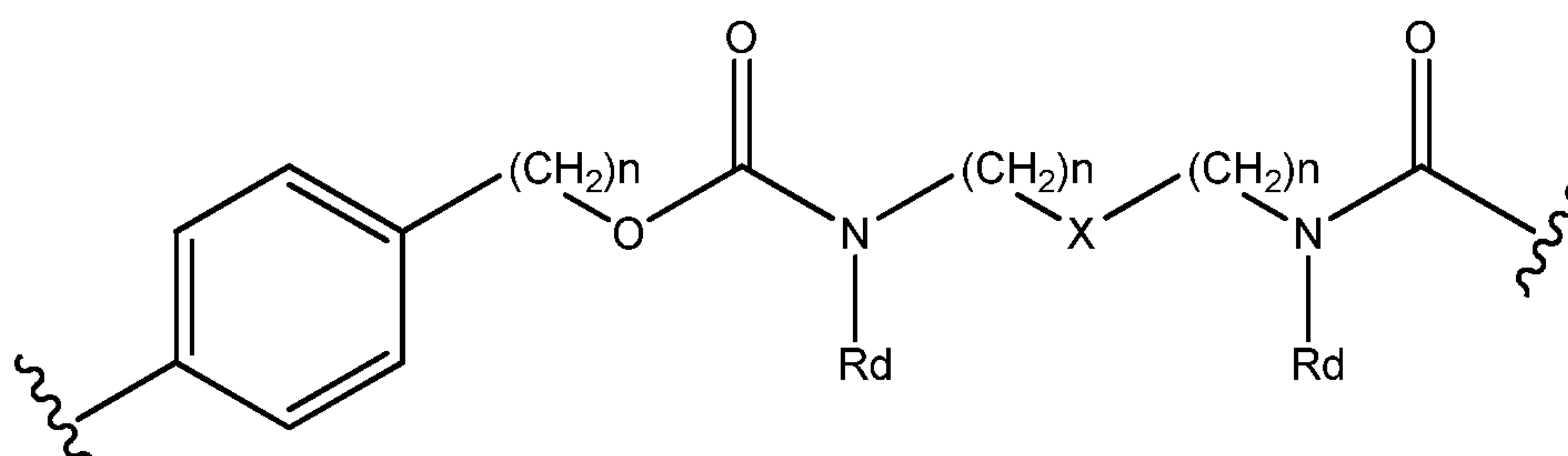
15 wherein

Str is a chemical moiety represented by the following formula:



wherein R^6 is selected from the group consisting of C_1 - C_{10} alkylene, and C_1 - C_{10} alkylene- $C(O)N(R^a)$ - C_2 - C_6 alkylene, where each alkylene may be substituted by one to five
20 substituents selected from the group consisting of halo, trifluoromethyl, difluoromethyl, amino, alkylamino, cyano, sulfonyl, sulfonamide, sulfoxide, hydroxy, alkoxy, ester, carboxylic acid, alkylthio, aryl, arylalkyl, C_3 - C_8 cycloalkyl, C_4 - C_7 heterocycloalkyl, heteroarylalkyl and heteroaryl each R^a is independently H or C_1 - C_6 alkyl;

Sp is $—Ar—R^b—$, wherein Ar is aryl or heteroaryl, R^b is $(C_1$ - C_{10} alkylene)O- or
25 Sp is the following formula



wherein

each n is independently 1-6;

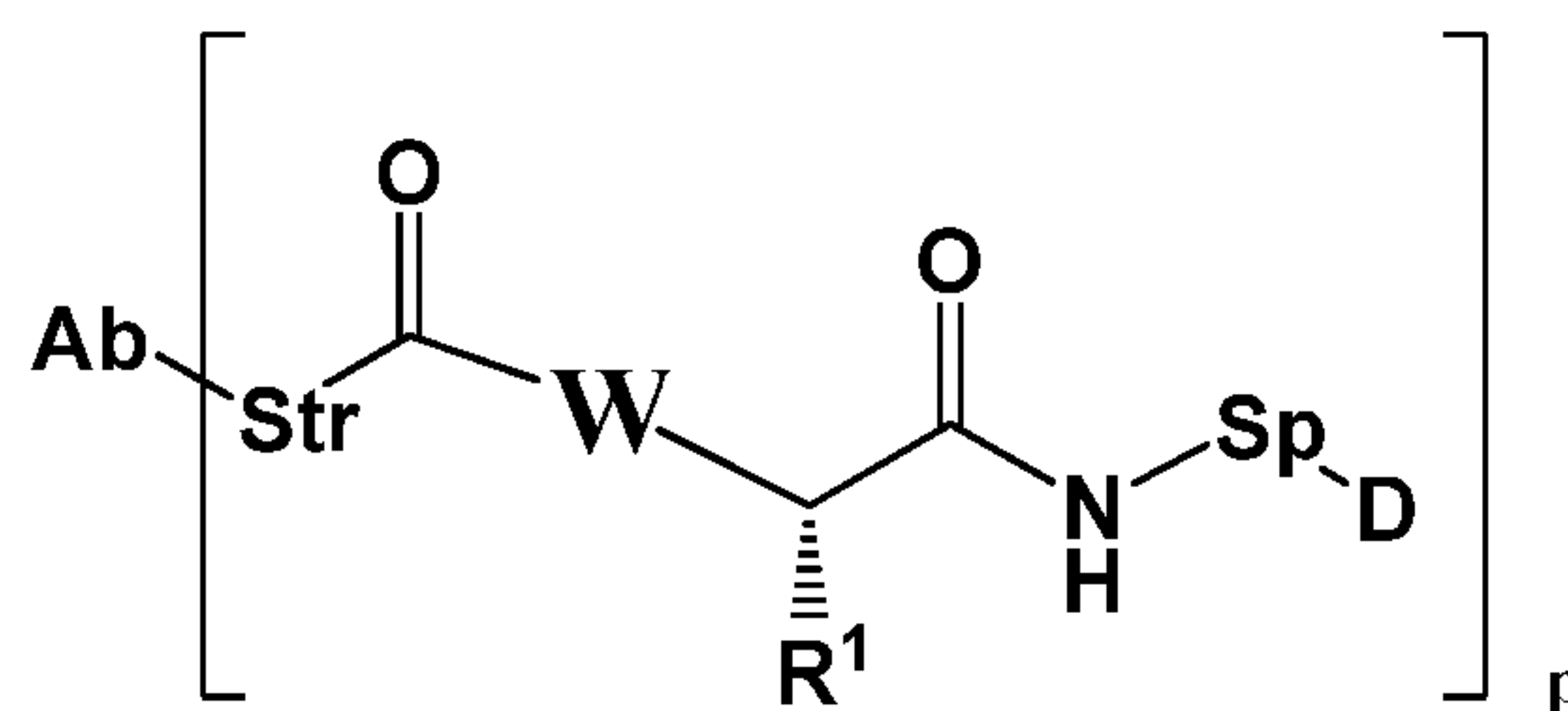
5 X is N, CH₂ or a bond;

each R^d is independently H or C₁-C₃alkyl; and

p is 1, 2, 3 or 4.

This invention also relates to any one of the above antibody-conjugates, wherein Y is heteroaryl, aryl or alkenyl; R⁶ is C₁-C₁₀alkylene.

10 This invention also relates to antibody-drug conjugates of Formula (I) represented by the following formula:

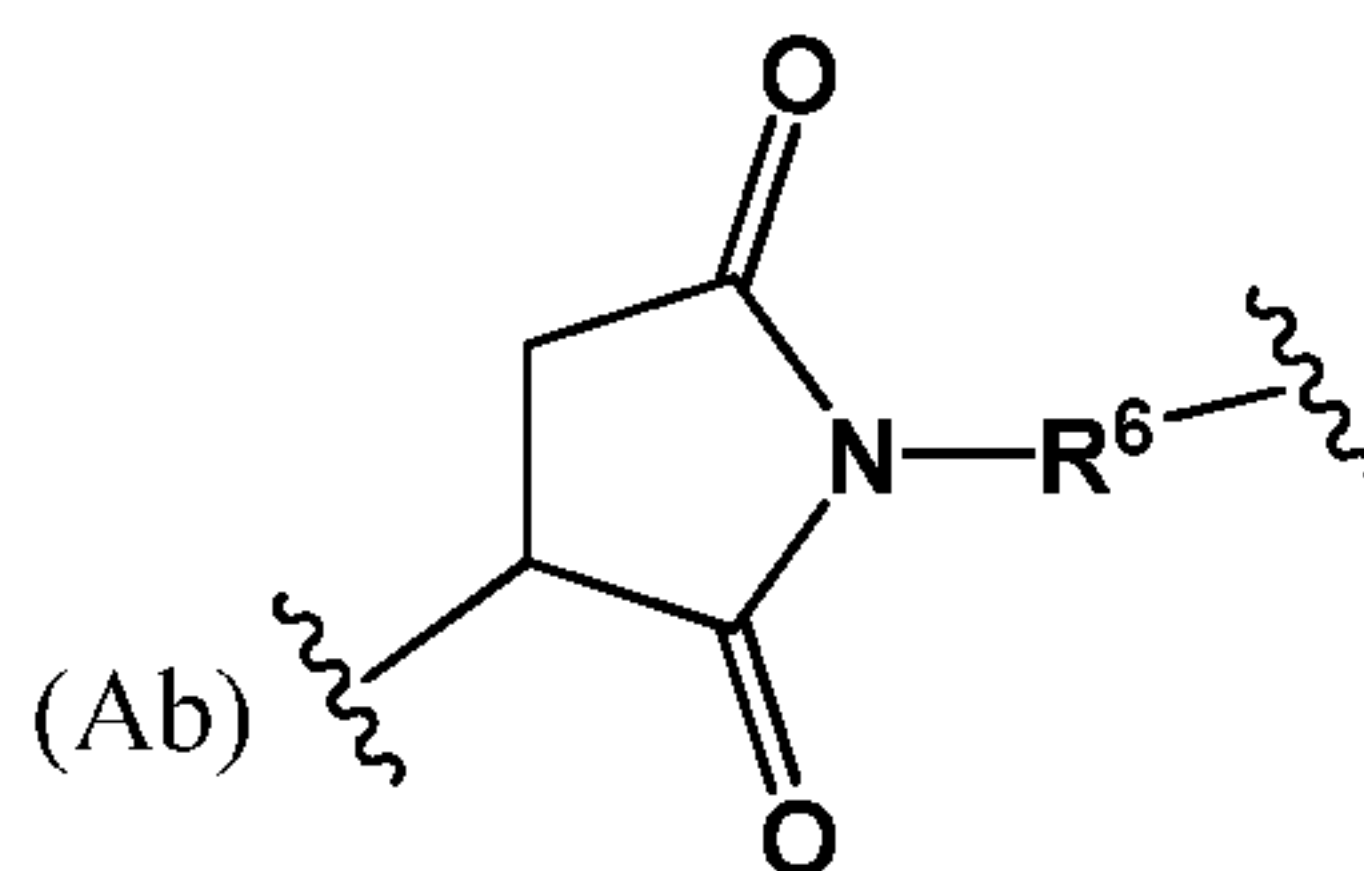


(I)(C1)

15

wherein

Str is a chemical moiety represented by the following formula:



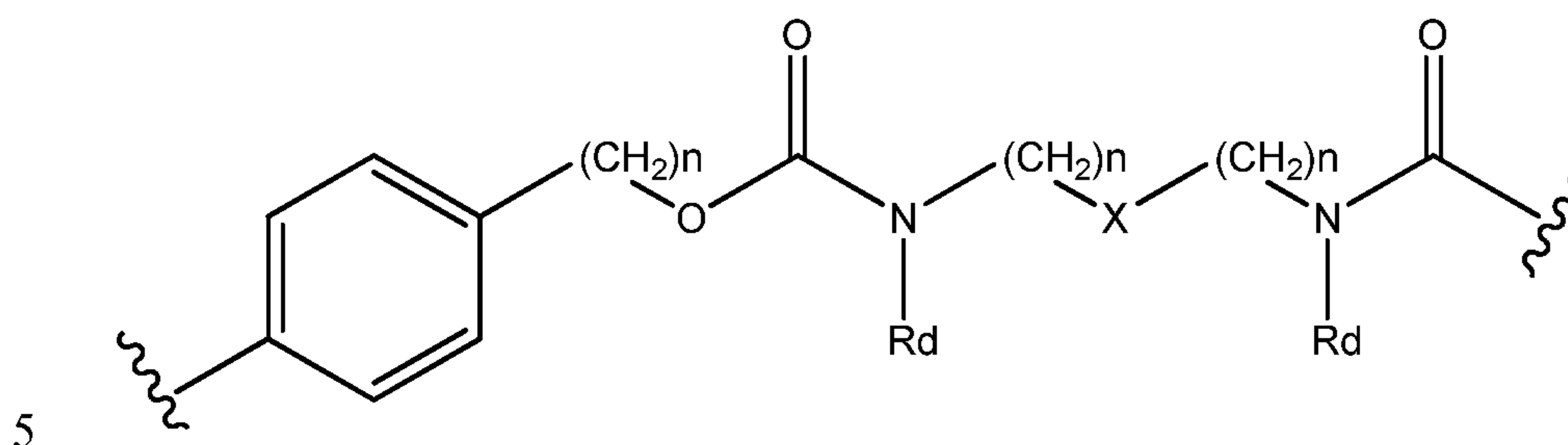
wherein R⁶ is selected from the group consisting of C₁-C₁₀alkylene, and C₁-

20 C₁₀alkylene-C(O)N(R^a)-C₂-C₆alkylene, where each alkylene may be substituted by one to five substituents selected from the group consisting of halo, trifluoromethyl, difluoromethyl, amino, alkylamino, cyano, sulfonyl, sulfonamide, sulfoxide, hydroxy, alkoxy, ester, carboxylic acid,

alkylthio, aryl, arylalkyl, C₃-C₈cycloalkyl, C₄-C₇heterocycloalkyl, aryl, arylalkyl, heteroarylalkyl and heteroaryl, each R^a is independently H or C₁-C₆alkyl;

Sp is —Ar—R^b—, wherein Ar is aryl or heteroaryl, R^b is (C₁-C₁₀alkylene)O- or

Sp is the following formula



wherein

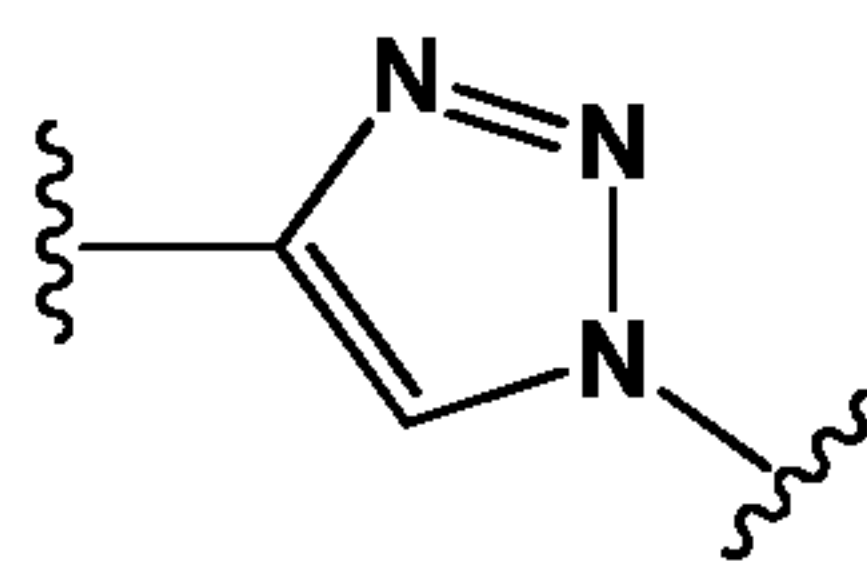
each n is independently 1-6;

X is N, CH₂ or a bond;

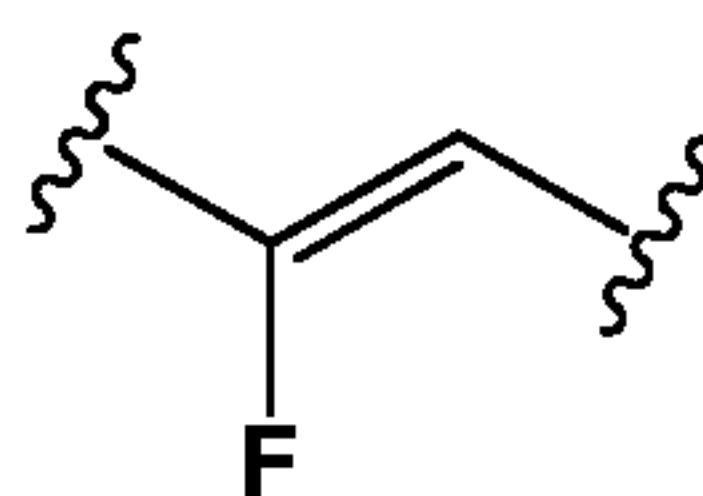
10 each R^d is independently H or C₁-C₃alkyl; and

p is 1, 2, 3 or 4.

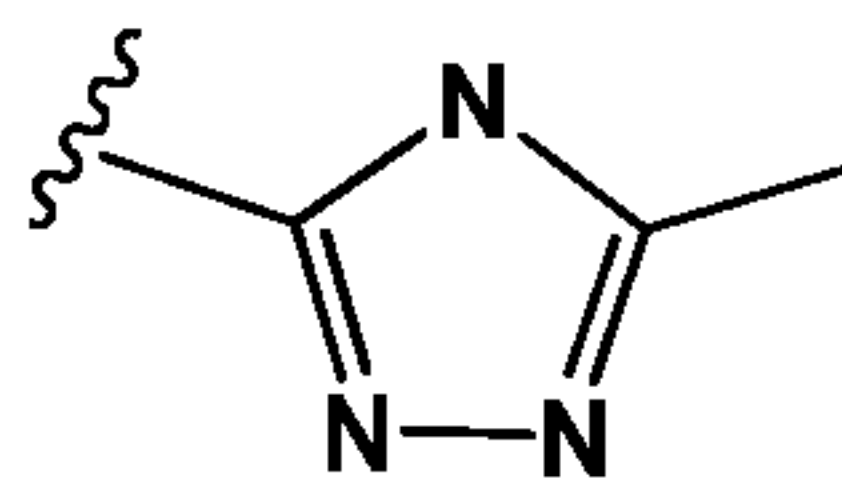
This invention also relates to any one of the above antibody-conjugates, wherein Y is



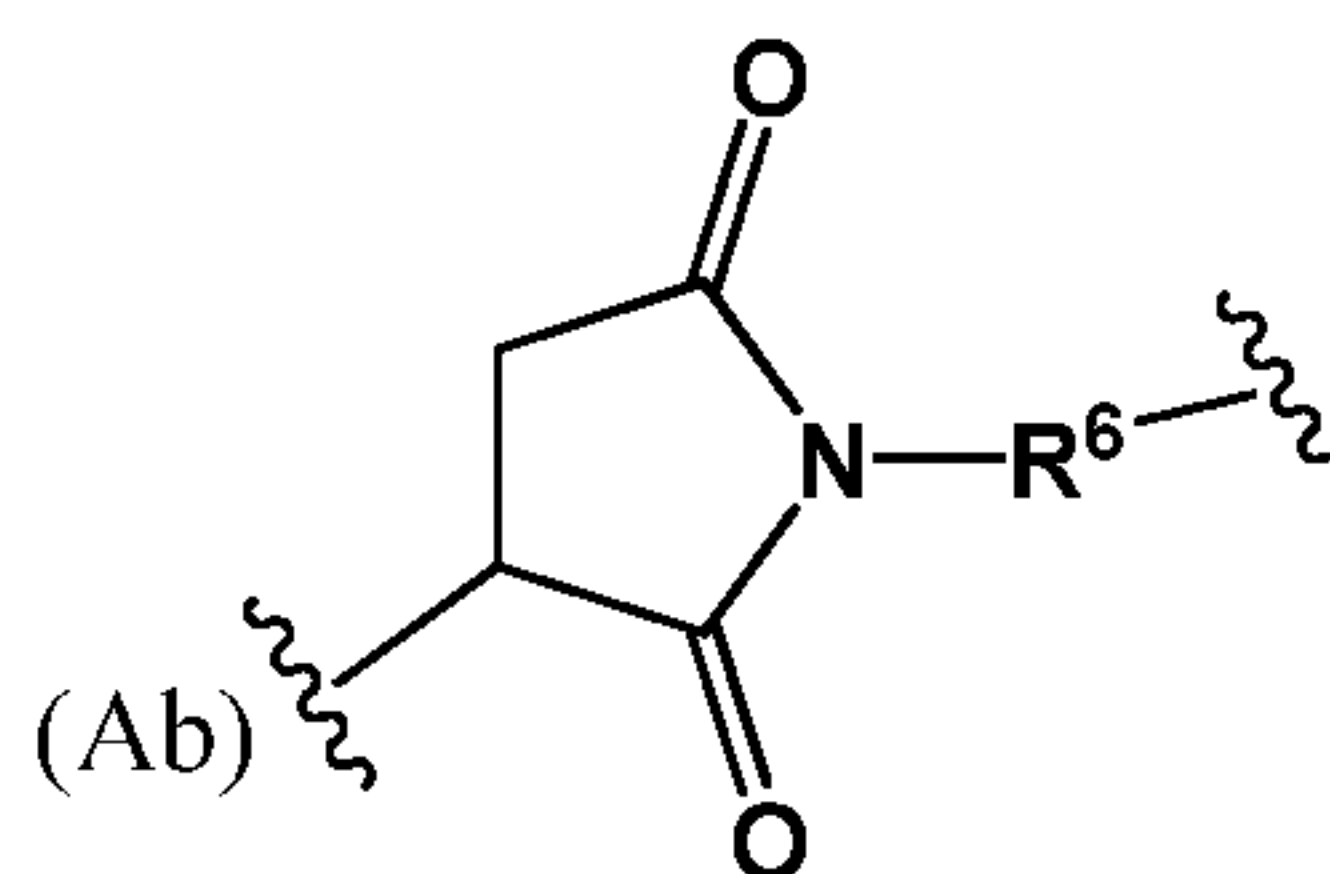
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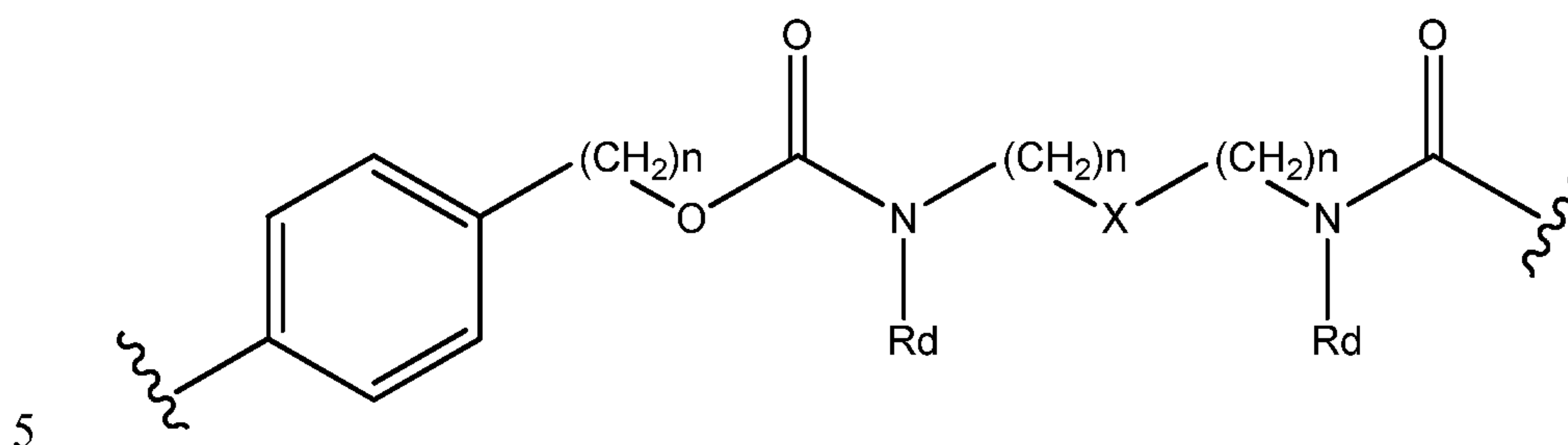


20 This invention also relates to any one of the above antibody-conjugates, wherein Str is a chemical moiety represented by the following formula:



R^6 is C_1 - C_6 alkylene;

Sp is $-\text{Ar}-R^b-$, wherein Ar is aryl or heteroaryl, R^b is $(C_1$ - C_{10} alkylene)O- or Sp is the following formula



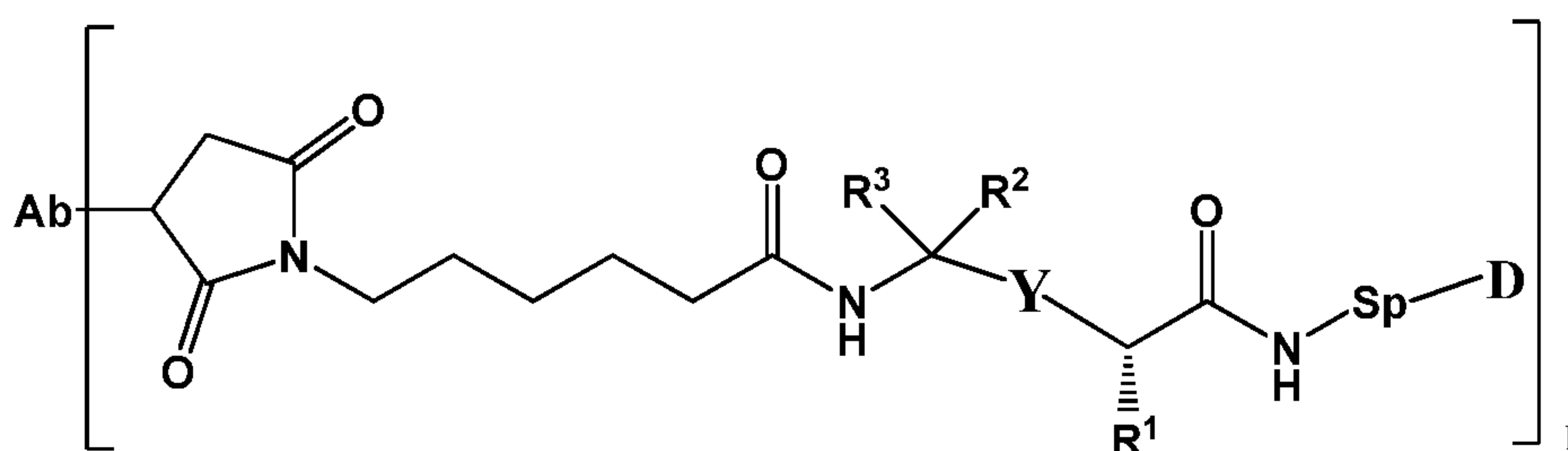
wherein

each n is independently 1-6;

X is N, CH_2 or a bond; and

10 each R^d is independently H or C_1 - C_3 alkyl.

This invention also relates to any one of the above antibody-conjugates (I), (I)(A1), represented by the following formula:



15

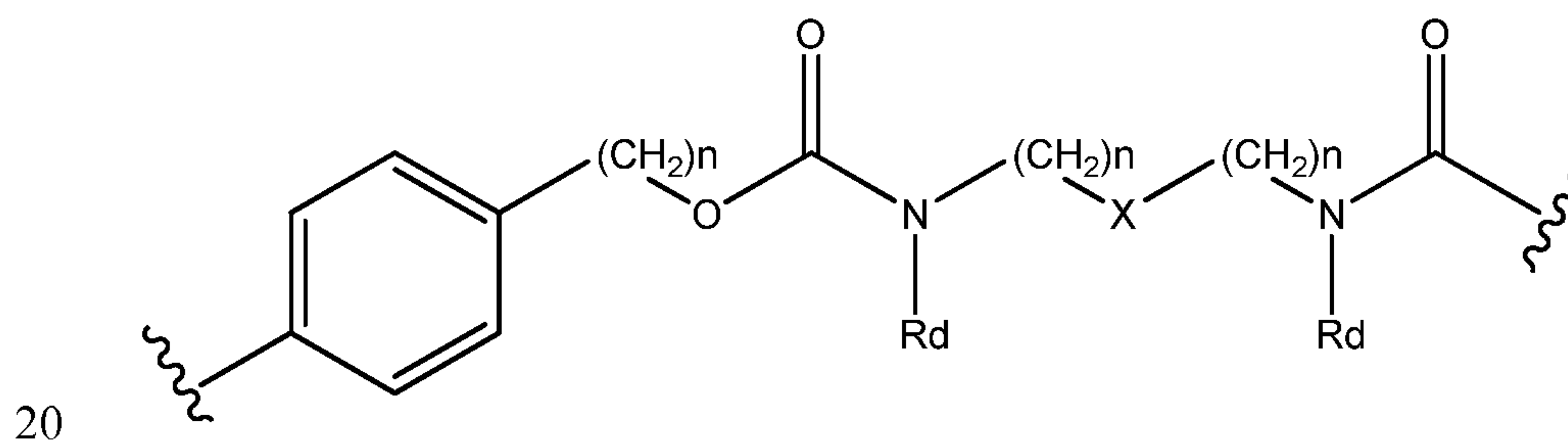
(I)(A2)

wherein

R^1 is C_1 - C_6 alkyl- NH_2 , $(C_1$ - C_6 alkyl) $\text{NHC}(\text{NH})\text{NH}_2$ or $(C_1$ - C_6 alkyl) $\text{NHC}(\text{O})\text{NH}_2$

p is 1, 2, 3 or 4;

Sp is the following formula



wherein

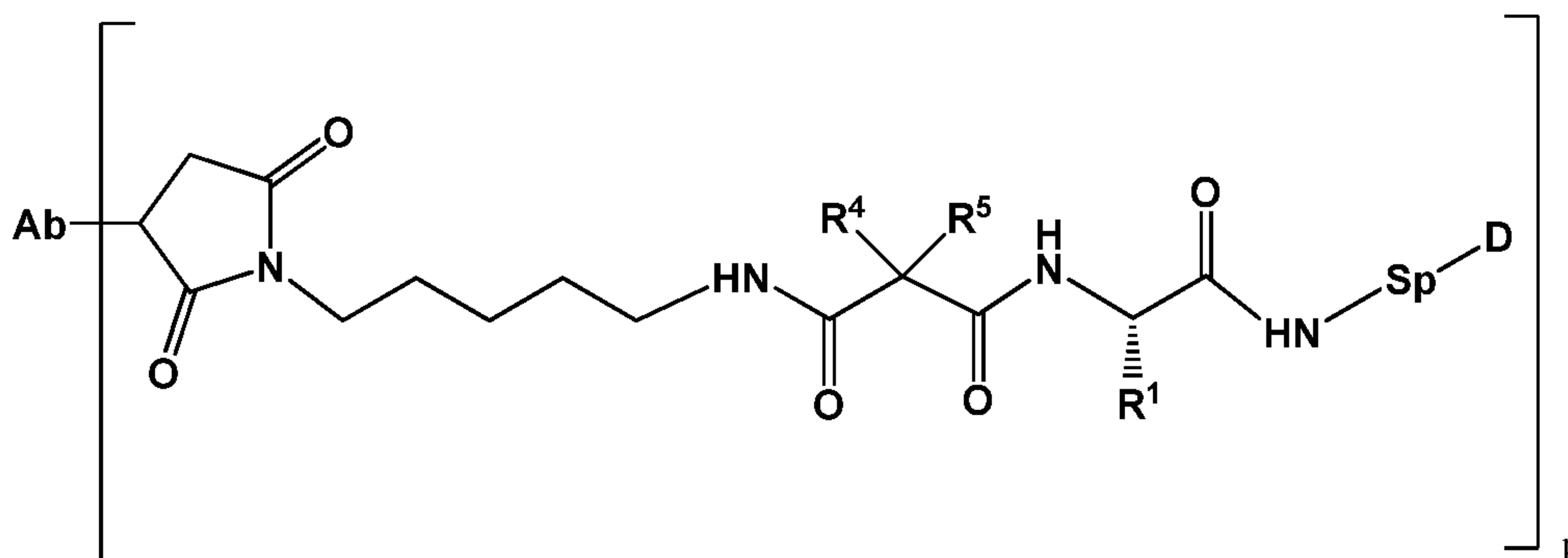
each n is independently 1-6;

X is N, CH₂ or a bond; and

each R^d is independently H or C₁-C₃alkyl.

5

This invention also relates to any one of the above antibody-conjugates (I), (I)(B1), represented by the following formula:



(I)(B2)

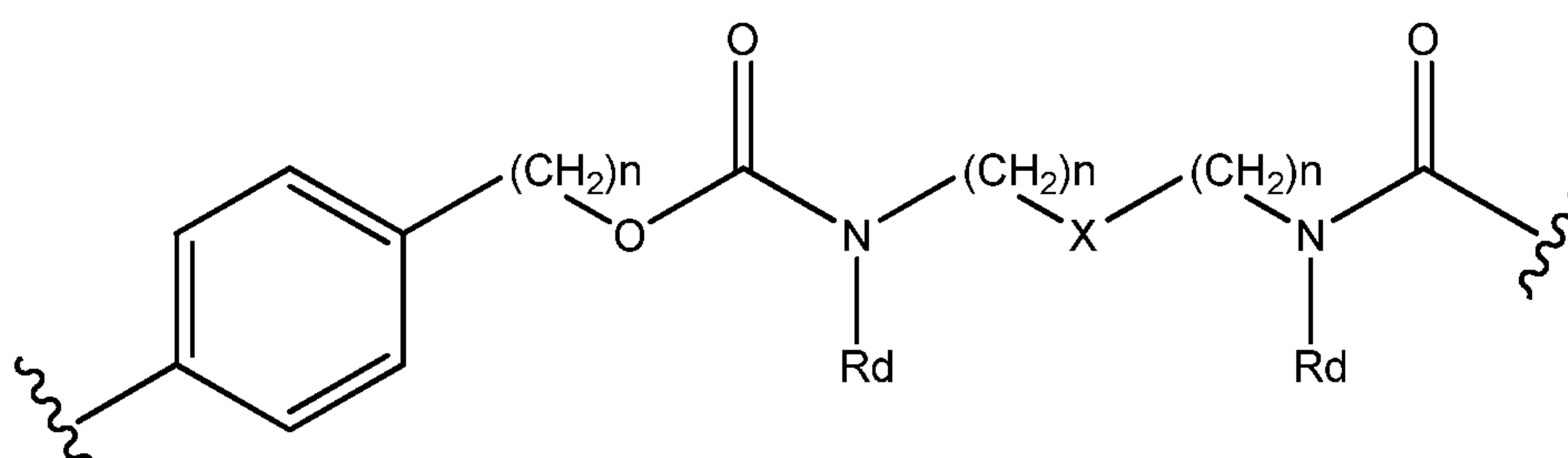
10 wherein

p is 1, 2, 3 or 4;

R¹ is C₁-C₆alkyl-NH₂, (C₁-C₆alkyl)NHC(NH)NH₂ or (C₁-C₆alkyl)NHC(O)NH₂;

R⁴ and R⁵ are each independently C₁-C₆alkyl, wherein said alkyl are unsubstituted, or R⁴ and R⁵ may form a C₃-C₇cycloalkyl ring; and

15 Sp is the following formula



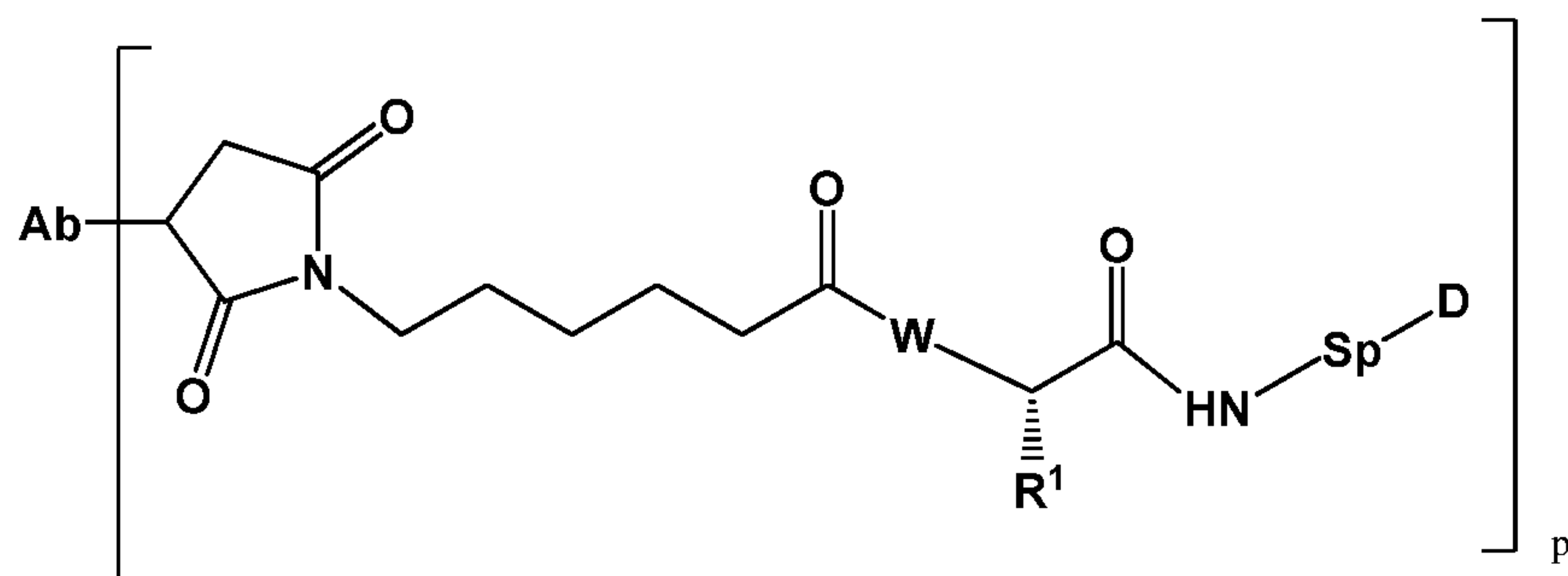
wherein

each n is independently 1-6;

20 X is N, CH₂ or a bond; and

each R^d is independently H or C₁-C₃alkyl.

This invention also relates to any one of the above antibody-conjugates (I) and (I)(C1), represented by the following formula:



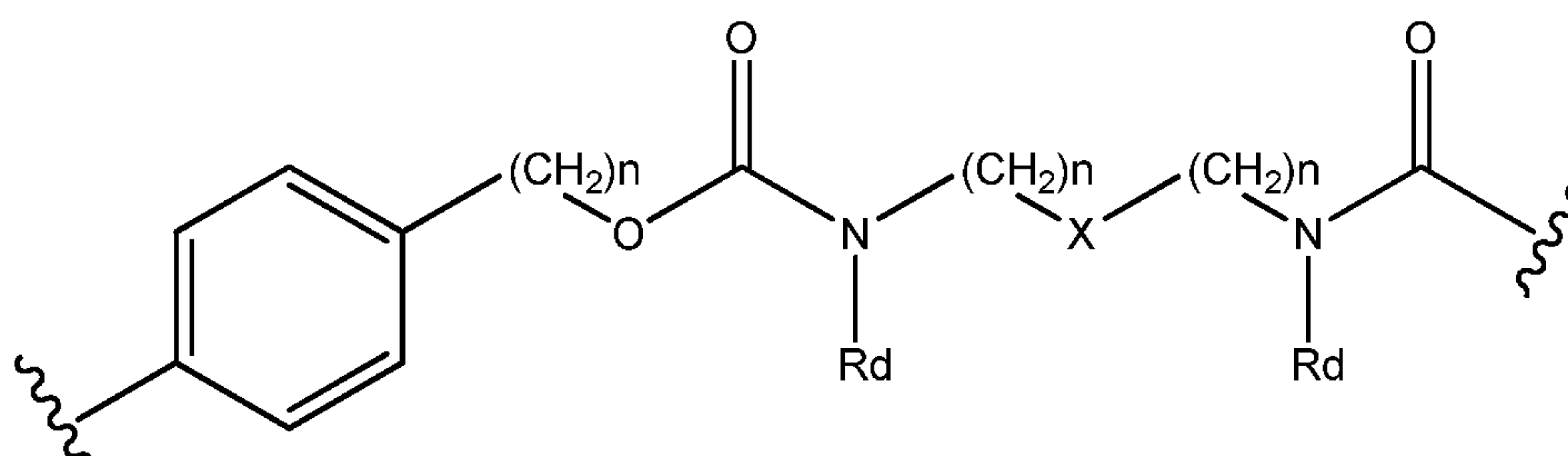
(I)(C2)

wherein

p is 1, 2, 3 or 4;

5 R^1 is C_1 - C_6 alkyl- NH_2 , $(C_1$ - C_6 alkyl) $NHC(NH)NH_2$ or $(C_1$ - C_6 alkyl) $NHC(O)NH_2$;

Sp is the following formula



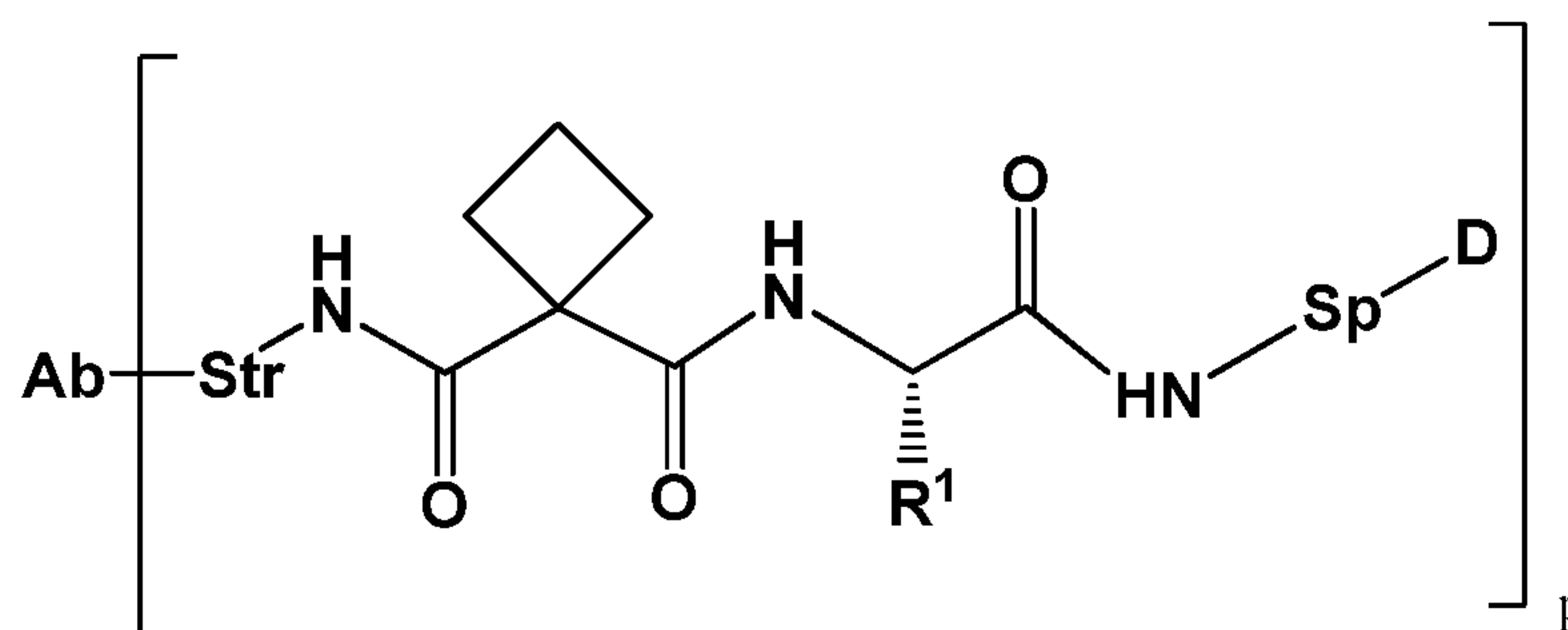
wherein

10 each n is independently 1-6;

X is N, CH_2 or a bond; and

each R^d is independently H or C_1 - C_3 alkyl.

This invention also relates to antibody-conjugates of formula (I), which is represented by the
15 following formula:



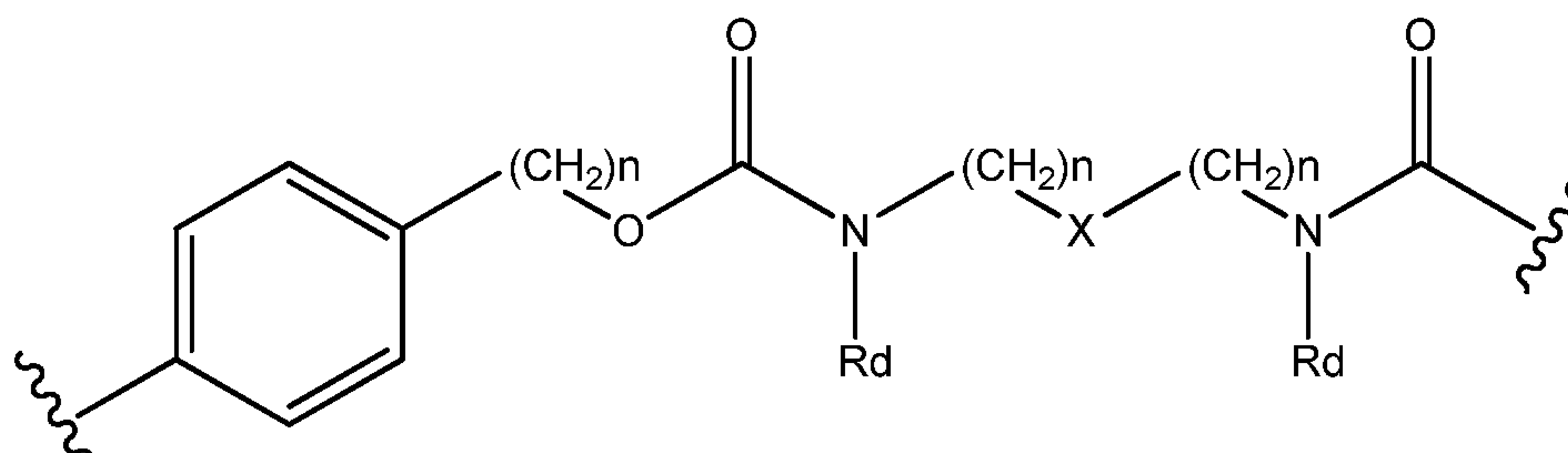
(I)(B3)

wherein

p is 1, 2, 3 or 4;

20 R^1 is C_1 - C_6 alkyl- NH_2 , $(C_1$ - C_6 alkyl) $NHC(NH)NH_2$ or $(C_1$ - C_6 alkyl) $NHC(O)NH_2$; and

Sp is the following formula

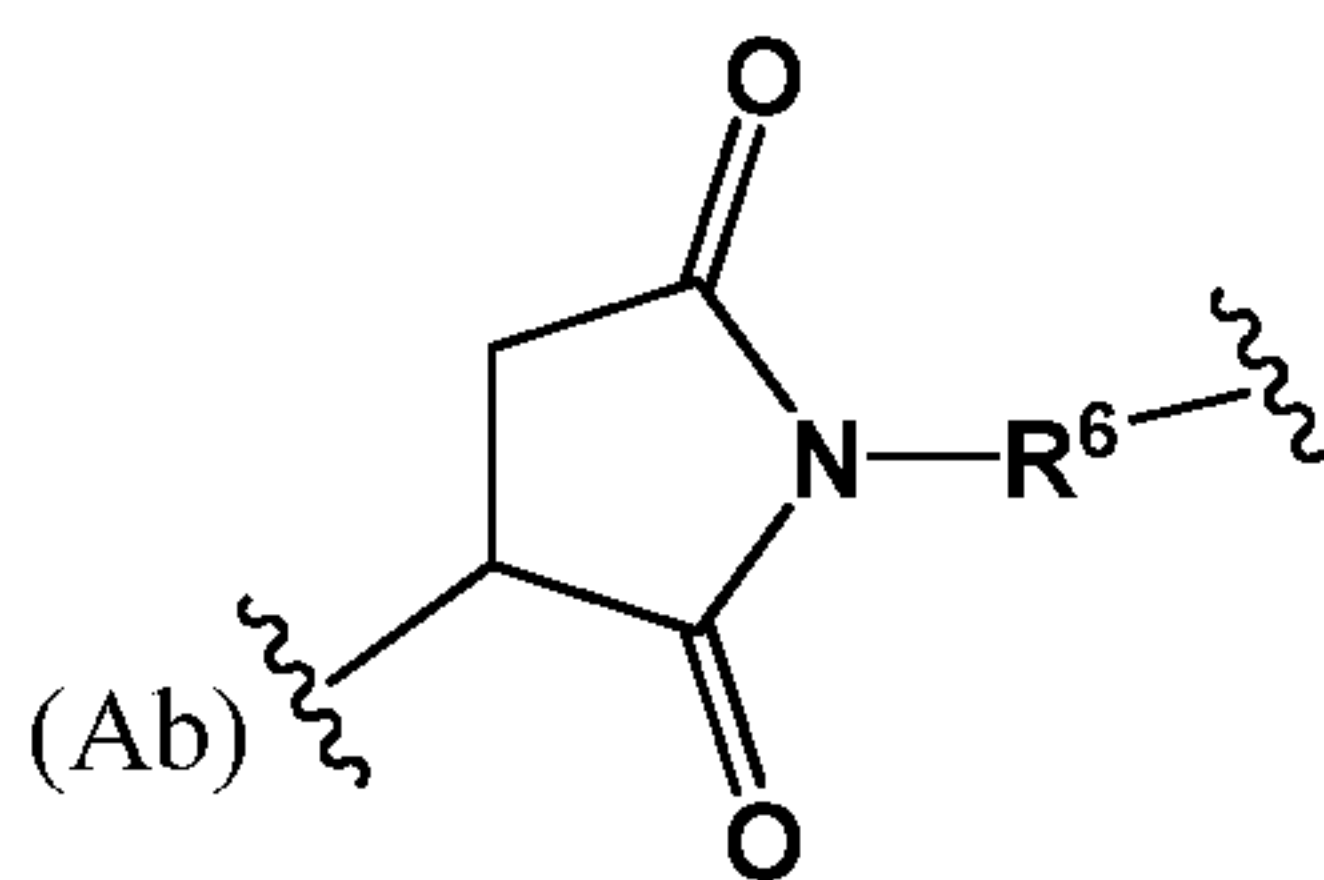


wherein

- 5 each n is independently 1-6;
 X is N, CH₂ or a bond; and
 each R^d is independently H or C₁-C₃alkyl.

This invention also relates to antibody-drug conjugates of (I)(B3), wherein

- 10 Str is a chemical moiety represented by the following formula:



R⁶ is C₁-C₆alkylene which may be substituted with 1-3 groups selected from aryl and heteroaryl;

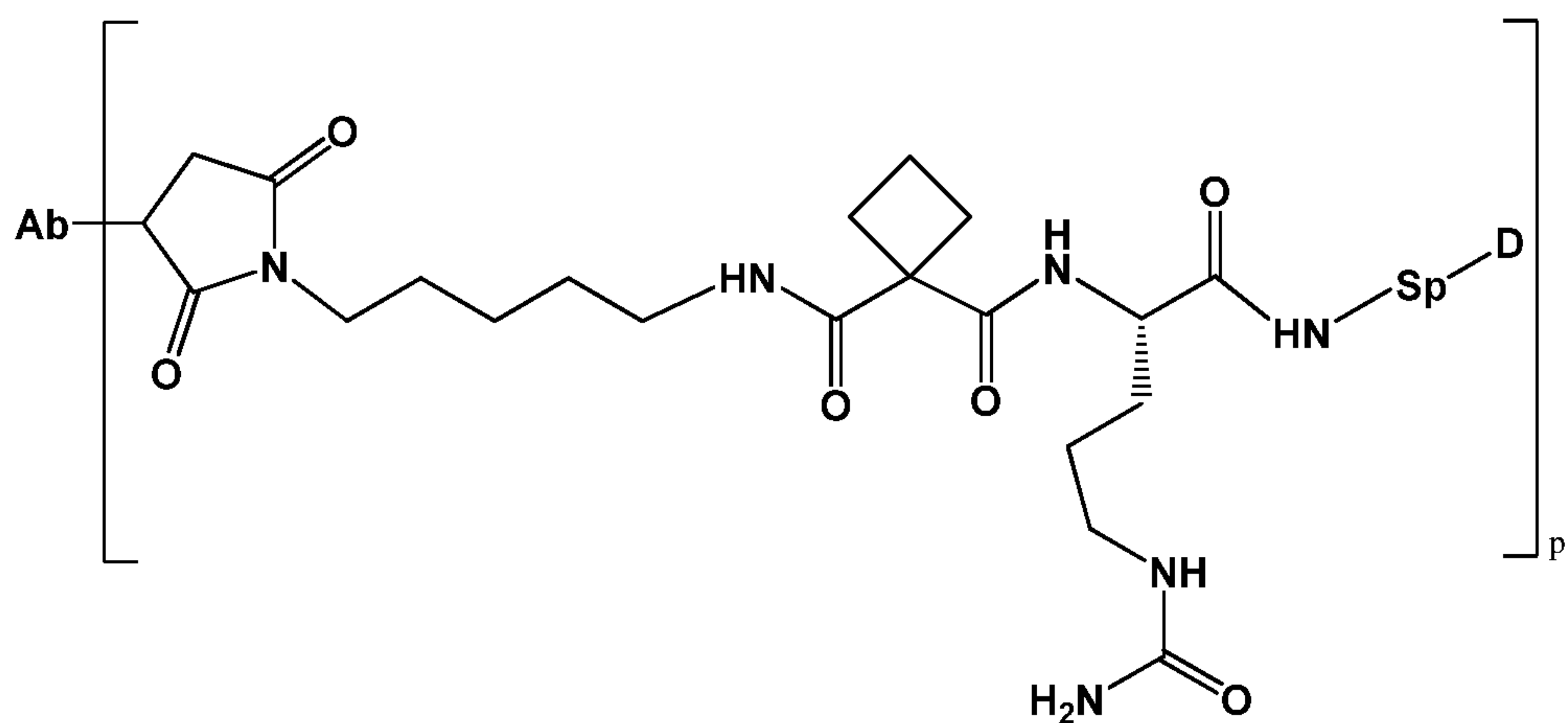
- 15 This invention also relates to antibody-drug conjugates of (I)(B3) wherein R¹ is (CH₂)₃NHC(O)NH₂.

This invention also relates to antibody-drug conjugates of (I)(B3) wherein R¹ is (CH₂)₄NH₂.

- 20 This invention also relates to antibody-drug conjugates of (I), (I)(B1), (I)(B2) and (I)(B3), wherein R¹ is (C₁-C₆alkyl)NHC(NH)NH₂.

This invention also relates to antibody-drug conjugates of formula (I), which is represented by the following formula:

25



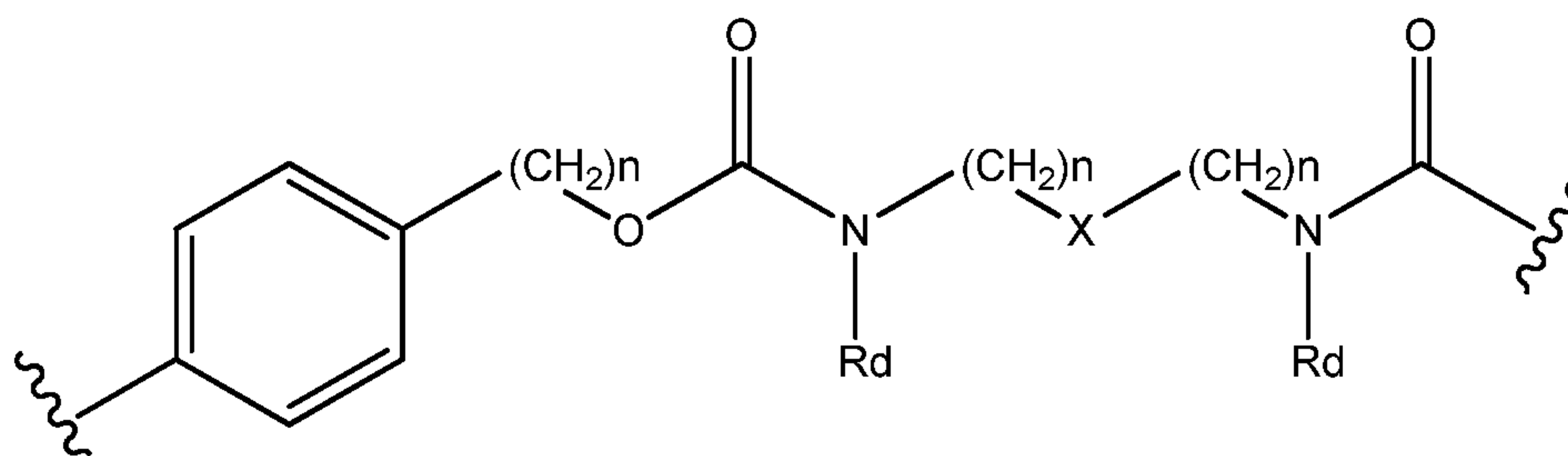
(I)(B4)

wherein,

Ab is an antibody that binds to a target selected from Her2, CLL1, CD33, CD22 and NaPi2b;

5 P is 1-4; and

Sp is the following formula



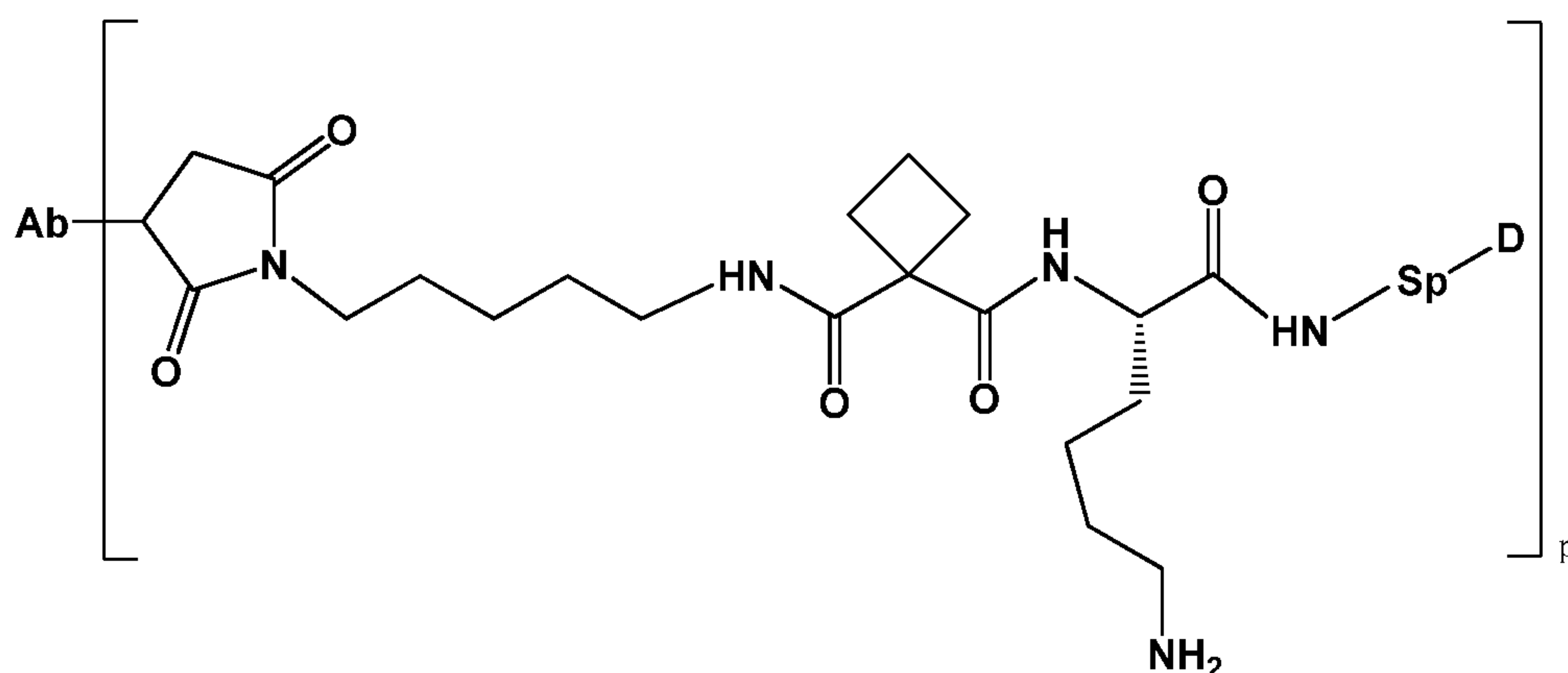
wherein

10 each n is independently 1-6;

X is N, CH₂ or a bond; and

each R^d is independently H or C₁-C₃alkyl.

15 This invention also relates to antibody-drug conjugates of formula (I), which is represented by the following formula:



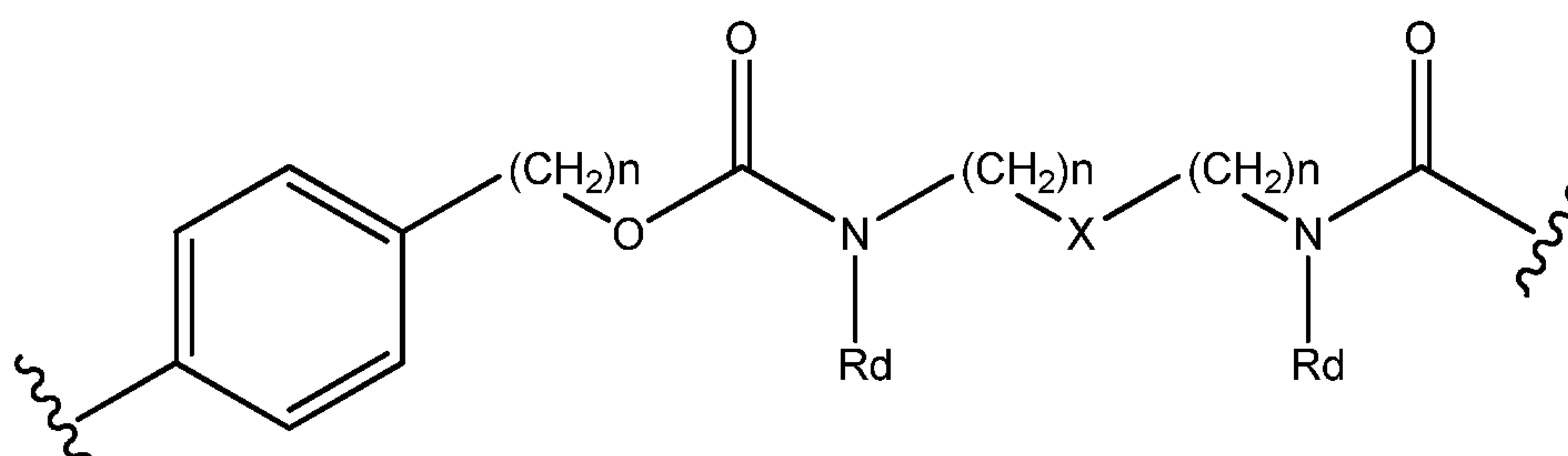
(I)(B5)

wherein,

Ab is an antibody that binds to a target selected from Her2, CLL1, CD33, CD22 and NaPi2b;

5 P is 1-4; and

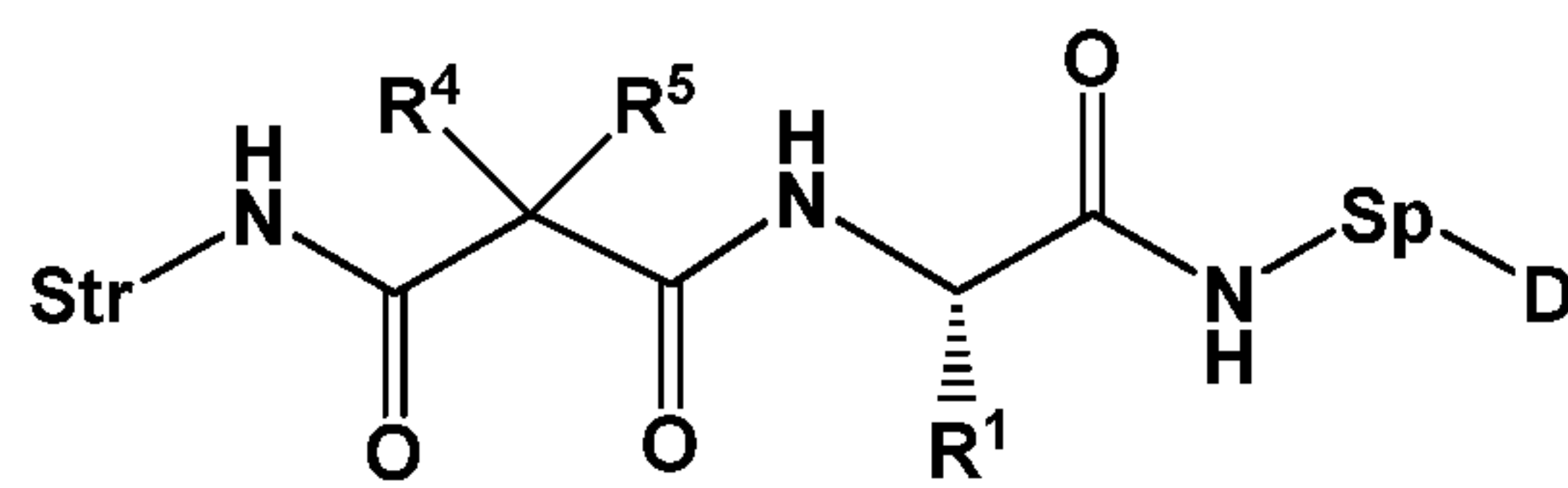
Sp is the following formula



wherein

10 each n is independently 1-6;

This invention also relates to non-peptide compounds of Formula (I)(B)(LD1):



(I)(B)(LD1)

15

wherein

Str is a stretcher unit which can be covalently attached to an antibody;

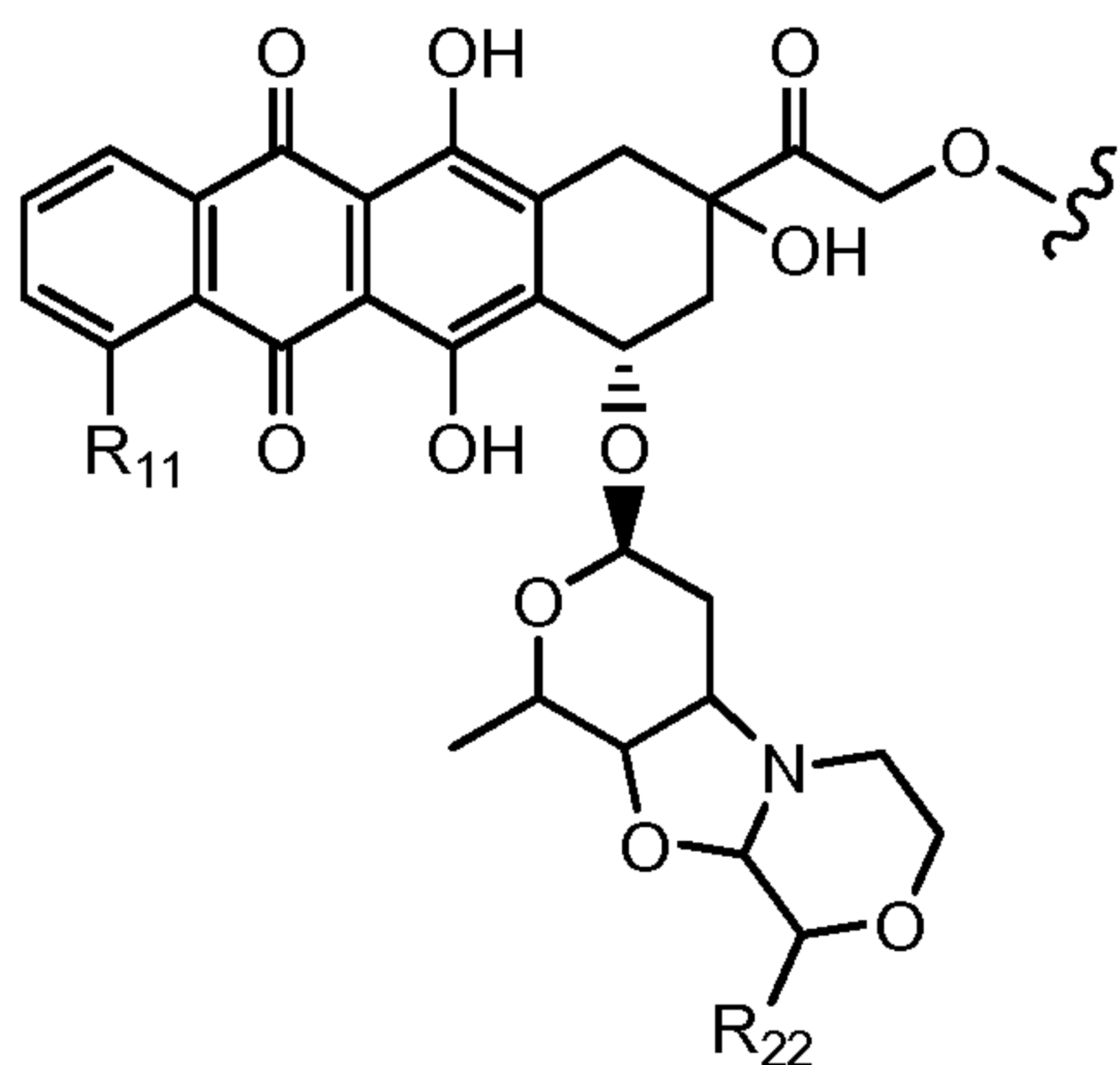
Sp is a bond or a spacer unit covalently attached to a drug moiety;

R¹ is C₁-C₁₀alkyl, (C₁-C₁₀alkyl)NHC(NH)NH₂ or (C₁-C₁₀alkyl)NHC(O)NH₂;

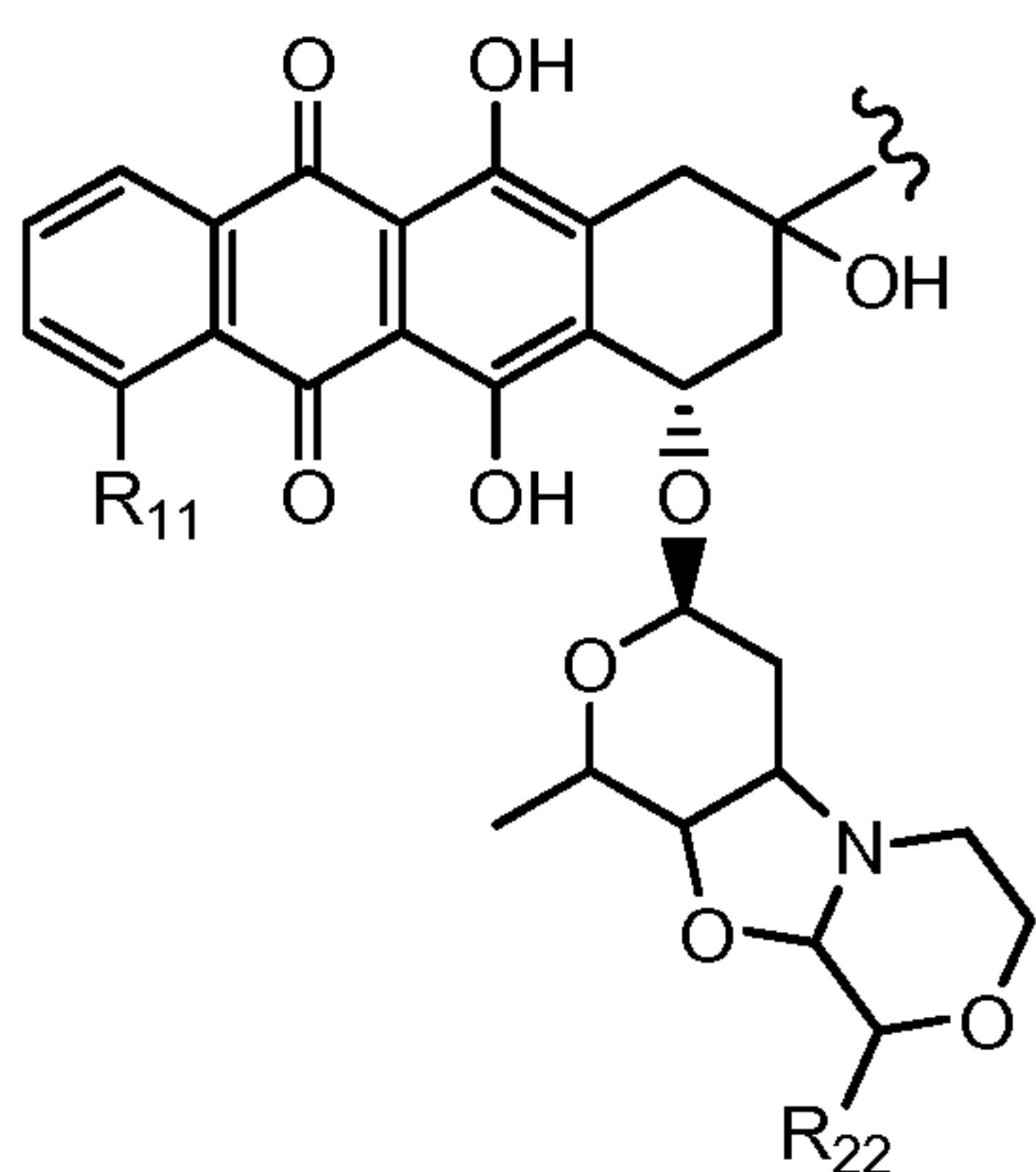
20 R⁴ and R⁵ are each independently C₁-C₁₀alkyl, arylalkyl, heteroarylalkyl, (C₁-C₁₀alkyl)OCH₂-, or

R⁴ and R⁵ may form a C₃-C₇cycloalkyl ring;

D is a drug moiety of Formula (IA) or (IB)



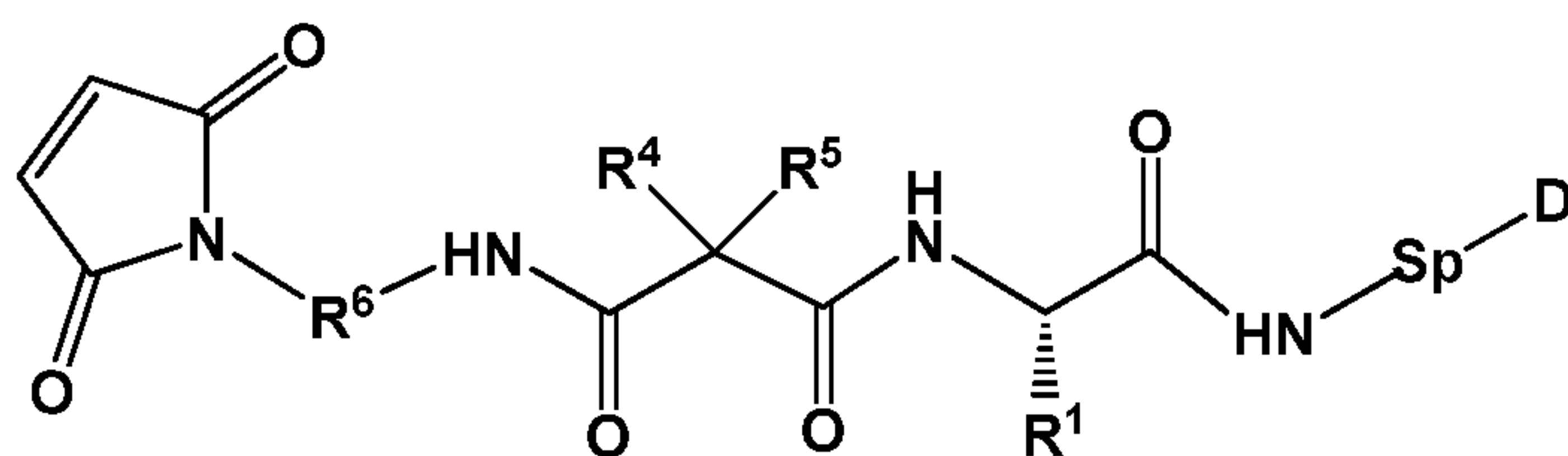
(IA)



5 (IB)

wherein R^{11} is hydrogen atom, hydroxy or methoxy group and R^{22} is a C_1 - C_5 alkoxy group, or a pharmaceutically acceptable salt thereof.

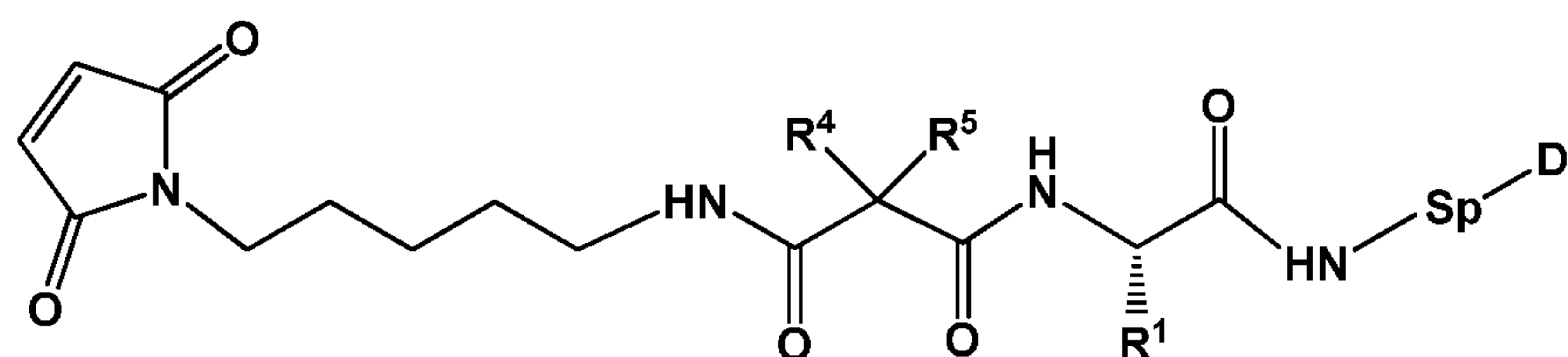
10 This invention also relates to non-peptide compounds represented by the following formula



(I)(B)(LD2)

wherein R_6 is C_1 - C_{10} alkylene; R^4 and R^5 together form a C_3 - C_7 cycloalkyl ring.

15 This invention also relates to non-peptide compounds represented by the following formula



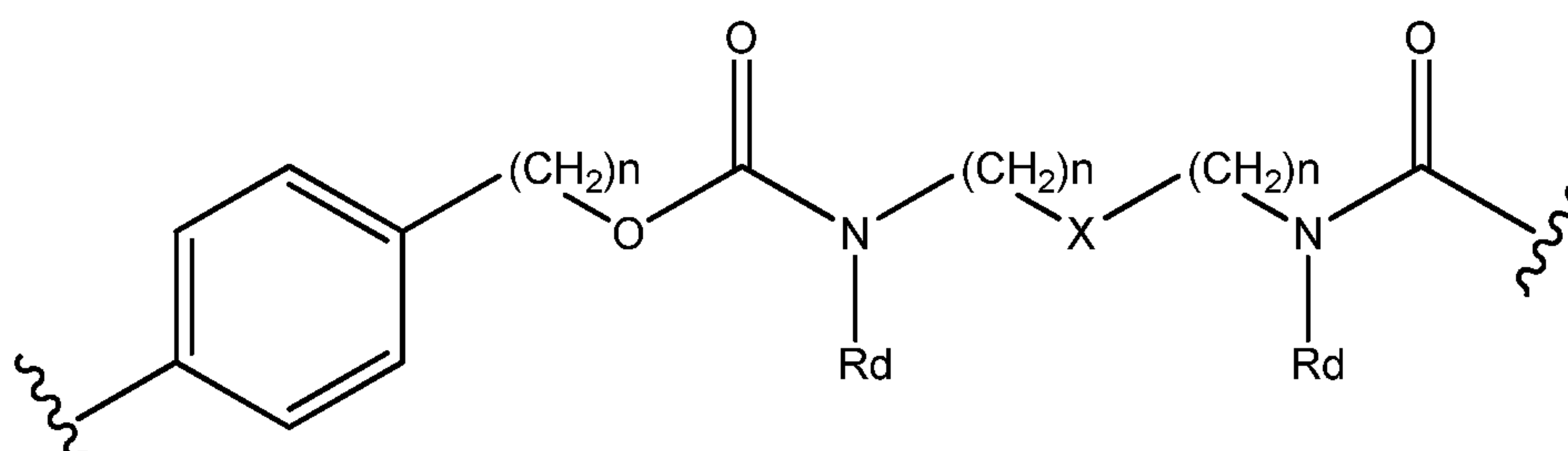
(I)(B)(LD3)

wherein

R^1 is C_1 - C_6 alkyl- NH_2 , $(C_1$ - C_6 alkyl) $NHC(NH)NH_2$ or $(C_1$ - C_6 alkyl) $NHC(O)NH_2$;

5 R^4 and R^5 are each independently C_1 - C_6 alkyl, wherein said alkyl are unsubstituted, or R^4 and R^5 may form a C_3 - C_7 cycloalkyl ring; and

Sp is the following formula



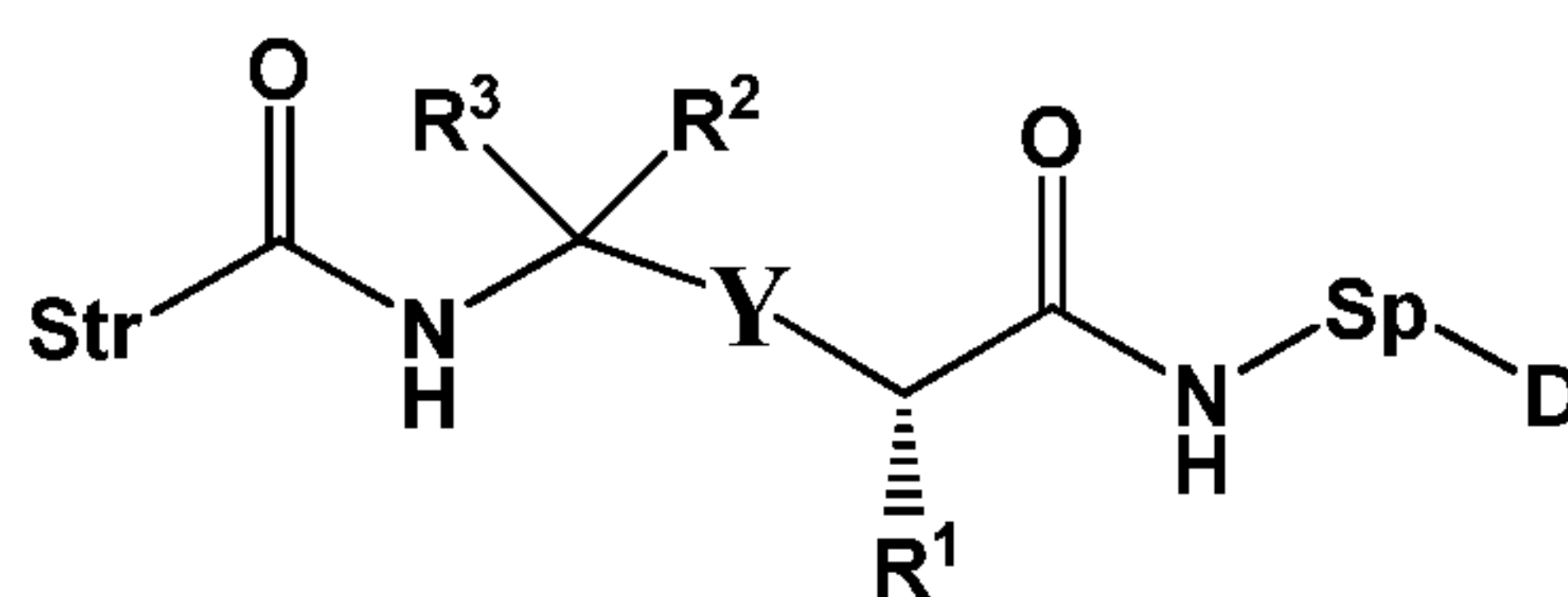
10 wherein

each n is independently 1-6;

X is N , CH_2 or a bond; and

each R^d is independently H or C_1 - C_3 alkyl.

15 This invention also relates to non-peptide compounds of Formula:



(I)(A)(LD1)

wherein

Str is a stretcher unit which can be covalently attached to an antibody;

20 Sp is an optional spacer unit covalently attached to a drug moiety;

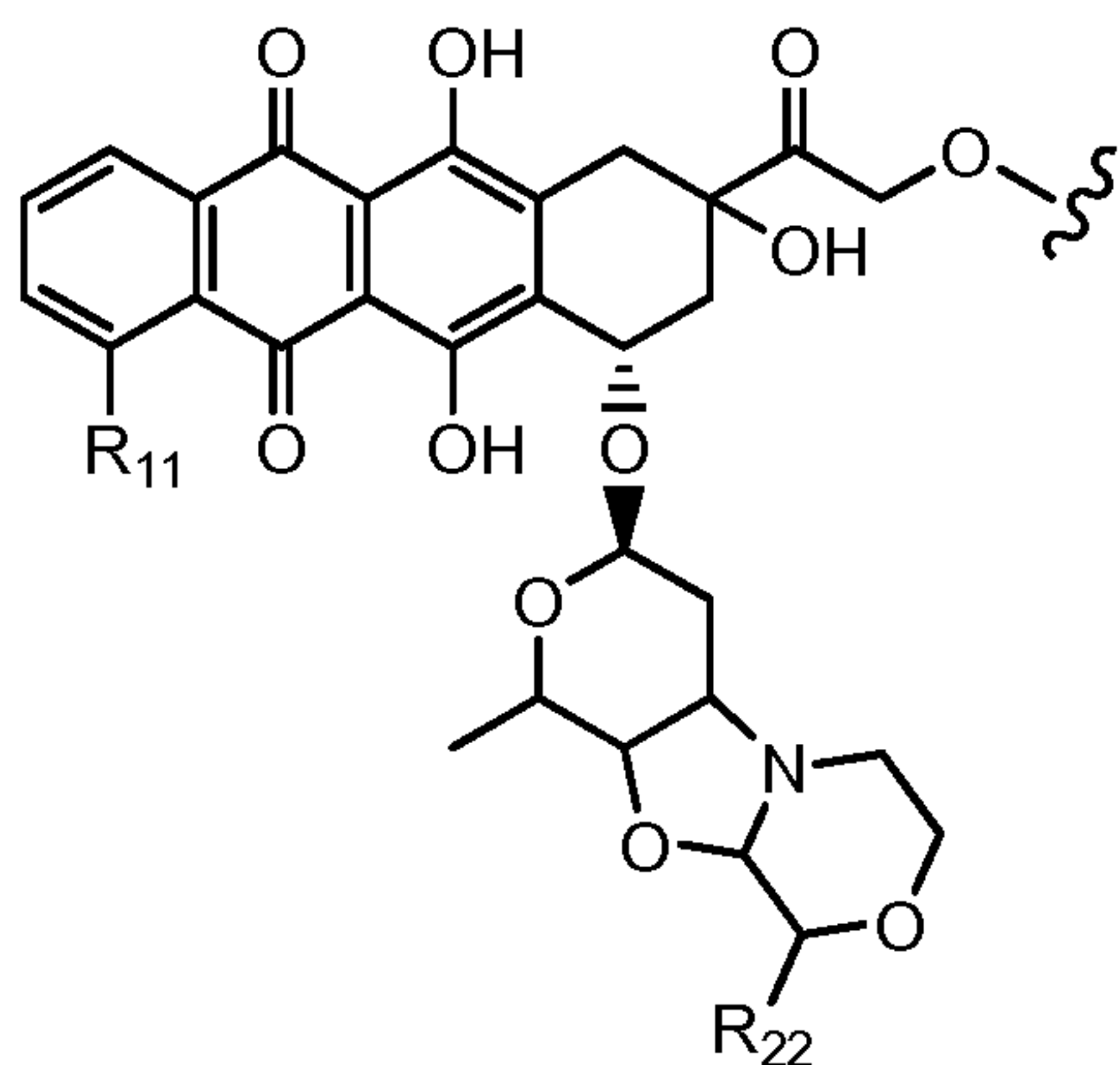
Y is heteroaryl, aryl, $-C(O)C_1$ - C_6 alkylene, C_1 - C_6 alkylene- NH_2 , C_1 - C_6 alkylene- $NH-CH_3$, C_1 - C_6 alkylene- $N-(CH_3)_2$, C_1 - C_6 alkenyl or C_1 - C_6 alkylenyl;

R^1 is C_1 - C_{10} alkyl, $(C_1$ - C_{10} alkyl) $NHC(NH)NH_2$ or $(C_1$ - C_{10} alkyl) $NHC(O)NH_2$;

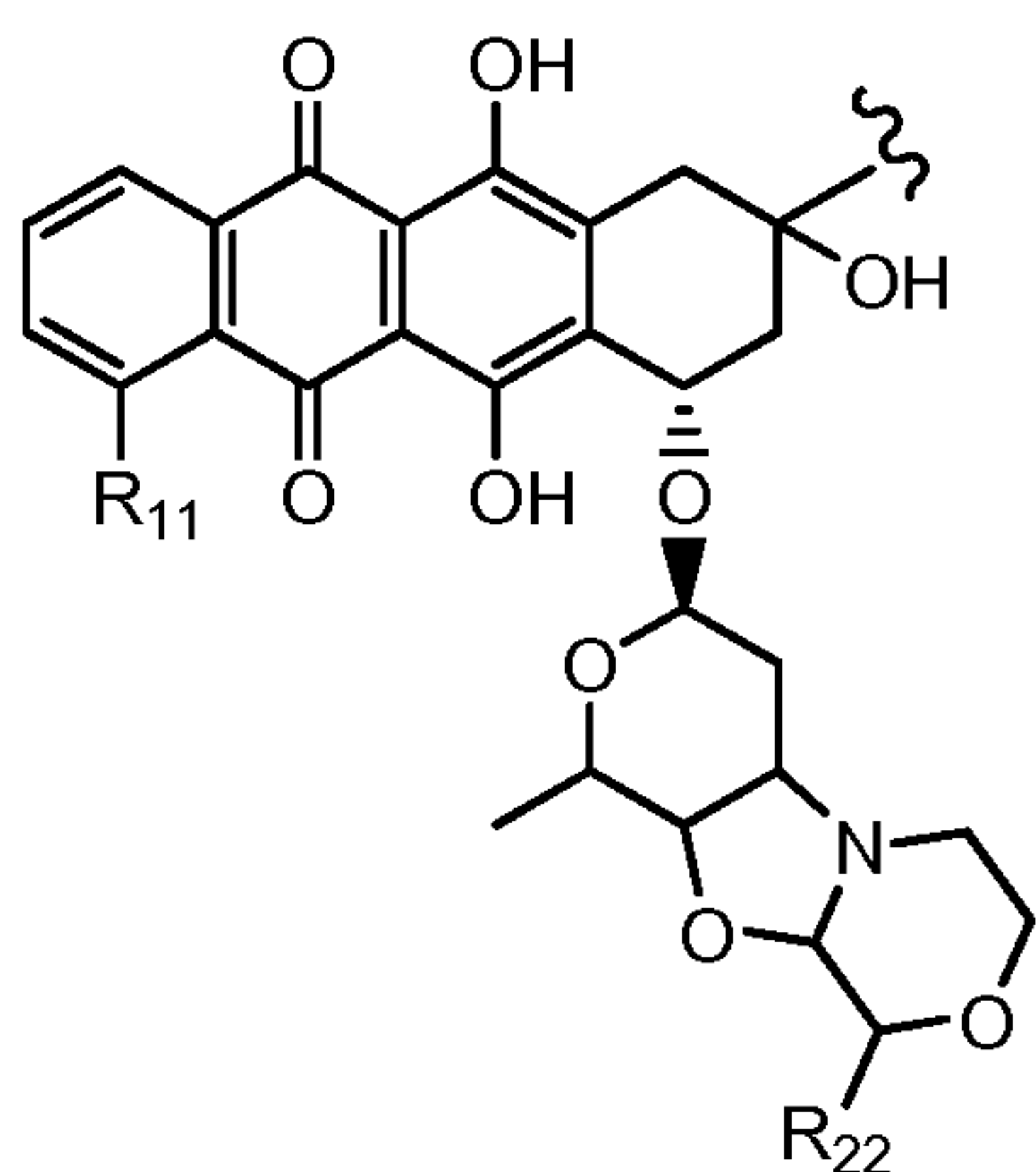
R^3 and R^2 are each independently H , C_1 - C_{10} alkyl, arylalkyl or heteroarylalkyl, or R^3 and R^2

25 together may form a C_3 - C_7 cycloalkyl;

D is a drug moiety of Formula (IA) or (IB)



(IA)

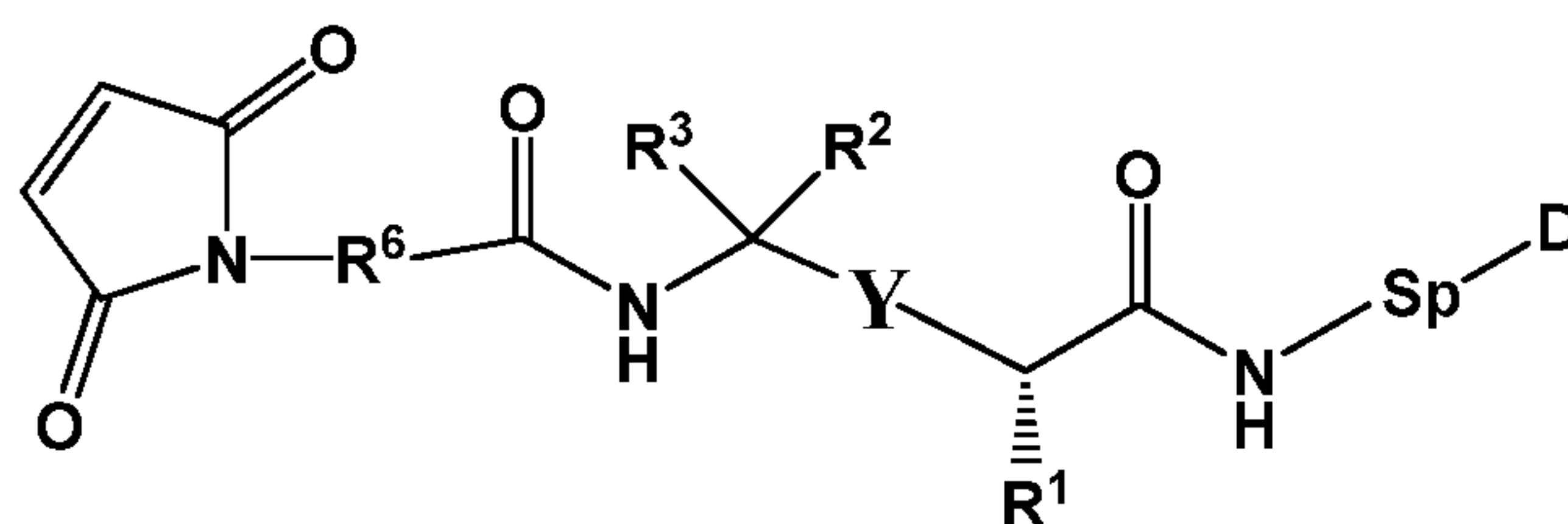


5 (IB)

wherein R^{11} is hydrogen atom, hydroxy or methoxy group and R^{22} is a C_1 - C_5 alkoxy group, or a pharmaceutically acceptable salt thereof.

This invention also relates to non-peptide compounds represented by the following formula:

10



(I)(A)(LD2)

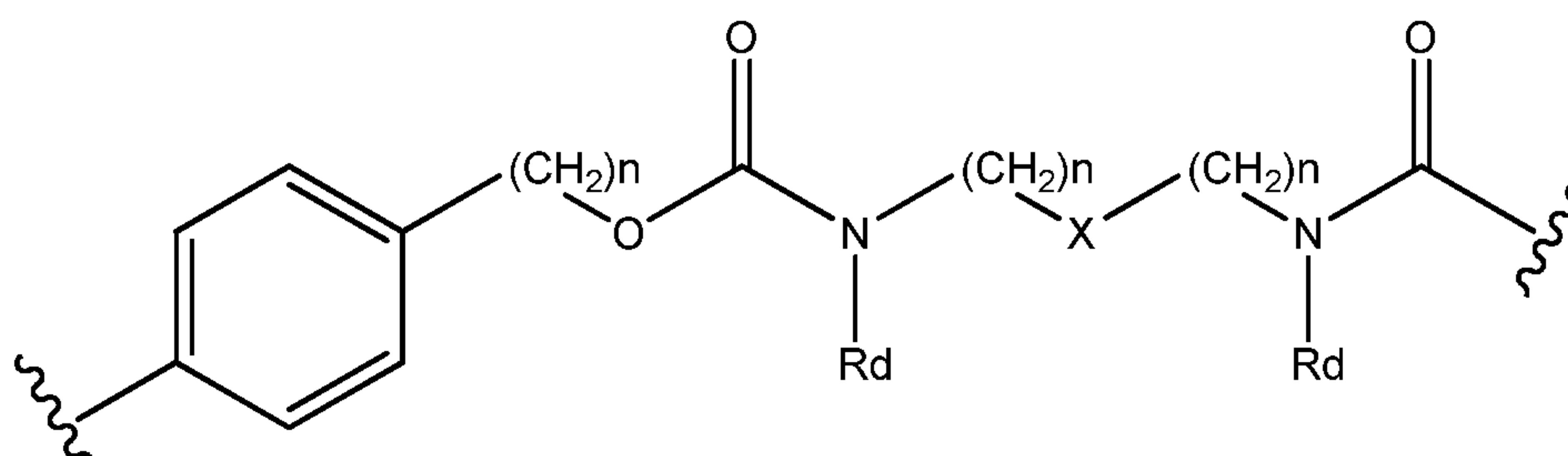
wherein

R^1 is C_1 - C_{10} alkyl, $(C_1$ - C_{10} alkyl) $NHC(NH)NH_2$ or $(C_1$ - C_{10} alkyl) $NHC(O)NH_2$;

15 R^3 and R^2 are each independently H, C_1 - C_{10} alkyl, arylalkyl or heteroarylalkyl, or R^3 and R^2 together may form a C_3 - C_7 cycloalkyl;

R_6 is C_1 - C_{10} alkylene; and

Sp is the following formula

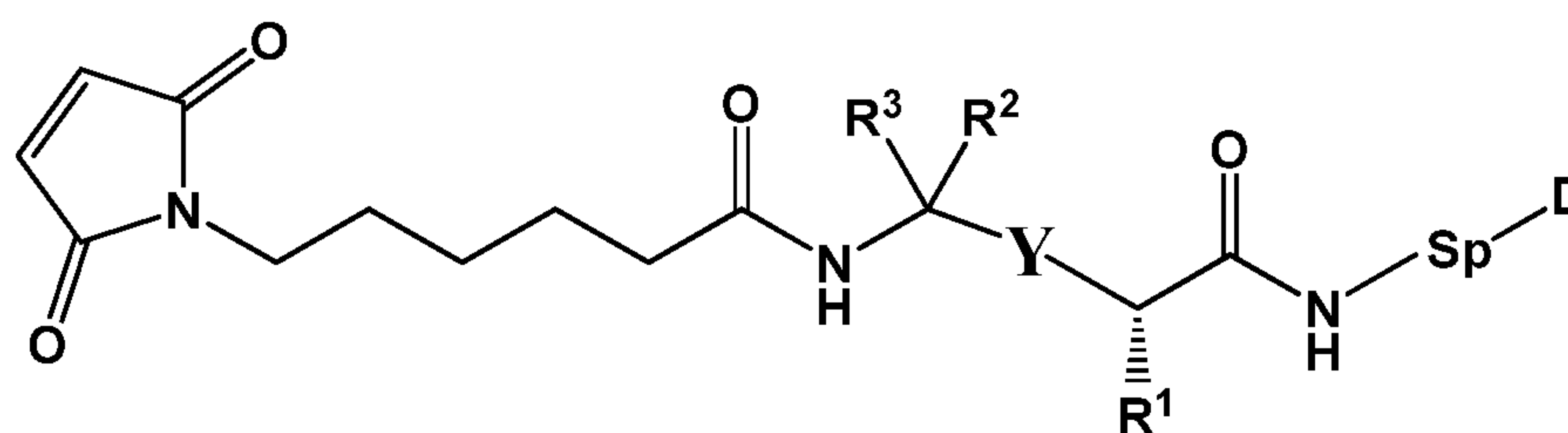


wherein

each n is independently 1-6;

- 5 X is N, CH₂ or a bond; and
each R^d is independently H or C₁-C₃alkyl.

This invention also relates to non-peptide compounds represented by the following formula:



10

(I)(A)(LD3)

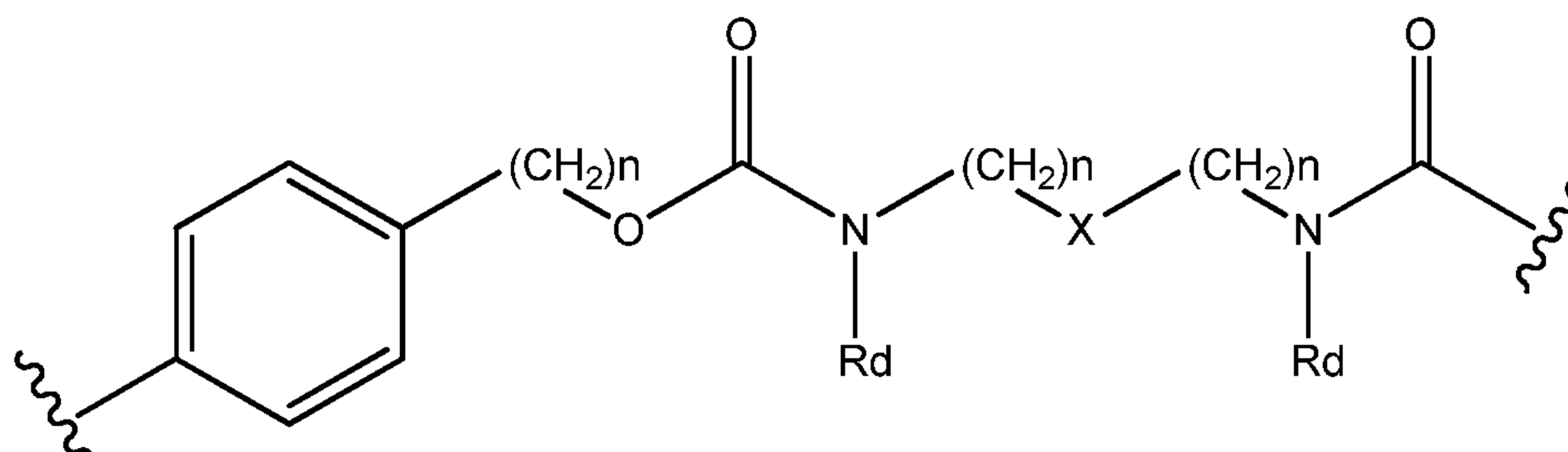
wherein

R¹ is C₁-C₁₀alkyl, (C₁-C₁₀alkyl)NHC(NH)NH₂ or (C₁-C₁₀alkyl)NHC(O)NH₂;

R³ and R² are each independently H, C₁-C₁₀alkyl, arylalkyl or heteroarylalkyl, or R³ and R² together may form a C₃-C₇cycloalkyl;

- 15 R₆ is C₁-C₁₀alkylene; and

Sp is the following formula



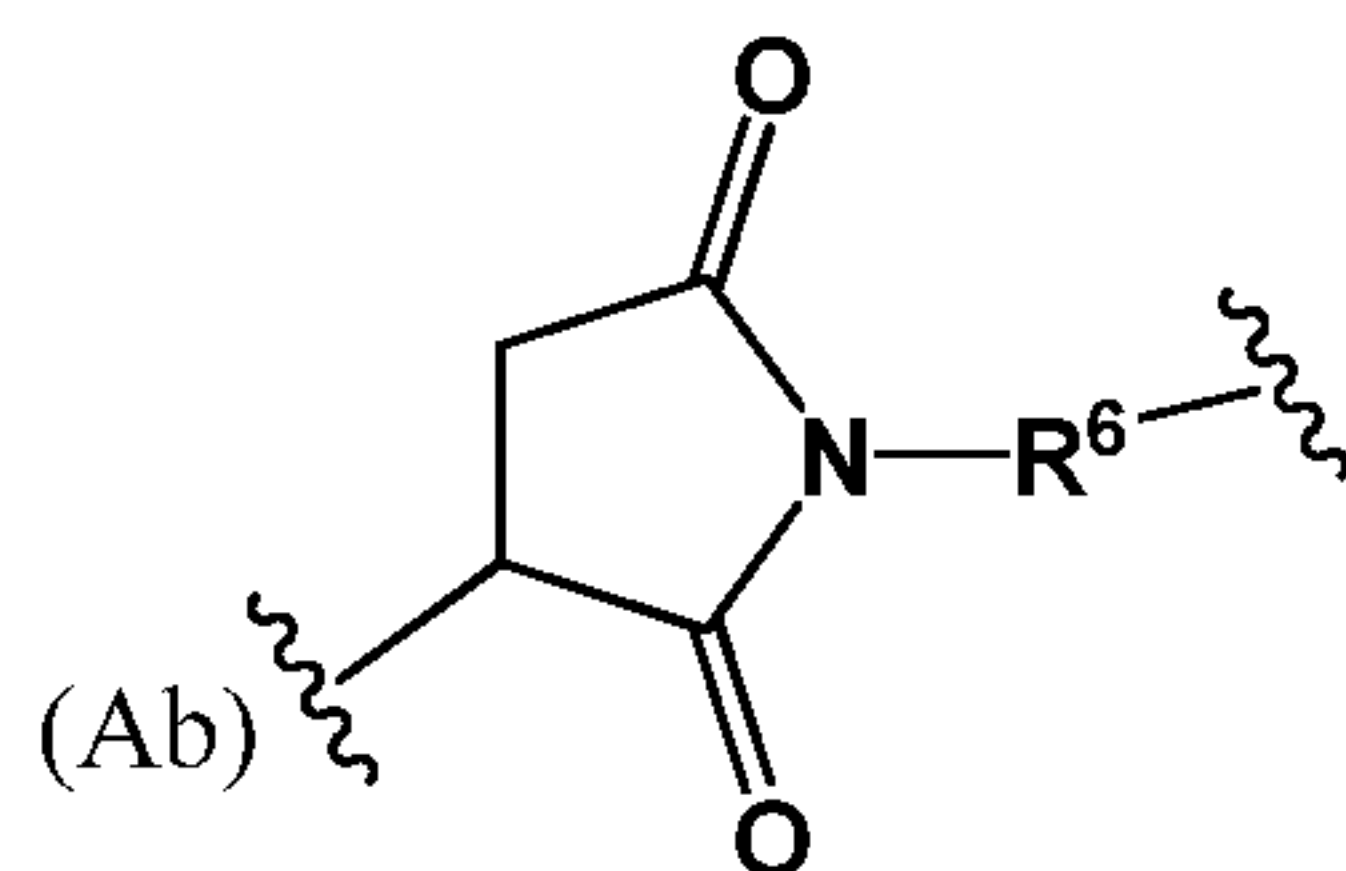
wherein

- 20 each n is independently 1-6;

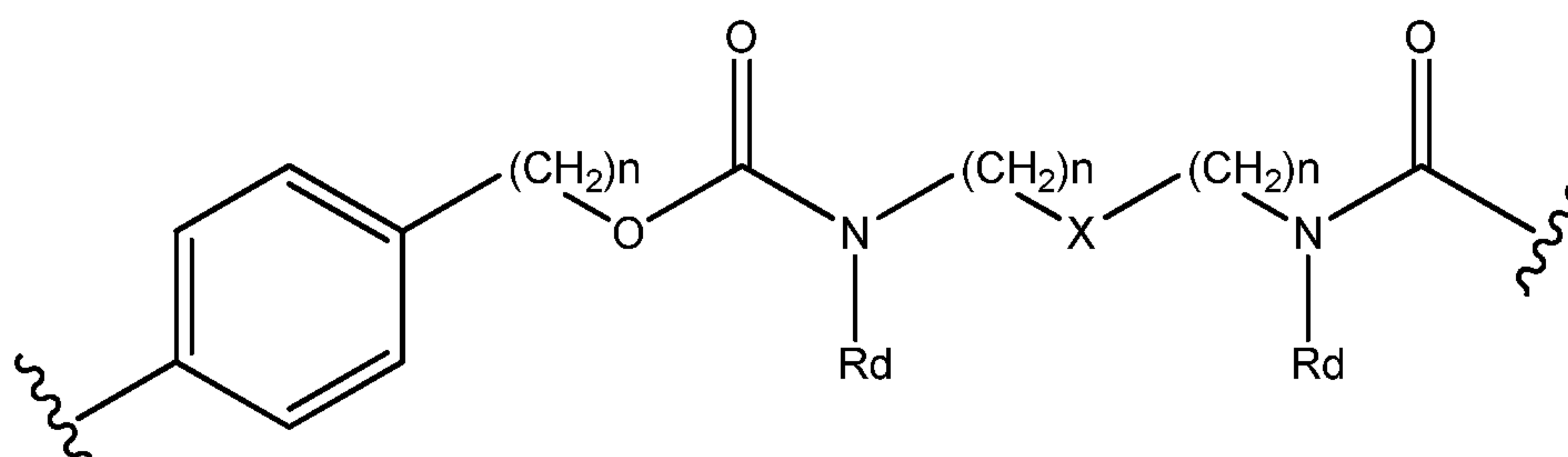
X is N, CH₂ or a bond; and

each R^d is independently H or C₁-C₃alkyl.

This invention also relates to any of the above non-peptide linker drug compounds, wherein Str has the following formula:

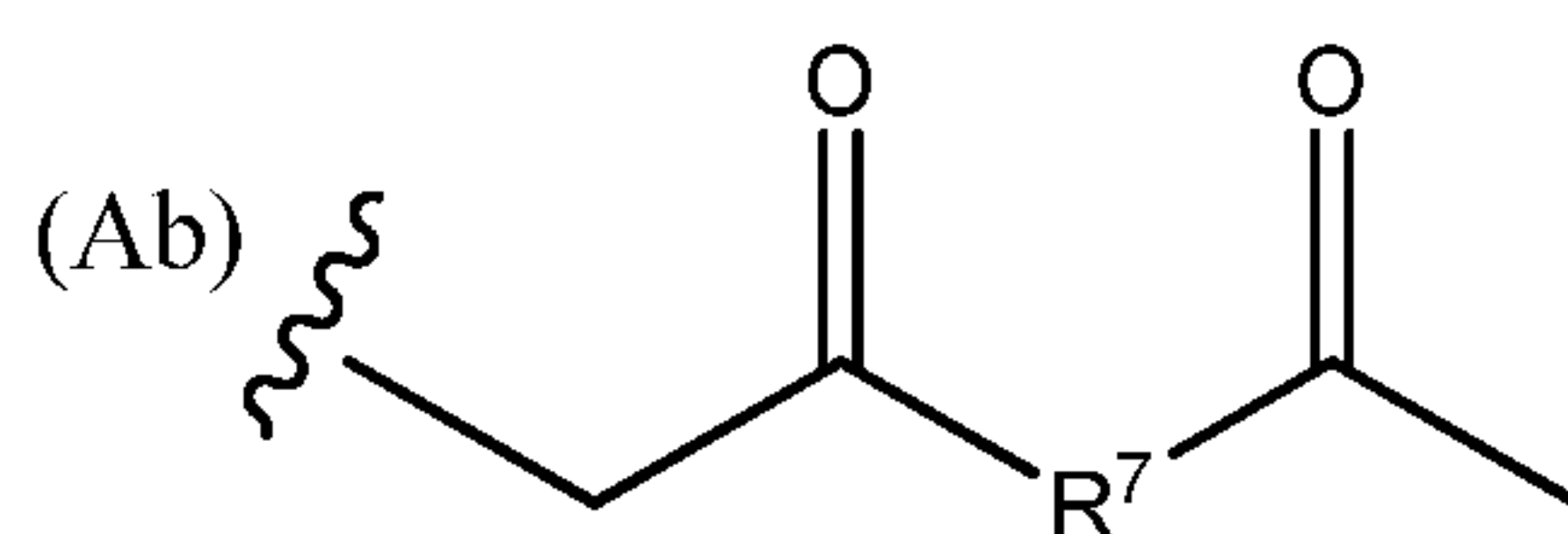


- 5 wherein R^6 is selected from the group consisting of C_1 - C_{10} alkylene, C_3 - C_8 cycloalkyl, O -(C_1 - C_8 alkylene), and C_1 - C_{10} alkylene- $C(O)N(R^a)$ - C_2 - C_6 alkylene, where each alkylene may be substituted by one to five substituents selected from the group consisting of halo, trifluoromethyl, difluoromethyl, amino, alkylamino, cyano, sulfonyl, sulfonamide, sulfoxide, hydroxy, alkoxy, ester, carboxylic acid, alkylthio, aryl, arylalkyl, C_3 - C_8 cycloalkyl, C_4 - C_7 heterocycloalkyl aryl, arylalkyl, heteroarylalkyl and heteroaryl; each R^a is independently H or C_1 - C_6 alkyl;
- 10 Sp is $—Ar—R^b—$, wherein Ar is aryl or heteroaryl, R^b is $(C_1$ - C_{10} alkylene) O - or Sp is the following formula



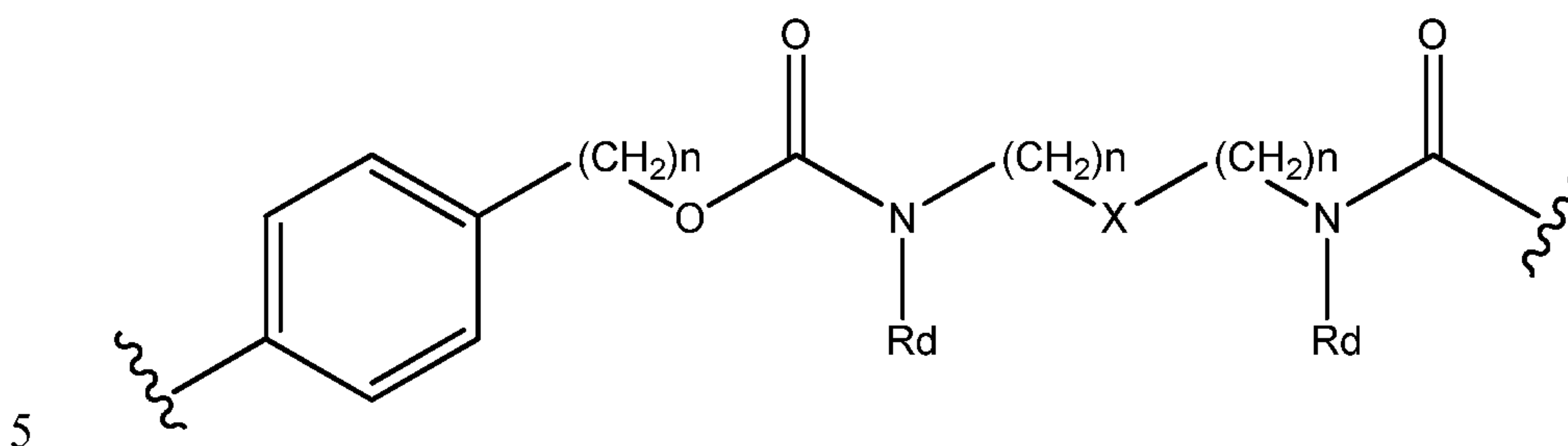
- 15 wherein
 each n is independently 1-6;
 X is N, CH_2 or a bond; and
 each R^d is independently H or C_1 - C_3 alkyl.
- 20 This invention also relates to non-peptide linker drug compounds, wherein R^6 is C_1 - C_{10} alkylene, Sp is $—Ar—R^b—$, wherein Ar is aryl R^b is $(C_1$ - C_6 alkylene) O -.

This invention also relates to non-peptide linker drug compounds, wherein Str has the formula:



wherein R^7 is selected from C_1 - C_{10} alkylene, C_1 - C_{10} alkylene-O, $N(R^c)$ -(C_2 - C_6 alkylene)- $N(R^c)$ and $N(R^c)$ -(C_2 - C_6 alkylene); where each R^c is independently H or C_1 - C_6 alkyl;

Sp is $-Ar-R^b-$, wherein Ar is aryl or heteroaryl, R^b is (C_1 - C_{10} alkylene)O- or Sp is the following formula



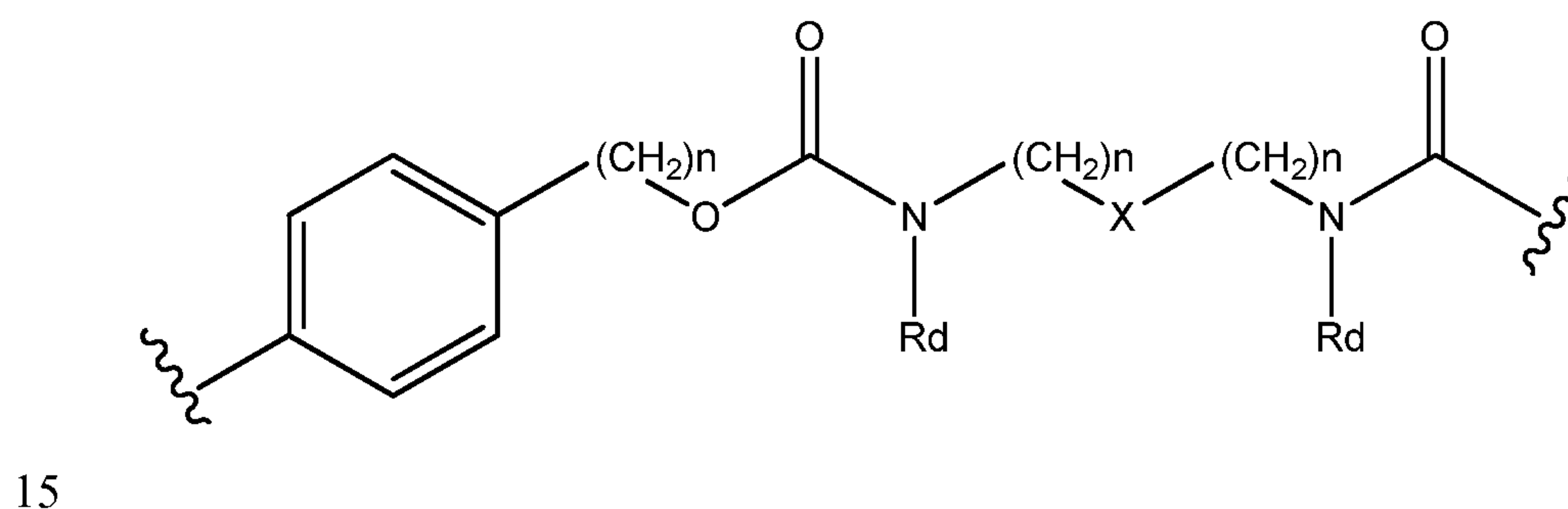
wherein

each n is independently 1-6;

X is N, CH_2 or a bond; and

10 each R^d is independently H or C_1 - C_3 alkyl.

This invention also relates to non-peptide linker drug compounds, wherein R^6 is C_1 - C_{10} alkylene, Sp is the following formula



wherein

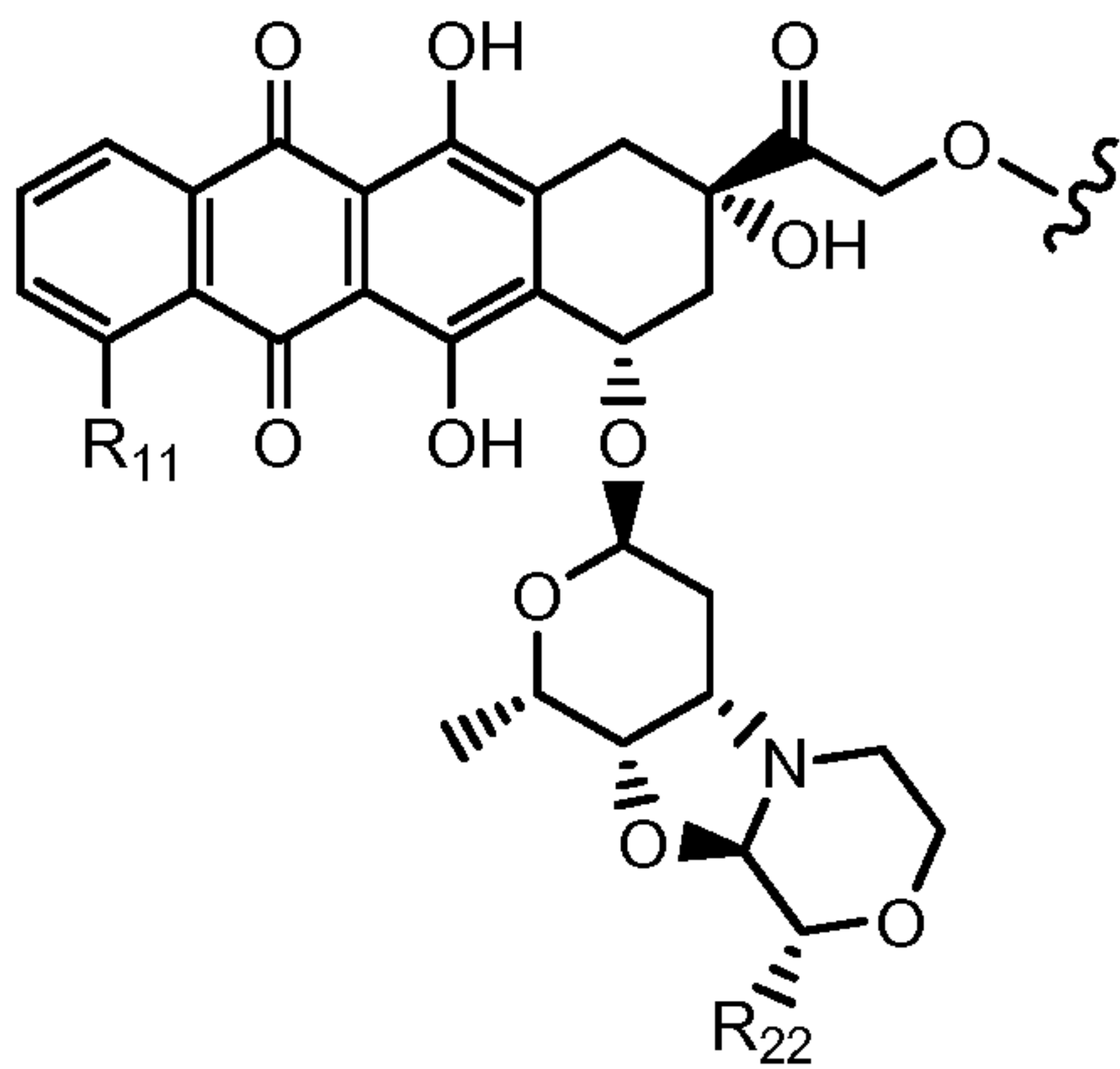
each n is independently 1-6;

X is N, CH_2 or a bond; and

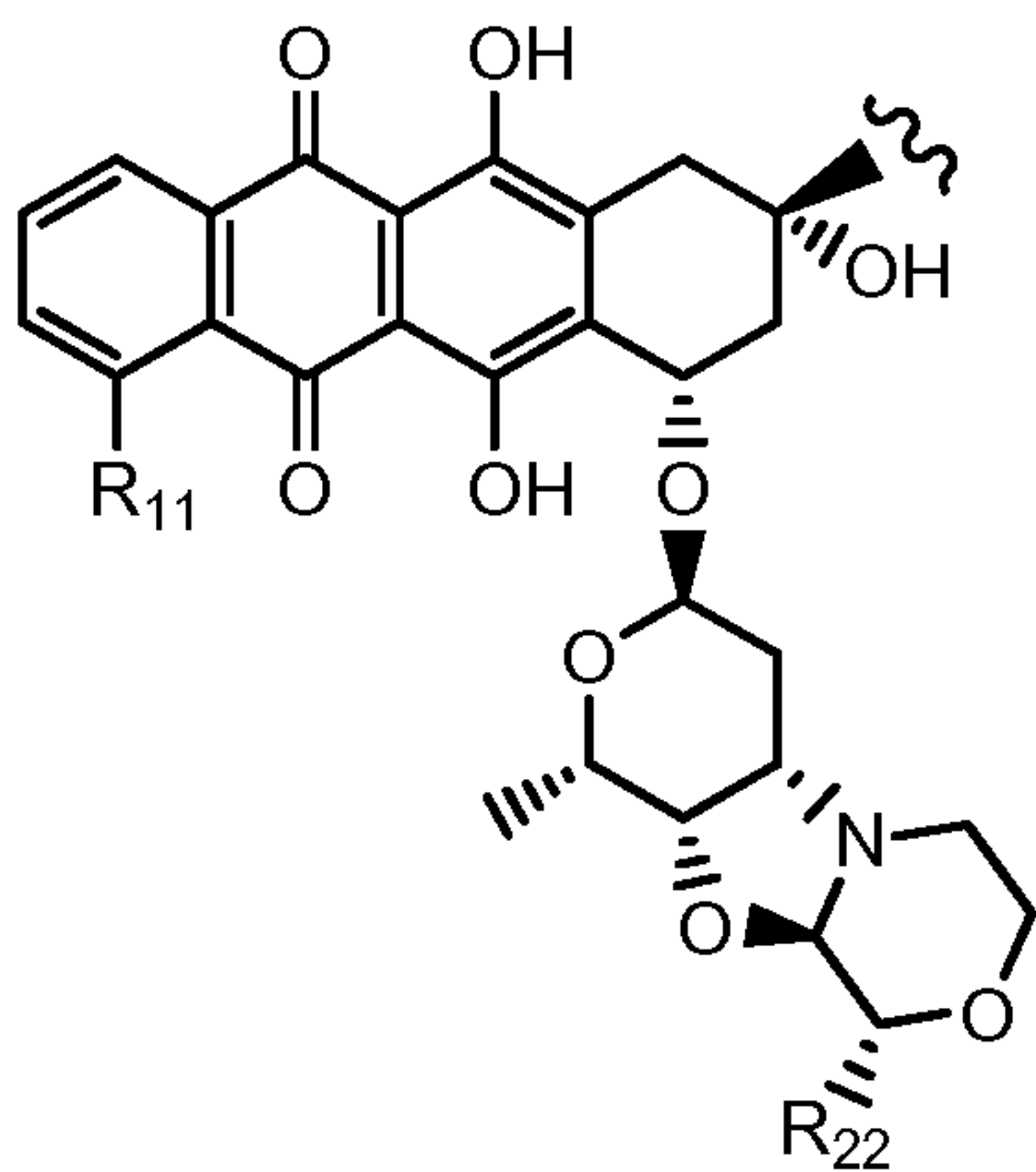
20 each R^d is independently H or C_1 - C_3 alkyl.

This invention also relates to any one of the above antibody-drug conjugates, wherein p is 2.

This invention also relates to linker drug compounds (I)(A)LD1) and (I)(B)(LD1) wherein (IA) is



(Ia) and (IB) is



(Ib)

5 This invention also relates to any one of the above antibody-drug conjugates, wherein the antibody binds to one or more of polypeptides selected from the group consisting of:

- CLL1;
- BMPR1B;
- E16;
- 10 STEAP1;
- 0772P;
- MPF;
- NaPi2b;
- Sema 5b;
- 15 PSCA hlg;
- ETBR;
- MSG783;
- STEAP2;
- TrpM4;
- 20 CRIPTO;
- CD21;

CD79b;
FcRH2;
HER2;
NCA;
5 MDP;
IL20R α ;
Brevican;
EphB2R;
ASLG659;
10 PSCA;
GEDA;
BAFF-R;
CD22;
CD79a;
15 CXCR5;
HLA-DOB;
P2X5 ;
CD72;
LY64;
20 FcRH1;
IRTA2;
TENB2;
PMEL17;
TMEFF1;
25 GDNF-Ra1;
Ly6E;
TMEM46;
Ly6G6D;
LGR5;
30 RET;
LY6K;
GPR19;
GPR54;
ASPHD1;
35 Tyrosinase;
TMEM118;
GPR172A;

MUC16 and
CD33.

This invention also relates to methods of treating a disease in a human in need thereof, comprising
5 administering to said human an effective amount of an Antibody-drug conjugate of claim 1.

This invention also relates to pharmaceutical compositions comprising a compound of claim 1 and
a pharmaceutically acceptable carrier thereof.

10 This invention also relates to any one of the above antibody-drug conjugates, wherein the antibody
binds to one or more of polypeptides selected from the group consisting of:

CLL1;
STEAP1;
NaPi2b;
15 STEAP2;
TrpM4;
CRIPTO;
CD21;
CD79b;
20 FcRH2;
HER2;
CD22;
CD79a;
CD72;
25 LY64;
Ly6E;
MUC16; and
CD33.

This invention also relates to any one of the above antibody-drug conjugates, wherein the
30 antibody binds to CD33.

This invention also relates to any one of the above antibody-drug conjugates, wherein the
antibody binds to CD22.

This invention also relates to any one of the above antibody-drug conjugates, wherein the
antibody binds to NaPi2b.

35 This invention also relates to any one of the above antibody-drug conjugates, wherein the
antibody binds to CLL1.

This invention also relates to any one of the above antibody-drug conjugates, wherein the antibody binds to Her2.

This invention also relates to any one of the above antibody-drug conjugates, wherein the
5 antibody binds to CD33 and the anti-CD33 antibody comprise an HVR-L1 comprising the amino
acid sequence of SEQ ID NO:11, an HVR-L2 comprising the amino acid sequence of SEQ ID
NO:12, an HVR-L3 comprising the amino acid sequence of SEQ ID NO:13, an HVR-H1
comprising the amino acid sequence of SEQ ID NO: 14, an HVR-H2 comprising the amino acid
sequence of SEQ ID NO:15, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:
10 16.

This invention also relates to any one of the above antibody-drug conjugates, wherein the
antibody binds to CD33 and the anti-CD33 antibody comprises a VL domain comprising the
amino acid sequence of SEQ ID NO:17 and a VH domain comprising the amino acid sequence of
15 SEQ ID NO:18.

In some embodiments, the antibody of the antibody-drug conjugate binds CD33. In some
embodiments, the antibody of the antibody-drug conjugate comprises (a) HVR-H1 comprising the
amino acid sequence of SEQ ID NO:22; (b) HVR-H2 comprising the amino acid sequence of SEQ
20 ID NO:23; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:24; (d) HVR-L1
comprising the amino acid sequence of SEQ ID NO:19; (e) HVR-L2 comprising the amino acid
sequence of SEQ ID NO:20; and (f) HVR-L3 comprising an amino acid sequence selected from
SEQ ID NO:21.

25 In some embodiments, the antibody comprises a VH as in any of the embodiments provided above,
and a VL as in any of the embodiments provided above. In one embodiment, the antibody
comprises the VL and VH sequences in SEQ ID NO:25 and SEQ ID NO:26, respectively,
including post-translational modifications of those sequences.

30 This invention also relates to any one of the above antibody-drug conjugates, wherein the
antibody binds to NaPi2b.

This invention also relates to any one of the above antibody-drug conjugates, wherein the
antibody binds to NaPi2b and the NaPi2b antibody comprise an HVR-L1 comprising the amino
35 acid sequence of SEQ ID NO:1, an HVR-L2 comprising the amino acid sequence of SEQ ID
NO:2, an HVR-L3 comprising the amino acid sequence of SEQ ID NO:3, an HVR-H1 comprising

the amino acid sequence of SEQ ID NO:4, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:5, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 6.

5 This invention also relates to any one of the above antibody-drug conjugates, wherein the antibody binds to NaPi2b and the NaPi2b antibody comprises a VL domain comprising the amino acid sequence of SEQ ID NO:7 and a VH domain comprising the amino acid sequence of SEQ ID NO:8.

10 This invention also relates to any one of the above antibody-drug conjugates, wherein the antibody binds to NaPi2b and the NaPi2b antibody comprises an amino acid sequence of SEQ ID NO:9 and an amino acid sequence of SEQ ID NO: 10.

This invention also relates to any one of the above antibody-drug conjugates, wherein the antibody binds to CD22.

15 This invention also relates to any one of the above antibody-drug conjugates, wherein the antibody binds to CD22 and the CD22 antibody comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:41, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:42, an HVR-L3 comprising the amino acid sequence of SEQ ID NO:43, an HVR-H1 comprising the amino acid sequence of SEQ ID NO:44, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:45, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 46.

20 This invention also relates to any one of the above antibody-drug conjugates, wherein the antibody binds to CD22 and the CD22 antibody comprises a VL domain comprising the amino acid sequence of SEQ ID NO:47 and a VH domain comprising the amino acid sequence of SEQ ID NO:48.

25 This invention also relates to any one of the above antibody-drug conjugates, wherein the antibody binds to CD22 and the CD22 antibody comprises an amino acid sequence of SEQ ID NO:49 and an amino acid sequence of SEQ ID NO: 50.

30

DEFINITIONS

Unless stated otherwise, the following terms and phrases as used herein are intended to have the following meanings: when trade names are used herein, applicants intend to independently include the trade name product formulation, the generic drug, and the active pharmaceutical ingredient(s) of the trade name product.

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The term “peptidomimetic” or PM as used herein means a non-peptide chemical moiety. Peptides are short chains of amino acid monomers linked by peptide (amide) bonds, the covalent chemical bonds formed when the carboxyl group of one amino acid reacts with the amino group of another. The shortest peptides are dipeptides, consisting of 2 amino acids joined by a single peptide bond, followed by tripeptides, tetrapeptides, etc. A peptidomimetic chemical moiety includes non-amino acid chemical moieties. A peptidomimetic chemical moiety may also include one or more amino acid that are separated by one or more non-amino acid chemical units. A peptidomimetic chemical moiety does not contain in any portion of its chemical structure two or more adjacent amino acids that are linked by peptide bonds.

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The term “amino acid” as used herein means glycine, alanine, valine, leucine, isoleucine, phenylalanine, proline, serine, threonine, tyrosine, cysteine, methionine, lysine, arginine, histidine, tryptophan, aspartic acid, glutamic acid, asparagine, glutamine or citrulline.

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The term “antibody” herein is used in the broadest sense and specifically covers monoclonal antibodies, polyclonal antibodies, dimers, multimers, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments, so long as they exhibit the desired biological activity (Miller et al (2003) *Jour. of Immunology* 170:4854-4861). Antibodies may be murine, human, humanized, chimeric, or derived from other species. An antibody is a protein generated by the immune system that is capable of recognizing and binding to a specific antigen. (Janeway, C., Travers, P., Walport, M., Shlomchik (2001) *Immuno Biology*, 5th Ed., Garland Publishing, New York). A target antigen generally has numerous binding sites, also called epitopes, recognized by CDRs on multiple antibodies. Each antibody that specifically binds to a different epitope has a different structure. Thus, one antigen may have more than one corresponding antibody. An

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antibody includes a full-length immunoglobulin molecule or an immunologically active portion of a full-length immunoglobulin molecule, i.e., a molecule that contains an antigen binding site that immunospecifically binds an antigen of a target of interest or part thereof, such targets including but not limited to, cancer cell or cells that produce autoimmune antibodies associated with an autoimmune disease. The immunoglobulin disclosed herein can be of any type (e.g., IgG, IgE, IgM, IgD, and IgA), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule. The immunoglobulins can be derived from any species. In one aspect, however, the immunoglobulin is of human, murine, or rabbit origin.

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The term "antibody fragment(s)" as used herein comprises a portion of a full length antibody, generally the antigen binding or variable region thereof. Examples of antibody fragments include Fab, Fab', F(ab')₂, and Fv fragments; diabodies; linear antibodies; minibodies (Olafsen et al (2004) *Protein Eng. Design & Sel.* 17(4):315-323), fragments produced by a Fab expression library, anti-

idiotypic (anti-Id) antibodies, CDR (complementary determining region), and epitope-binding fragments of any of the above which immunospecifically bind to cancer cell antigens, viral antigens or microbial antigens, single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

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The term “monoclonal antibody” as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site.

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Furthermore, in contrast to polyclonal antibody preparations which include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they may be synthesized uncontaminated by other antibodies. The modifier “monoclonal” indicates the character of the antibody as being obtained from a substantially

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homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler et al (1975) *Nature*, 256:495, or may be made by recombinant DNA methods (see for example: US 4816567; US 5807715). The monoclonal antibodies may also be isolated from phage
20 antibody libraries using the techniques described in Clackson et al (1991) *Nature*, 352:624-628; Marks et al (1991) *J. Mol. Biol.*, 222:581-597; for example.

The monoclonal antibodies herein specifically include “chimeric” antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in
25 antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (US 4816567; and Morrison et al (1984) *Proc. Natl. Acad. Sci. USA*, 81:6851-6855). Chimeric
30 antibodies of interest herein include “primatized” antibodies comprising variable domain antigen-binding sequences derived from a non-human primate (*e.g.*, Old World Monkey, Ape, etc.) and human constant region sequences.

The term “intact antibody” as used herein is one comprising a VL and VH domains, as well as a
35 light chain constant domain (CL) and heavy chain constant domains, CH1, CH2 and CH3. The constant domains may be native sequence constant domains (*e.g.*, human native sequence constant domains) or amino acid sequence variant thereof. The intact antibody may have one or more

“effector functions” which refer to those biological activities attributable to the Fc constant region (a native sequence Fc region or amino acid sequence variant Fc region) of an antibody. Examples of antibody effector functions include C1q binding; complement dependent cytotoxicity; Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; and down
5 regulation of cell surface receptors such as B cell receptor and BCR.

The term “Fc region” as used herein means a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region. The term includes native sequence Fc regions and variant Fc regions. In one embodiment, a human IgG heavy chain Fc region extends
10 from Cys226, or from Pro230, to the carboxyl-terminus of the heavy chain. However, the C-terminal lysine (Lys447) of the Fc region may or may not be present. Unless otherwise specified herein, numbering of amino acid residues in the Fc region or constant region is according to the EU numbering system, also called the EU index, as described in Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health,
15 Bethesda, MD, 1991.

The term “framework” or “FR” as used herein refers to variable domain residues other than hypervariable region (HVR) residues. The FR of a variable domain generally consists of four FR domains: FR1, FR2, FR3, and FR4. Accordingly, the HVR and FR sequences generally appear in
20 the following sequence in VH (or VL): FR1-H1(L1)-FR2-H2(L2)-FR3-H3(L3)-FR4. Depending on the amino acid sequence of the constant domain of their heavy chains, intact antibodies can be assigned to different “classes.” There are five major classes of intact immunoglobulin antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into “subclasses” (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA, and IgA2. The heavy-
25 chain constant domains that correspond to the different classes of antibodies are called α , δ , ϵ , γ , and μ , respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known. Ig forms include hinge-modifications or hingeless forms (Roux et al (1998) J. Immunol. 161:4083-4090; Lund et al (2000) Eur. J. Biochem. 267:7246-7256; US 2005/0048572; US 2004/0229310).

30 The term “human antibody” as used herein refers to an antibody which possesses an amino acid sequence which corresponds to that of an antibody produced by a human or a human cell or derived from a non-human source that utilizes human antibody repertoires or other human antibody-encoding sequences. This definition of a human antibody specifically excludes a
35 humanized antibody comprising non-human antigen-binding residues.

The term “human consensus framework” as used herein refers to a framework which represents the most commonly occurring amino acid residues in a selection of human immunoglobulin VL or VH framework sequences. Generally, the selection of human immunoglobulin VL or VH sequences is from a subgroup of variable domain sequences. Generally, the subgroup of sequences is a
5 subgroup as in Kabat et al., *Sequences of Proteins of Immunological Interest*, Fifth Edition, NIH Publication 91-3242, Bethesda MD (1991), vols. 1-3. In one embodiment, for the VL, the subgroup is subgroup kappa I as in Kabat et al., *supra*. In one embodiment, for the VH, the subgroup is subgroup III as in Kabat et al., *supra*.

10 The term “humanized antibody” as used herein refers to a chimeric antibody comprising amino acid residues from non-human HVRs and amino acid residues from human FRs. In certain embodiments, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the HVRs (e.g., CDRs) correspond to those of a non-human antibody, and all or substantially all of the FRs correspond to those of a
15 human antibody. A humanized antibody optionally may comprise at least a portion of an antibody constant region derived from a human antibody. A “humanized form” of an antibody, e.g., a non-human antibody, refers to an antibody that has undergone humanization.

The term “hypervariable region” or “HVR,” as used herein, refers to each of the regions of an
20 antibody variable domain which are hypervariable in sequence and/or form structurally defined loops (“hypervariable loops”). Generally, native four-chain antibodies comprise six HVRs; three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). HVRs generally comprise amino acid residues from the hypervariable loops and/or from the “complementarity determining regions” (CDRs), the latter being of highest sequence variability and/or involved in antigen recognition.
25 Exemplary hypervariable loops occur at amino acid residues 26-32 (L1), 50-52 (L2), 91-96 (L3), 26-32 (H1), 53-55 (H2), and 96-101 (H3). (Chothia and Lesk, *J. Mol. Biol.* 196:901-917 (1987).) Exemplary CDRs (CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2, and CDR-H3) occur at amino acid residues 24-34 of L1, 50-56 of L2, 89-97 of L3, 31-35B of H1, 50-65 of H2, and 95-102 of
30 H3. (Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991).) With the exception of CDR1 in VH, CDRs generally comprise the amino acid residues that form the hypervariable loops. CDRs also comprise “specificity determining residues,” or “SDRs,” which are residues that contact antigen. SDRs are contained within regions of the CDRs called abbreviated-CDRs, or a-CDRs. Exemplary a-CDRs (a-CDR-L1, a-CDR-L2, a-CDR-L3, a-CDR-H1, a-CDR-H2, and a-CDR-H3) occur at
35 amino acid residues 31-34 of L1, 50-55 of L2, 89-96 of L3, 31-35B of H1, 50-58 of H2, and 95-102 of H3. (See Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008).) Unless otherwise

indicated, HVR residues and other residues in the variable domain (e.g., FR residues) are numbered herein according to Kabat et al., *supra*.

5 The term “variable region” or “variable domain” as used herein refers to the domain of an antibody heavy or light chain that is involved in binding the antibody to antigen. The variable domains of the heavy chain and light chain (VH and VL, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three hypervariable regions (HVRs). (See, e.g., Kindt et al. *Kuby Immunology*, 6th ed., W.H. Freeman and Co., page 91 (2007).) A single VH or VL domain may be sufficient to confer
10 antigen-binding specificity. Furthermore, antibodies that bind a particular antigen may be isolated using a VH or VL domain from an antibody that binds the antigen to screen a library of complementary VL or VH domains, respectively. See, e.g., Portolano et al., *J. Immunol.* 150:880-887 (1993); Clarkson et al., *Nature* 352:624-628 (1991).

15 The term “vector” as used herein, refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are capable of directing the expression of nucleic acids to which they are operatively linked. Such vectors are referred to herein as “expression vectors.”

20 The term “free cysteine amino acid” as used herein refers to a cysteine amino acid residue which has been engineered into a parent antibody, has a thiol functional group (-SH), and is not paired as an intramolecular or intermolecular disulfide bridge.

25 The term “Linker”, “Linker Unit”, or “link” as used herein means a chemical moiety comprising a chain of atoms that covalently attaches a drug moiety to an antibody. In various embodiments, a linker is a divalent radical, specified as L.

30 The term “drug moiety” as used herein means a substance that that inhibits or prevents a cellular function and/or causes cell death or destruction. Cytotoxic agents include, but are not limited to, radioactive isotopes (e.g., At²¹¹, I¹³¹, I¹²⁵, Y⁹⁰, Re¹⁸⁶, Re¹⁸⁸, Sm¹⁵³, Bi²¹², P³², Pb²¹² and radioactive isotopes of Lu); chemotherapeutic agents or drugs (e.g., methotrexate, adriamycin, vinca alkaloids (vincristine, vinblastine, etoposide), doxorubicin, melphalan, mitomycin C, chlorambucil, daunorubicin or other intercalating agents); growth inhibitory agents; enzymes and fragments
35 thereof such as nucleolytic enzymes; and the various antitumor or anticancer agents disclosed below.

As used herein, unless defined otherwise in a claim, the term “acyl” refers to the group -C(O)R', where R' is alkyl, C₃-C₆cycloalkyl, or heterocyclyl, as each is defined herein.

As used herein, unless defined otherwise in a claim, the term “alkoxy” refers to the group -OR', where R' is C₁-C₄alkyl or C₃-C₆cycloalkyl as defined above. Examples of “alkoxy” include methoxy, ethoxy, isopropoxy, propoxy, butoxy, t-butoxy, isobutoxy, cyclopropoxy, and cyclobutoxy, and halogenated forms thereof, e.g. fluoromethoxy and difluoromethoxy.

As used herein, unless defined otherwise in a claim, the term “alkyl” refers to a straight or branched, monovalent or divalent hydrocarbon chain radical having from one to twelve (C₁-C₁₂) carbon atoms, which may be unsubstituted or substituted with multiple degrees of substitution, for example one, two, three, four, five or six included within the present invention. Examples of substituents are selected from the group consisting of halo, trifluoromethyl, difluoromethyl, amino, alkylamino, cyano, sulfonyl, sulfonamide, sulfoxide, hydroxy, alkoxy, ester, carboxylic acid and alkylthio. Examples of “alkyl” as used herein include, but are not limited to, methyl (Me, -CH₃), ethyl (Et, -CH₂CH₃), 1-propyl (n-Pr, n-propyl, -CH₂CH₂CH₃), 2-propyl (i-Pr, i-propyl, -CH(CH₃)₂), 1-butyl (n-Bu, n-butyl, -CH₂CH₂CH₂CH₃), 2-methyl-1-propyl (i-Bu, i-butyl, -CH₂CH(CH₃)₂), 2-butyl (s-Bu, s-butyl, -CH(CH₃)CH₂CH₃), 2-methyl-2-propyl (t-Bu, t-butyl, -C(CH₃)₃), 1-pentyl (n-pentyl, -CH₂CH₂CH₂CH₂CH₃), 2-pentyl (-CH(CH₃)CH₂CH₂CH₃), 3-pentyl (-CH(CH₂CH₃)₂), 2-methyl-2-butyl (-C(CH₃)₂CH₂CH₃), 3-methyl-2-butyl (-CH(CH₃)CH(CH₃)₂), 3-methyl-1-butyl (-CH₂CH₂CH(CH₃)₂), 2-methyl-1-butyl (-CH₂CH(CH₃)CH₂CH₃), 1-hexyl (-CH₂CH₂CH₂CH₂CH₂CH₃), 2-hexyl (-CH(CH₃)CH₂CH₂CH₂CH₃), 3-hexyl (-CH(CH₂CH₃)(CH₂CH₂CH₃)), 2-methyl-2-pentyl (-C(CH₃)₂CH₂CH₂CH₃), 3-methyl-2-pentyl (-CH(CH₃)CH(CH₃)CH₂CH₃), 4-methyl-2-pentyl (-CH(CH₃)CH₂CH(CH₃)₂), 3-methyl-3-pentyl (-C(CH₃)(CH₂CH₃)₂), 2-methyl-3-pentyl (-CH(CH₂CH₃)CH(CH₃)₂), 2,3-dimethyl-2-butyl (-C(CH₃)₂CH(CH₃)₂), 3,3-dimethyl-2-butyl (-CH(CH₃)C(CH₃)₃), as well as the divalent (“alkylene”) and substituted versions thereof.

Examples of substituted alkyl include but are not limited to, hydroxymethyl, difluoromethyl and trifluoromethyl.

As used herein unless otherwise defined in a claim, the term “alkenyl” means a linear or branched, monovalent or divalent hydrocarbon chain radical of any length from two to eight carbon atoms (C₂-C₁₀) with at least one site of unsaturation, i.e., a carbon-carbon, sp² double bond, wherein the alkenyl radical may be optionally substituted independently with one or more substituents described above in the definition of “alkyl”, and includes radicals having “cis” and “trans”

orientations, or alternatively, “E” and “Z” orientations. Examples of alkenyl include, but are not limited to, ethenyl or vinyl ($-\text{CH}=\text{CH}_2$), prop-1-enyl ($-\text{CH}=\text{CHCH}_3$), prop-2-enyl ($-\text{CH}_2\text{CH}=\text{CH}_2$), 2-methylprop-1-enyl, but-1-enyl, but-2-enyl, but-3-enyl, buta-1,3-dienyl, 2-methylbuta-1,3-diene, hex-1-enyl, hex-2-enyl, hex-3-enyl, hex-4-enyl, hexa-1,3-dienyl as well as the divalent
5 (“alkenylene”) and substituted versions thereof.

As used herein unless otherwise defined in a claim, the term “alkynyl” refers to a linear or branched, monovalent or divalent hydrocarbon radical of any length from two to eight carbon atoms ($\text{C}_2\text{--C}_{10}$) with at least one site of unsaturation, i.e., a carbon-carbon, sp triple bond, wherein
10 the alkynyl radical may be optionally substituted independently with one or more substituents described above in the definition of alkyl, examples of alkynyl includes, but not limited to, ethynyl ($-\text{C}\equiv\text{CH}$), prop-1-ynyl ($-\text{C}\equiv\text{CCH}_3$), prop-2-ynyl (propargyl, $-\text{CH}_2\text{C}\equiv\text{CH}$), but-1-ynyl, but-2-ynyl and but-3-ynyl, as well as the divalent (“alkynylene”) and substituted versions thereof.

15 As used herein, unless defined otherwise in a claim, the term “alkylamino” refers to the group $-\text{NR}'\text{R}''$, wherein R' is H, $\text{C}_1\text{--C}_6$ alkyl or $\text{C}_3\text{--C}_6$ cycloalkyl, and R'' is $\text{C}_1\text{--C}_6$ alkyl or $\text{C}_3\text{--C}_6$ cycloalkyl, examples of alkylamino include, but are not limited to, methylamino, dimethylamino, ethylamino, diethylamino, propylamino and cyclopropylamino.

20 As used herein, unless defined otherwise in a claim, the term “amide” refers to the group $-\text{C}(\text{O})\text{NR}'\text{R}''$, wherein R' and R'' are each independently H, $\text{C}_1\text{--C}_6$ alkyl, or $\text{C}_3\text{--C}_6$ cycloalkyl; examples of amide include, but are not limited to, $-\text{C}(\text{O})\text{NH}_2$, $-\text{C}(\text{O})\text{NHCH}_3$, and $-\text{C}(\text{O})\text{N}(\text{CH}_3)_2$.

As used herein, unless defined otherwise in a claim, the term “aryl” refers to an aromatic,
25 hydrocarbon, ring system. The ring system may be monocyclic or fused polycyclic (e.g., bicyclic, tricyclic, etc.), substituted or unsubstituted. In various embodiments, the monocyclic aryl ring is $\text{C}_5\text{--C}_{10}$, or $\text{C}_5\text{--C}_7$, or $\text{C}_5\text{--C}_6$, where these carbon numbers refer to the number of carbon atoms that form the ring system. A C_6 ring system, i.e. a phenyl ring, is an aryl group. In various
embodiments, the polycyclic ring is a bicyclic aryl group, where examples of bicyclic aryl groups
30 include are $\text{C}_8\text{--C}_{12}$, or $\text{C}_9\text{--C}_{10}$. A naphthyl ring, which has 10 carbon atoms, is a polycyclic aryl group. Examples of substituents for aryl are described below in the definition of “optionally substituted”.

As used herein, unless defined otherwise in a claim, the term “cyano” refers to the group $-\text{CN}$.

35 As used herein, unless defined otherwise in a claim, “cycloalkyl” refers to a non-aromatic, substituted or unsubstituted, saturated or partially unsaturated hydrocarbon ring group. Examples

of substituents are described in the definition of “optionally substituted”. In one example, the cycloalkyl group is 3 to 12 carbon atoms (C₃-C₁₂). In other examples, cycloalkyl is C₃-C₈, C₃-C₁₀ or C₅-C₁₀. In other examples, the cycloalkyl group, as a monocycle, is C₃-C₈, C₃-C₆ or C₅-C₆. In another example, the cycloalkyl group, as a bicycle, is C₇-C₁₂. In another example, the cycloalkyl group, as a spiro system, is C₅-C₁₂. Examples of monocyclic cycloalkyl include cyclopropyl, cyclobutyl, cyclopentyl, 1-cyclopent-1-enyl, 1-cyclopent-2-enyl, 1-cyclopent-3-enyl, cyclohexyl, perdeuteriocyclohexyl, 1-cyclohex-1-enyl, 1-cyclohex-2-enyl, 1-cyclohex-3-enyl, cyclohexadienyl, cycloheptyl, cyclooctyl, cyclononyl, cyclodecyl, cycloundecyl and cyclododecyl. Exemplary arrangements of bicyclic cycloalkyls having 7 to 12 ring atoms include, but are not limited to, [4,4], [4,5], [5,5], [5,6] or [6,6] ring systems. Exemplary bridged bicyclic cycloalkyls include, but are not limited to, bicyclo[2.2.1]heptane, bicyclo[2.2.2]octane and bicyclo[3.2.2]nonane. Examples of spiro cycloalkyl include, spiro[2.2]pentane, spiro[2.3]hexane, spiro[2.4]heptane, spiro[2.5]octane and spiro[4.5]decane.

As used herein, unless defined otherwise in a claim, the term “ester” refers to the group -C(O)OR’, where R’ is C₁-C₆alkyl, or C₃-C₆cycloalkyl.

As used herein, unless defined otherwise in a claim, the term “heterocycle” “heterocycloalkyl” or “heterocyclyl” refers to unsubstituted and substituted mono- or polycyclic non-aromatic ring system containing 2 to 12 ring carbon atoms and 1 to 3 ring hetero atoms. Polycyclic ring systems can be fused bi- or tri-cyclic, spiro or bridged. Examples of heteroatoms include N, O, and S, including N-oxides, sulfur oxides, and dioxides. In one embodiment, the ring is three to eight-membered and is either fully saturated or has one or more degrees of unsaturation. Multiple degrees of substitution are included within the present definition. Examples of substituents are defined hereunder. Examples of “heterocyclic” groups include, but are not limited to tetrahydrofuranyl, pyranal, 1,4-dioxanyl, 1,3-dioxanyl, oxolanyl, oxetanyl, 2-oxa-6-azaspiro[3.3]heptan-6-yl, piperidinyl, pyrrolidinyl, morpholinyl, azetidyl, piperazinyl, pyrrolidinonyl, piperazinonyl, pyrazolidinyl, imidazolyl, imidazolidinyl, and their various tautomers.

As used herein, unless defined otherwise in a claim, the term “heteroaryl”, unless defined otherwise in a claim, refers to an aromatic ring system containing 1 to 9 carbon(s) and at least one heteroatom. Examples of heteroatoms include N, O, and S. Heteroaryl may be monocyclic or polycyclic, substituted or unsubstituted. A monocyclic heteroaryl group may have 2 to 6 ring carbon atoms and 1 to 3 ring hetero atoms in the ring, while a polycyclic heteroaryl may contain 3 to 9 ring carbon atoms and 1 to 5 ring hetero atoms. A polycyclic heteroaryl ring may contain fused, spiro or bridged ring junctions, for example, bicyclic heteroaryl is a polycyclic heteroaryl.

Bicyclic heteroaryl rings may contain from 8 to 12 member atoms. Monocyclic heteroaryl rings may contain from 5 to 8 member atoms (carbons and heteroatoms). Exemplary heteroaryl groups include but are not limited to: benzofuranyl, benzothiophenyl, furanyl, imidazolyl, indolyl, azaindolyl, azabenzimidazolyl, benzoxazolyl, benzthiazolyl, benzothiadiazolyl, benzotriazolyl, 5 benzoimidazolyl, tetrazinyl, tetrazolyl, isothiazolyl, oxazolyl, isoxazolyl, pyrazinyl, pyrazolyl, pyridazinyl, pyridinyl, pyrimidinyl, pyrrolyl, quinolinyl, quinazolinyl, quinoxalinyl, triazinyl, triazolyl, thiazolyl and thiophenyl. Examples of substituents for heteroaryl are described below in the definition of “optionally substituted”.

10 As used herein, unless defined otherwise in a claim, the term “heteroarylalkyl” means the group (heteroaryl) C_1 - C_3 alkyl.

As used herein, unless defined otherwise in a claim, the term “arylalkyl” means the group (aryl) C_1 - C_3 alkyl.

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As used herein, unless defined otherwise in a claim, the term “urea” refers to the group – NR’C(O)NR”, wherein R’ and R” are each independently H, C_1 - C_6 alkyl, or C_3 - C_6 cycloalkyl.

As used herein, unless defined otherwise in a claim, the term “optionally” means that the subsequently described event(s) may or may not occur, and includes both event(s) that occur and 20 event(s) that do not occur.

As used herein, unless defined otherwise, the phrase “optionally substituted”, “substituted” or variations thereof denote an optional substitution, including multiple degrees of substitution, with one or more substituent group, for example, one, two or three. The phrase should not be interpreted 25 as duplicative of the substitutions herein described and depicted. Exemplary optional substituent groups include acyl, C_1 - C_6 alkyl, sulfonyl, amino, sulfonamide, sulfoxide, alkoxy, cyano, halo, urea, ester, carboxylic acid, amide, hydroxy, oxo, and nitro.

As used herein, unless defined otherwise in a claim, the term “treatment” refers to alleviating the specified condition, eliminating or reducing one or more symptoms of the condition, slowing or 30 eliminating the progression of the condition

As used herein, unless defined otherwise in a claim, the term "effective amount" means that amount of a drug or pharmaceutical agent that will elicit the biological or medical response of a 35 tissue, system, animal, or human that is being sought, for instance, by a researcher or clinician.

As used herein, unless defined otherwise in a claim, the term “therapeutically effective amount” means any amount which, as compared to a corresponding subject who has not received such

amount, results in treatment of a disease, disorder, or side effect, or a decrease in the rate of advancement of a disease or disorder. The term also includes within its scope amounts effective to enhance normal physiological function. For use in therapy, therapeutically effective amounts of a compound of Formula I, as well as salts thereof, may be administered as the raw chemical.

5 Additionally, the active ingredient may be presented as a pharmaceutical composition.

This invention also relates to any one of the examples in the Experimental section.

The phrase "pharmaceutically acceptable salt," as used herein, refers to pharmaceutically acceptable organic or inorganic salts of an antibody-drug conjugate (ADC) or a linker-drug moiety. Exemplary salts include, but are not limited, to sulfate, citrate, acetate, oxalate, chloride, bromide, iodide, nitrate, bisulfate, phosphate, acid phosphate, isonicotinate, lactate, salicylate, acid citrate, tartrate, oleate, tannate, pantothenate, bitartrate, ascorbate, succinate, maleate, gentisinate, fumarate, gluconate, glucuronate, saccharate, formate, benzoate, glutamate, methanesulfonate, ethanesulfonate, benzenesulfonate, *p*-toluenesulfonate, and pamoate (*i.e.*, 1,1'-methylene-bis - (2-hydroxy-3-naphthoate)) salts. A pharmaceutically acceptable salt may involve the inclusion of another molecule such as an acetate ion, a succinate ion or other counterion. The counterion may be any organic or inorganic moiety that stabilizes the charge on the parent compound.

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20 Furthermore, a pharmaceutically acceptable salt may have more than one charged atom in its structure. Instances where multiple charged atoms are part of the pharmaceutically acceptable salt can have multiple counter ions. Hence, a pharmaceutically acceptable salt can have one or more charged atoms and/or one or more counterion.

25 Other salts, which are not pharmaceutically acceptable, may be useful in the preparation of compounds of this invention and these should be considered to form a further aspect of the invention. These salts, such as oxalic or trifluoroacetate, while not in themselves pharmaceutically acceptable, may be useful in the preparation of salts useful as intermediates in obtaining the compounds of the invention and their pharmaceutically acceptable salts.

30 Compounds of the present invention may exist in solid or liquid form. In the solid state, it may exist in crystalline or noncrystalline form, or as a mixture thereof. The skilled artisan will appreciate that pharmaceutically acceptable solvates may be formed for crystalline or non-crystalline compounds. In crystalline solvates, solvent molecules are incorporated into the crystalline lattice during crystallization. Solvates may involve non-aqueous solvents such as, but not limited to, ethanol, isopropanol, DMSO, acetic acid, ethanolamine, or ethyl acetate, or they may involve water as the solvent that is incorporated into the crystalline lattice. Solvates wherein water is the solvent incorporated into the crystalline lattice are typically referred to as "hydrates."

Hydrates include stoichiometric hydrates as well as compositions containing variable amounts of water. The invention includes all such solvates.

The skilled artisan will further appreciate that certain compounds of the invention that exist in crystalline form, including the various solvates thereof, may exhibit polymorphism (i.e. the capacity to occur in different crystalline structures). These different crystalline forms are typically known as "polymorphs." The invention includes all such polymorphs. Polymorphs have the same chemical composition but differ in packing, geometrical arrangement, and other descriptive properties of the crystalline solid state. Polymorphs, therefore, may have different physical properties such as shape, density, hardness, deformability, stability, and dissolution properties. Polymorphs typically exhibit different melting points, IR spectra, and X-ray powder diffraction patterns, which may be used for identification. The skilled artisan will appreciate that different polymorphs may be produced, for example, by changing or adjusting the reaction conditions or reagents, used in making the compound. For example, changes in temperature, pressure, or solvent may result in polymorphs. In addition, one polymorph may spontaneously convert to another polymorph under certain conditions.

Compounds of the present invention or a salt thereof may exist in stereoisomeric forms (e.g., it contains one or more asymmetric carbon atoms). The individual stereoisomers (enantiomers and diastereomers) and mixtures of these are included within the scope of the present invention. Likewise, it is understood that a compound or salt of Formula (I) may exist in tautomeric forms other than that shown in the formula and these are also included within the scope of the present invention. It is to be understood that the present invention includes all combinations and subsets of the particular groups defined hereinabove. The scope of the present invention includes mixtures of stereoisomers as well as purified enantiomers or enantiomerically/diastereomerically enriched mixtures. It is to be understood that the present invention includes all combinations and subsets of the particular groups defined hereinabove.

The subject invention also includes isotopically-labelled forms of the compounds of the present invention, but for the fact that one or more atoms are replaced by an atom having an atomic mass or mass number different from the atomic mass or mass number usually found in nature. Examples of isotopes that can be incorporated into compounds of the invention and pharmaceutically acceptable salts thereof include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorous, sulphur, fluorine, iodine, and chlorine, such as 2H , 3H , 11C , 13C , 14C , 15N , 17O , 18O , 31P , 32P , 35S , 18F , 36Cl , 123I and 125I .

Compounds of the present invention and pharmaceutically acceptable salts of said compounds that contain the aforementioned isotopes and/or other isotopes of other atoms are within the scope of the present invention. Isotopically-labelled compounds of the present invention, for example those into which radioactive isotopes such as ^3H , ^{14}C are incorporated, are useful in drug and/or substrate tissue distribution assays. Tritiated, i.e., ^3H , and carbon-14, i.e., ^{14}C , isotopes are commonly used for their ease of preparation and detectability. ^{11}C and ^{18}F isotopes are useful in PET (positron emission tomography), and ^{125}I isotopes are useful in SPECT (single photon emission computerized tomography), all useful in brain imaging. Further, substitution with heavier isotopes such as deuterium, i.e., ^2H , can afford certain therapeutic advantages resulting from greater metabolic stability, for example increased in vivo half-life or reduced dosage requirements and, hence, may be preferred in some circumstances. Isotopically labelled compounds of formula I and following of this invention can generally be prepared by carrying out the procedures disclosed in the Schemes and/or in the Examples below, by substituting a readily available isotopically labelled reagent for a non-isotopically labelled reagent.

15

PHARMACEUTICAL COMPOSITION OF ADCS

Pharmaceutical formulations of therapeutic antibody-drug conjugates (ADC) of the invention are typically prepared for parenteral administration, i.e. bolus, intravenous, intratumor injection with a pharmaceutically acceptable parenteral vehicle and in a unit dosage injectable form. An antibody-drug conjugate (ADC) having the desired degree of purity is optionally mixed with pharmaceutically acceptable diluents, carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences (1980) 16th edition, Osol, A. Ed.), in the form of a lyophilized formulation or an aqueous solution.

25 CYSTEINE ENGINEERED ANTIBODIES

The compounds of the invention include antibody-drug conjugates comprising cysteine engineered antibodies where one or more amino acids of a wild-type or parent antibody are replaced with a cysteine amino acid. Any form of antibody may be so engineered, i.e. mutated. For example, a parent Fab antibody fragment may be engineered to form a cysteine engineered Fab, referred to herein as "ThioFab." Similarly, a parent monoclonal antibody may be engineered to form a "ThioMab." It should be noted that a single site mutation yields a single engineered cysteine residue in a ThioFab, while a single site mutation yields two engineered cysteine residues in a ThioMab, due to the dimeric nature of the IgG antibody. Mutants with replaced ("engineered") cysteine (Cys) residues are evaluated for the reactivity of the newly introduced, engineered cysteine thiol groups. The thiol reactivity value is a relative, numerical term in the range of 0 to 1.0 and can be measured for any cysteine engineered antibody. Thiol reactivity values of cysteine engineered antibodies of the invention are in the ranges of 0.6 to 1.0; 0.7 to 1.0; or 0.8 to 1.0.

35

To prepare a cysteine engineered antibody by mutagenesis, DNA encoding an amino acid sequence variant of the starting polypeptide is prepared by a variety of methods known in the art. These methods include, but are not limited to, preparation by site-directed (or oligonucleotide-mediated) mutagenesis, PCR mutagenesis, and cassette mutagenesis of an earlier prepared DNA
5 encoding the polypeptide. Variants of recombinant antibodies may be constructed also by restriction fragment manipulation or by overlap extension PCR with synthetic oligonucleotides. Mutagenic primers encode the cysteine codon replacement(s). Standard mutagenesis techniques can be employed to generate DNA encoding such mutant cysteine engineered antibodies. General guidance can be found in Sambrook et al Molecular Cloning, A Laboratory Manual, Cold Spring
10 Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989; and Ausubel et al Current Protocols in Molecular Biology, Greene Publishing and Wiley-Interscience, New York, N.Y., 1993.

Cysteine amino acids may be engineered at reactive sites in an antibody and which do not form intrachain or intermolecular disulfide linkages (Junutula, et al., 2008b Nature Biotech., 26(8):925-932; Dorman et al (2009) Blood 114(13):2721-2729; US 7521541; US 7723485; WO2009/052249,
15 Shen et al (2012) Nature Biotech., 30(2):184-191; Junutula et al (2008) Jour of Immun. Methods 332:41-52). The engineered cysteine thiols may react with linker reagents or the linker-drug intermediates of the present invention which have thiol-reactive, electrophilic groups such as maleimide or alpha-halo amides to form ADC with cysteine engineered antibodies (ThioMabs) and the drug (D) moiety. The location of the drug moiety can thus be designed, controlled, and known.
20 The drug loading can be controlled since the engineered cysteine thiol groups typically react with thiol-reactive linker reagents or linker-drug intermediates in high yield. Engineering an antibody to introduce a cysteine amino acid by substitution at a single site on the heavy or light chain gives two new cysteines on the symmetrical antibody. A drug loading near 2 can be achieved and near homogeneity of the conjugation product ADC.

25 Cysteine engineered antibodies of the invention preferably retain the antigen binding capability of their wild type, parent antibody counterparts. Thus, cysteine engineered antibodies are capable of binding, preferably specifically, to antigens. Such antigens include, for example, tumor-associated antigens (TAA), cell surface receptor proteins and other cell surface molecules, transmembrane
30 proteins, signaling proteins, cell survival regulatory factors, cell proliferation regulatory factors, molecules associated with (for e.g., known or suspected to contribute functionally to) tissue development or differentiation, lymphokines, cytokines, molecules involved in cell cycle regulation, molecules involved in vasculogenesis and molecules associated with (for e.g., known or suspected to contribute functionally to) angiogenesis. The tumor-associated antigen may be a
35 cluster differentiation factor (i.e., a CD protein). An antigen to which a cysteine engineered antibody is capable of binding may be a member of a subset of one of the above-mentioned

categories, wherein the other subset(s) of said category comprise other molecules/antigens that have a distinct characteristic (with respect to the antigen of interest).

Cysteine engineered antibodies are prepared for conjugation with linker-drug intermediates by reduction and reoxidation of intrachain disulfide groups.

5

TUMOR-ASSOCIATED ANTIGENS:

Antibodies, including but not limited to cysteine engineered antibodies, which may be useful in the antibody-drug conjugates of the invention in the treatment of cancer include, but are not limited to, antibodies against cell surface receptors and tumor-associated antigens (TAA). Certain tumor-associated antigens are known in the art, and can be prepared for use in generating antibodies using methods and information which are well known in the art. In attempts to discover effective cellular targets for cancer diagnosis and therapy, researchers have sought to identify transmembrane or otherwise tumor-associated polypeptides that are specifically expressed on the surface of one or more particular type(s) of cancer cell as compared to on one or more normal non-cancerous cell(s). Often, such tumor-associated polypeptides are more abundantly expressed on the surface of the cancer cells as compared to on the surface of the non-cancerous cells. The identification of such tumor-associated cell surface antigen polypeptides has given rise to the ability to more specifically target cancer cells for destruction via antibody-based therapies.

Examples of tumor-associated antigens TAA include, but are not limited to, those listed below. For convenience, information relating to these antigens, all of which are known in the art, is listed below and includes names, alternative names, Genbank accession numbers and primary reference(s), following nucleic acid and protein sequence identification conventions of the National Center for Biotechnology Information (NCBI). Nucleic acid and protein sequences corresponding to TAA listed below are available in public databases such as GenBank. Tumor-associated antigens targeted by antibodies include all amino acid sequence variants and isoforms possessing at least about 70%, 80%, 85%, 90%, or 95% sequence identity relative to the sequences identified in the cited references, and/or which exhibit substantially the same biological properties or characteristics as a TAA having a sequence found in the cited references. For example, a TAA having a variant sequence generally is able to bind specifically to an antibody that binds specifically to the TAA with the corresponding sequence listed. The sequences and disclosure in the reference specifically recited herein are expressly incorporated by reference.

(1) BMPR1B (bone morphogenetic protein receptor-type IB, Genbank accession no. NM_001203) ten Dijke,P., et al Science 264 (5155):101-104 (1994), Oncogene 14 (11):1377-1382 (1997)); WO2004063362 (Claim 2); WO2003042661 (Claim 12); US2003134790-A1 (Page 38-39); WO2002102235 (Claim 13; Page 296); WO2003055443 (Page 91-92); WO200299122 (Example 2; Page 528-530); WO2003029421 (Claim 6);

35

- WO2003024392 (Claim 2; Fig 112); WO200298358 (Claim 1; Page 183);
 WO200254940 (Page 100-101); WO200259377 (Page 349-350); WO200230268 (Claim
 27; Page 376); WO200148204 (Example; Fig 4)
 NP_001194 bone morphogenetic protein receptor, type IB /pid=NP_001194.1 -
 5 Cross-references: MIM:603248; NP_001194.1; AY065994
- (2) E16 (LAT1, SLC7A5, Genbank accession no. NM_003486)
 Biochem. Biophys. Res. Commun. 255 (2), 283-288 (1999), Nature 395 (6699):288-291 (1998),
 Gaugitsch, H.W., et al (1992) J. Biol. Chem. 267 (16):11267-11273); WO2004048938 (Example
 10 2); WO2004032842 (Example IV); WO2003042661 (Claim 12); WO2003016475 (Claim 1);
 WO200278524 (Example 2); WO200299074 (Claim 19; Page 127-129); WO200286443 (Claim
 27; Pages 222, 393); WO2003003906 (Claim 10; Page 293); WO200264798 (Claim 33; Page 93-
 95); WO200014228 (Claim 5; Page 133-136); US2003224454 (Fig 3); WO2003025138 (Claim
 12; Page 150);
 15 NP_003477 solute carrier family 7 (cationic amino acid transporter, y+
 system), member 5 /pid=NP_003477.3 - Homo sapiens
 Cross-references: MIM:600182; NP_003477.3; NM_015923; NM_003486_1
- (3) STEAP1 (six transmembrane epithelial antigen of prostate, Genbank accession no.
 20 NM_012449)
 Cancer Res. 61 (15), 5857-5860 (2001), Hubert, R.S., et al (1999) Proc. Natl. Acad. Sci. U.S.A. 96
 (25):14523-14528); WO2004065577 (Claim 6); WO2004027049 (Fig 1L); EP1394274 (Example
 11); WO2004016225 (Claim 2); WO2003042661 (Claim 12); US2003157089 (Example 5);
 US2003185830 (Example 5); US2003064397 (Fig 2); WO200289747 (Example 5; Page 618-619);
 25 WO2003022995 (Example 9; Fig 13A, Example 53; Page 173, Example 2; Fig 2A);
 NP_036581 six transmembrane epithelial antigen of the prostate
 Cross-references: MIM:604415; NP_036581.1; NM_012449_1
- (4) 0772P (CA125, MUC16, Genbank accession no. AF361486)
 30 J. Biol. Chem. 276 (29):27371-27375 (2001)); WO2004045553 (Claim 14);
 WO200292836 (Claim 6; Fig 12); WO200283866 (Claim 15; Page 116-121);
 US2003124140 (Example 16); Cross-references: GI:34501467; AAK74120.3;
 AF361486_1
- (5) MPF (MPF, MSLN, SMR, megakaryocyte potentiating factor, mesothelin, Genbank
 accession no. NM_005823) Yamaguchi, N., et al Biol. Chem. 269 (2), 805-808 (1994),
 Proc. Natl. Acad. Sci. U.S.A. 96 (20):11531-11536 (1999), Proc. Natl. Acad. Sci.

- U.S.A. 93 (1):136-140 (1996), J. Biol. Chem. 270 (37):21984-21990 (1995));
 WO2003101283 (Claim 14); (WO2002102235 (Claim 13; Page 287-288);
 WO2002101075 (Claim 4; Page 308-309); WO200271928 (Page 320-321);
 WO9410312 (Page 52-57); Cross-references: MIM:601051; NP_005814.2;
 5 NM_005823_1
- (6) NaPi2b (NAPI-3B, NPTIIb, SLC34A2, solute carrier family 34 (sodium phosphate),
 member 2, type II sodium-dependent phosphate transporter 3b, Genbank accession no.
 NM_006424)
- 10 J. Biol. Chem. 277 (22):19665-19672 (2002), Genomics 62 (2):281-284 (1999), Feild, J.A., et al
 (1999) Biochem. Biophys. Res. Commun. 258 (3):578-582); WO2004022778 (Claim 2);
 EP1394274 (Example 11); WO2002102235 (Claim 13; Page 326); EP875569 (Claim 1; Page 17-
 19); WO200157188 (Claim 20; Page 329); WO2004032842 (Example IV); WO200175177
 (Claim 24; Page 139-140);
- 15 Cross-references: MIM:604217; NP_006415.1; NM_006424_1
- (7) Sema 5b (FLJ10372, KIAA1445, Mm.42015, SEMA5B, SEMAG, Semaphorin 5b Hlog, sema
 domain, seven thrombospondin repeats (type 1 and type 1-like), transmembrane domain (TM) and
 short cytoplasmic domain, (semaphorin) 5B, Genbank accession no. AB040878)
- 20 Nagase T., et al (2000) DNA Res. 7 (2):143-150); WO2004000997 (Claim 1); WO2003003984
 (Claim 1); WO200206339 (Claim 1; Page 50); WO200188133 (Claim 1; Page 41-43, 48-58);
 WO2003054152 (Claim 20); WO2003101400 (Claim 11);
 Accession: Q9P283; EMBL; AB040878; BAA95969.1. Genew; HGNC:10737;
- 25 (8) PSCA hlg (2700050C12Rik, C530008O16Rik, RIKEN cDNA 2700050C12, RIKEN cDNA
 2700050C12 gene, Genbank accession no. AY358628); Ross et al (2002) Cancer Res. 62:2546-
 2553; US2003129192 (Claim 2); US2004044180 (Claim 12); US2004044179 (Claim 11);
 US2003096961 (Claim 11); US2003232056 (Example 5); WO2003105758 (Claim 12);
 US2003206918 (Example 5); EP1347046 (Claim 1); WO2003025148 (Claim 20);
- 30 Cross-references: GI:37182378; AAQ88991.1; AY358628_1
- (9) ETBR (Endothelin type B receptor, Genbank accession no. AY275463);
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M., et al Gene 228, 43-49, 1999; Strausberg R.L., et al Proc. Natl. Acad. Sci. U.S.A. 99, 16899-16903, 2002; Bourgeois C., et al J. Clin. Endocrinol. Metab. 82, 3116-3123, 1997; Okamoto Y., et al Biol. Chem. 272, 21589-21596, 1997; Verheij J.B., et al Am. J. Med. Genet. 108, 223-225, 2002; Hofstra R.M.W., et al Eur. J. Hum. Genet. 5, 180-185, 1997; Puffenberger E.G., et al Cell 5 79, 1257-1266, 1994; Attie T., et al, Hum. Mol. Genet. 4, 2407-2409, 1995; Auricchio A., et al Hum. Mol. Genet. 5:351-354, 1996; Amiel J., et al Hum. Mol. Genet. 5, 355-357, 1996; Hofstra R.M.W., et al Nat. Genet. 12, 445-447, 1996; Svensson P.J., et al Hum. Genet. 103, 145-148, 1998; Fuchs S., et al Mol. Med. 7, 115-124, 2001; Pingault V., et al (2002) Hum. Genet. 111, 198-206; WO2004045516 (Claim 1); WO2004048938 (Example 2); WO2004040000 (Claim 10 151); WO2003087768 (Claim 1); WO2003016475 (Claim 1); WO2003016475 (Claim 1); WO200261087 (Fig 1); WO2003016494 (Fig 6); WO2003025138 (Claim 12; Page 144); WO200198351 (Claim 1; Page 124-125); EP522868 (Claim 8; Fig 2); WO200177172 (Claim 1; Page 297-299); US2003109676; US6518404 (Fig 3); US5773223 (Claim 1a; Col 31-34); WO2004001004;

15 (10) MSG783 (RNF124, hypothetical protein FLJ20315, Genbank accession no. NM_017763); WO2003104275 (Claim 1); WO2004046342 (Example 2); WO2003042661 (Claim 12); WO2003083074 (Claim 14; Page 61); WO2003018621 (Claim 1); WO2003024392 (Claim 2; Fig 93); WO200166689 (Example 6);

20 Cross-references: LocusID:54894; NP_060233.2; NM_017763_1

(11) STEAP2 (HGNC_8639, IPCA-1, PCANAP1, STAMP1, STEAP2, STMP, prostate cancer associated gene 1, prostate cancer associated protein 1, six transmembrane epithelial antigen of prostate 2, six transmembrane prostate protein, Genbank accession no. AF455138)

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30 Cross-references: GI:22655488; AAN04080.1; AF455138_1

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WO200230268 (Claim 27; Page 391); US2003219806 (Claim 4); WO200162794 (Claim 14; Fig 1A-D);

Cross-references: MIM:606936; NP_060106.2; NM_017636_1

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Cross-references: MIM:187395; NP_003203.1; NM_003212_1

(14) CD21 (CR2 (Complement receptor 2) or C3DR (C3d/Epstein Barr virus receptor)

15 or Hs.73792 Genbank accession no. M26004)

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(Claim 9); WO2004045520 (Example 4); WO9102536 (Fig 9.1-9.9); WO2004020595

(Claim 1);

Accession: P20023; Q13866; Q14212; EMBL; M26004; AAA35786.1.

25 (15) CD79b (CD79B, CD79 β , Igb (immunoglobulin-associated beta), B29, Genbank accession no. NM_000626 or 11038674)

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et al (1992) Eur. J. Immunol. 22 (6):1621-1625); WO2004016225 (claim 2, Fig 140);

WO2003087768, US2004101874 (claim 1, page 102); WO2003062401 (claim 9); WO200278524

30 (Example 2); US2002150573 (claim 5, page 15); US5644033; WO2003048202 (claim 1, pages

306 and 309); WO 99/558658, US6534482 (claim 13, Fig 17A/B); WO200055351 (claim 11,

pages 1145-1146);

Cross-references: MIM:147245; NP_000617.1; NM_000626_1

35 (16) FcRH2 (IFGP4, IRTA4, SPAP1A (SH2 domain containing phosphatase anchor protein 1a), SPAP1B, SPAP1C, Genbank accession no. NM_030764, AY358130)

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- 5 WO2003089624 (Claim 25);
Cross-references: MIM:606509; NP_110391.2; NM_030764_1
- (17) HER2 (ErbB2, Genbank accession no. M11730)
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107); WO200212341 (Claim 68; Fig 7); WO200213847 (Page 71-74); WO200214503
(Page 114-117); WO200153463 (Claim 2; Page 41-46); WO200141787 (Page 15);
WO200044899 (Claim 52; Fig 7); WO200020579 (Claim 3; Fig 2); US5869445 (Claim
20 3; Col 31-38); WO9630514 (Claim 2; Page 56-61); EP1439393 (Claim 7);
WO2004043361 (Claim 7); WO2004022709; WO200100244 (Example 3; Fig 4);
Accession: P04626; EMBL; M11767; AAA35808.1. EMBL; M11761; AAA35808.1.
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WO200260317 (Claim 2);
30 Accession: P40199; Q14920; EMBL; M29541; AAA59915.1. EMBL; M18728;
- (19) MDP (DPEP1, Genbank accession no. BC017023)
Proc. Natl. Acad. Sci. U.S.A. 99 (26):16899-16903 (2002)); WO2003016475 (Claim 1);
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Accession: Q9UHF4; Q6UWA9; Q96SH8; EMBL; AF184971; AAF01320.1.
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Gary S.C., et al *Gene* 256, 139-147, 2000; Clark H.F., et al *Genome Res.* 13, 2265-2270, 2003; Strausberg R.L., et al *Proc. Natl. Acad. Sci. U.S.A.* 99, 16899-16903, 2002; US2003186372 (Claim 11); US2003186373 (Claim 11); US2003119131 (Claim 1; Fig 15 52); US2003119122 (Claim 1; Fig 52); US2003119126 (Claim 1); US2003119121 (Claim 1; Fig 52); US2003119129 (Claim 1); US2003119130 (Claim 1); US2003119128 (Claim 1; Fig 52); US2003119125 (Claim 1); WO2003016475 (Claim 1); WO200202634 (Claim 1);
- 20 (22) EphB2R (DRT, ERK, Hek5, EPHT3, Tyro5, Genbank accession no. NM_004442)
Chan, J. and Watt, V.M., *Oncogene* 6 (6), 1057-1061 (1991) *Oncogene* 10 (5):897-905 (1995), *Annu. Rev. Neurosci.* 21:309-345 (1998), *Int. Rev. Cytol.* 196:177-244 (2000)); WO2003042661 (Claim 12); WO200053216 (Claim 1; Page 41); WO2004065576 (Claim 1); WO2004020583 (Claim 9); WO2003004529 (Page 128-132); WO200053216 (Claim 1; Page 42);
25 Cross-references: MIM:600997; NP_004433.2; NM_004442_1
- (23) ASLG659 (B7h, Genbank accession no. AX092328)
US20040101899 (Claim 2); WO2003104399 (Claim 11); WO2004000221 (Fig 3);
US2003165504 (Claim 1); US2003124140 (Example 2); US2003065143 (Fig 60);
30 WO2002102235 (Claim 13; Page 299); US2003091580 (Example 2); WO200210187 (Claim 6; Fig 10); WO200194641 (Claim 12; Fig 7b); WO200202624 (Claim 13; Fig 1A-1B); US2002034749 (Claim 54; Page 45-46); WO200206317 (Example 2; Page 320-321, Claim 34; Page 321-322); WO200271928 (Page 468-469); WO200202587 (Example 1; Fig 1);
WO200140269 (Example 3; Pages 190-192); WO200036107 (Example 2; Page 205-207);
35 WO2004053079 (Claim 12); WO2003004989 (Claim 1); WO200271928 (Page 233-234, 452-453); WO 0116318;

- (24) PSCA (Prostate stem cell antigen precursor, Genbank accession no. AJ297436)
Reiter R.E., et al Proc. Natl. Acad. Sci. U.S.A. 95, 1735-1740, 1998; Gu Z., et al
Oncogene 19, 1288-1296, 2000; Biochem. Biophys. Res. Commun. (2000) 275(3):783-
788; WO2004022709; EP1394274 (Example 11); US2004018553 (Claim 17);
5 WO2003008537 (Claim 1); WO200281646 (Claim 1; Page 164); WO2003003906
(Claim 10; Page 288); WO200140309 (Example 1; Fig 17); US2001055751 (Example
1; Fig 1b); WO200032752 (Claim 18; Fig 1); WO9851805 (Claim 17; Page 97);
WO9851824 (Claim 10; Page 94); WO9840403 (Claim 2; Fig 1B);
Accession: O43653; EMBL; AF043498; AAC39607.1.
- 10
- (25) GEDA (Genbank accession No. AY260763);
AAP14954 lipoma HMGIC fusion-partner-like protein /pid=AAP14954.1 - Homo sapiens
Species: Homo sapiens (human)
WO2003054152 (Claim 20); WO2003000842 (Claim 1); WO2003023013 (Example 3, Claim
15 20); US2003194704 (Claim 45);
Cross-references: GI:30102449; AAP14954.1; AY260763_1
- (26) BAFF-R (B cell -activating factor receptor, BLyS receptor 3, BR3, Genbank accession No.
AF116456); BAFF receptor /pid=NP_443177.1 - Homo sapiens
20 Thompson, J.S., et al Science 293 (5537), 2108-2111 (2001); WO2004058309; WO2004011611;
WO2003045422 (Example; Page 32-33); WO2003014294 (Claim 35; Fig 6B); WO2003035846
(Claim 70; Page 615-616); WO200294852 (Col 136-137); WO200238766 (Claim 3; Page 133);
WO200224909 (Example 3; Fig 3);
Cross-references: MIM:606269; NP_443177.1; NM_052945_1; AF132600
- 25
- (27) CD22 (B-cell receptor CD22-B isoform, BL-CAM, Lyb-8, Lyb8, SIGLEC-2, FLJ22814,
Genbank accession No. AK026467);
Wilson et al (1991) J. Exp. Med. 173:137-146; WO2003072036 (Claim 1; Fig 1);
Cross-references: MIM:107266; NP_001762.1; NM_001771_1
- 30
- (28) CD79a (CD79A, CD79 α , immunoglobulin-associated alpha, a B cell-specific protein that
covalently interacts with Ig beta (CD79B) and forms a complex on the surface with Ig M
molecules, transduces a signal involved in B-cell differentiation), pI: 4.84, MW: 25028 TM: 2 [P]
Gene Chromosome: 19q13.2, Genbank accession No. NP_001774.10)
35 WO2003088808, US20030228319; WO2003062401 (claim 9); US2002150573 (claim 4, pages
13-14); WO9958658 (claim 13, Fig 16); WO9207574 (Fig 1); US5644033; Ha et al (1992) J.
Immunol. 148(5):1526-1531; Mueller et al (1992) Eur. J. Biochem. 22:1621-1625; Hashimoto et

al (1994) Immunogenetics 40(4):287-295; Preud'homme et al (1992) Clin. Exp. Immunol. 90(1):141-146; Yu et al (1992) J. Immunol. 148(2) 633-637; Sakaguchi et al (1988) EMBO J. 7(11):3457-3464;

- 5 (29) CXCR5 (Burkitt's lymphoma receptor 1, a G protein-coupled receptor that is activated by the CXCL13 chemokine, functions in lymphocyte migration and humoral defense, plays a role in HIV-2 infection and perhaps development of AIDS, lymphoma, myeloma, and leukemia); 372 aa, pI: 8.54 MW: 41959 TM: 7 [P] Gene Chromosome: 11q23.3, Genbank accession No. NP_001707.1)
- 10 WO2004040000; WO2004015426; US2003105292 (Example 2); US6555339 (Example 2); WO200261087 (Fig 1); WO200157188 (Claim 20, page 269); WO200172830 (pages 12-13); WO200022129 (Example 1, pages 152-153, Example 2, pages 254-256); WO9928468 (claim 1, page 38); US5440021 (Example 2, col 49-52); WO9428931 (pages 56-58); WO9217497 (claim 7, Fig 5); Dobner et al (1992) Eur. J. Immunol. 22:2795-2799; Barella et al (1995) Biochem. J.
- 15 309:773-779;

- (30) HLA-DOB (Beta subunit of MHC class II molecule (Ia antigen) that binds peptides and presents them to CD4+ T lymphocytes); 273 aa, pI: 6.56 MW: 30820 TM: 1 [P] Gene Chromosome: 6p21.3, Genbank accession No. NP_002111.1)
- 20 Tonnelle et al (1985) EMBO J. 4(11):2839-2847; Jonsson et al (1989) Immunogenetics 29(6):411-413; Beck et al (1992) J. Mol. Biol. 228:433-441; Strausberg et al (2002) Proc. Natl. Acad. Sci USA 99:16899-16903; Servenius et al (1987) J. Biol. Chem. 262:8759-8766; Beck et al (1996) J. Mol. Biol. 255:1-13; Naruse et al (2002) Tissue Antigens 59:512-519; WO9958658 (claim 13, Fig 15); US6153408 (Col 35-38); US5976551 (col 168-170); US6011146 (col 145-
- 25 146); Kasahara et al (1989) Immunogenetics 30(1):66-68; Larhammar et al (1985) J. Biol. Chem. 260(26):14111-14119;

- (31) P2X5 (Purinergic receptor P2X ligand-gated ion channel 5, an ion channel gated by extracellular ATP, may be involved in synaptic transmission and neurogenesis, deficiency may contribute to the pathophysiology of idiopathic detrusor instability); 422 aa, pI: 7.63, MW: 47206
- 30 TM: 1 [P] Gene Chromosome: 17p13.3, Genbank accession No. NP_002552.2)
- Le et al (1997) FEBS Lett. 418(1-2):195-199; WO2004047749; WO2003072035 (claim 10); Touchman et al (2000) Genome Res. 10:165-173; WO200222660 (claim 20); WO2003093444 (claim 1); WO2003087768 (claim 1); WO2003029277 (page 82);
- 35

- (32) CD72 (B-cell differentiation antigen CD72, Lyb-2) PROTEIN SEQUENCE Full
maeaity...tafrfpd (1..359; 359 aa), pI: 8.66, MW: 40225 TM: 1 [P] Gene Chromosome: 9p13.3,
Genbank accession No. NP_001773.1)
WO2004042346 (claim 65); WO2003026493 (pages 51-52, 57-58); WO200075655 (pages 105-
5 106); Von Hoegen et al (1990) J. Immunol. 144(12):4870-4877; Strausberg et al (2002) Proc.
Natl. Acad. Sci USA 99:16899-16903;
- (33) LY64 (Lymphocyte antigen 64 (RP105), type I membrane protein of the leucine rich repeat
10 (LRR) family, regulates B-cell activation and apoptosis, loss of function is associated with
increased disease activity in patients with systemic lupus erythematosus); 661 aa, pI: 6.20, MW:
74147 TM: 1 [P] Gene Chromosome: 5q12, Genbank accession No. NP_005573.1)
US2002193567; WO9707198 (claim 11, pages 39-42); Miura et al (1996) Genomics 38(3):299-
304; Miura et al (1998) Blood 92:2815-2822; WO2003083047; WO9744452 (claim 8, pages 57-
61); WO200012130 (pages 24-26);
- 15
- (34) FcRH1 (Fc receptor-like protein 1, a putative receptor for the immunoglobulin Fc domain
that contains C2 type Ig-like and ITAM domains, may have a role in B-lymphocyte
differentiation); 429 aa, pI: 5.28, MW: 46925 TM: 1 [P] Gene Chromosome: 1q21-1q22,
Genbank accession No. NP_443170.1)
20 WO2003077836; WO200138490 (claim 6, Fig 18E-1-18-E-2); Davis et al (2001) Proc. Natl.
Acad. Sci USA 98(17):9772-9777; WO2003089624 (claim 8); EP1347046 (claim 1);
WO2003089624 (claim 7);
- (35) IRTA2 (Immunoglobulin superfamily receptor translocation associated 2, a putative
25 immunoreceptor with possible roles in B cell development and lymphomagenesis; deregulation of
the gene by translocation occurs in some B cell malignancies); 977 aa, pI: 6.88 MW: 106468
TM: 1 [P] Gene Chromosome: 1q21, Genbank accession No. Human:AF343662, AF343663,
AF343664, AF343665, AF369794, AF397453, AK090423, AK090475, AL834187, AY358085;
Mouse:AK089756, AY158090, AY506558; NP_112571.1
30 WO2003024392 (claim 2, Fig 97); Nakayama et al (2000) Biochem. Biophys. Res. Commun.
277(1):124-127; WO2003077836; WO200138490 (claim 3, Fig 18B-1-18B-2);
- (36) TENB2 (TMEFF2, tomoregulin, TPEF, HPP1, TR, putative transmembrane proteoglycan,
related to the EGF/heregulin family of growth factors and follistatin); 374 aa, NCBI Accession:
35 AAD55776, AAF91397, AAG49451, NCBI RefSeq: NP_057276; NCBI Gene: 23671; OMIM:
605734; SwissProt Q9UIK5; Genbank accession No. AF179274; AY358907, CAF85723,
CQ782436

- WO2004074320 (SEQ ID NO 810); JP2004113151 (SEQ ID NOS 2, 4, 8); WO2003042661 (SEQ ID NO 580); WO2003009814 (SEQ ID NO 411); EP1295944 (pages 69-70); WO200230268 (page 329); WO200190304 (SEQ ID NO 2706); US2004249130; US2004022727; WO2004063355; US2004197325; US2003232350; US2004005563; US2003124579; Horie et al (2000) *Genomics* 67:146-152; Uchida et al (1999) *Biochem. Biophys. Res. Commun.* 266:593-602; Liang et al (2000) *Cancer Res.* 60:4907-12; Glynn-Jones et al (2001) *Int J Cancer.* Oct 15;94(2):178-84;
- (37) PMEL17 (silver homolog; SILV; D12S53E; PMEL17; (SI); (SIL); ME20; gp100) BC001414; BT007202; M32295; M77348; NM_006928; McGlinchey, R.P. et al (2009) *Proc. Natl. Acad. Sci. U.S.A.* 106 (33), 13731-13736; Kummer, M.P. et al (2009) *J. Biol. Chem.* 284 (4), 2296-2306;
- (38) TMEFF1 (transmembrane protein with EGF-like and two follistatin-like domains 1; Tomoregulin-1; H7365; C9orf2; C9ORF2; U19878; X83961) NM_080655; NM_003692; Harms, P.W. (2003) *Genes Dev.* 17 (21), 2624-2629; Gery, S. et al (2003) *Oncogene* 22 (18):2723-2727;
- (39) GDNF-Ra1 (GDNF family receptor alpha 1; GFRA1; GDNFR; GDNFRA; RETL1; TRNR1; RET1L; GDNFR-alpha1; GFR-ALPHA-1; U95847; BC014962; NM_145793) NM_005264; Kim, M.H. et al (2009) *Mol. Cell. Biol.* 29 (8), 2264-2277; Treanor, J.J. et al (1996) *Nature* 382 (6586):80-83;
- (40) Ly6E (lymphocyte antigen 6 complex, locus E; Ly67,RIG-E,SCA-2,TSA-1) NP_002337.1; NM_002346.2; de Nooij-van Dalen, A.G. et al (2003) *Int. J. Cancer* 103 (6), 768-774; Zammit, D.J. et al (2002) *Mol. Cell. Biol.* 22 (3):946-952;
- (41) TMEM46 (shisa homolog 2 (*Xenopus laevis*); SHISA2) NP_001007539.1; NM_001007538.1; Furushima, K. et al (2007) *Dev. Biol.* 306 (2), 480-492; Clark, H.F. et al (2003) *Genome Res.* 13 (10):2265-2270;
- (42) Ly6G6D (lymphocyte antigen 6 complex, locus G6D; Ly6-D, MEGT1) NP_067079.2; NM_021246.2; Mallya, M. et al (2002) *Genomics* 80 (1):113-123; Ribas, G. et al (1999) *J. Immunol.* 163 (1):278-287;
- (43) LGR5 (leucine-rich repeat-containing G protein-coupled receptor 5; GPR49, GPR67) NP_003658.1; NM_003667.2; Salanti, G. et al (2009) *Am. J. Epidemiol.* 170 (5):537-545; Yamamoto, Y. et al (2003) *Hepatology* 37 (3):528-533;

- (44) RET (ret proto-oncogene; MEN2A; HSCR1; MEN2B; MTC1; (PTC); CDHF12; Hs.168114; RET51; RET-ELE1) NP_066124.1; NM_020975.4; Tsukamoto, H. et al (2009) Cancer Sci. 100 (10):1895-1901; Narita, N. et al (2009) Oncogene 28 (34):3058-3068;
- 5 (45) LY6K (lymphocyte antigen 6 complex, locus K; LY6K; HSJ001348; FLJ35226) NP_059997.3; NM_017527.3; Ishikawa, N. et al (2007) Cancer Res. 67 (24):11601-11611; de Nooij-van Dalen, A.G. et al (2003) Int. J. Cancer 103 (6):768-774;
- (46) GPR19 (G protein-coupled receptor 19; Mm.4787) NP_006134.1; NM_006143.2; Montpetit, 10 A. and Sinnett, D. (1999) Hum. Genet. 105 (1-2):162-164; O'Dowd, B.F. et al (1996) FEBS Lett. 394 (3):325-329;
- (47) GPR54 (KISS1 receptor; KISS1R; GPR54; HOT7T175; AXOR12) NP_115940.2; NM_032551.4; Navenot, J.M. et al (2009) Mol. Pharmacol. 75 (6):1300-1306; Hata, K. et al 15 (2009) Anticancer Res. 29 (2):617-623;
- (48) ASPHD1 (aspartate beta-hydroxylase domain containing 1; LOC253982) NP_859069.2; NM_181718.3; Gerhard, D.S. et al (2004) Genome Res. 14 (10B):2121-2127;
- 20 (49) Tyrosinase (TYR; OCAIA; OCA1A; tyrosinase; SHEP3) NP_000363.1; NM_000372.4; Bishop, D.T. et al (2009) Nat. Genet. 41 (8):920-925; Nan, H. et al (2009) Int. J. Cancer 125 (4):909-917;
- (50) TMEM118 (ring finger protein, transmembrane 2; RNFT2; FLJ14627) NP_001103373.1; 25 NM_001109903.1; Clark, H.F. et al (2003) Genome Res. 13 (10):2265-2270; Scherer, S.E. et al (2006) Nature 440 (7082):346-351
- (51) GPR172A (G protein-coupled receptor 172A; GPCR41; FLJ11856; D15Ert4747e) NP_078807.1; NM_024531.3; Ericsson, T.A. et al (2003) Proc. Natl. Acad. Sci. U.S.A. 100 30 (11):6759-6764; Takeda, S. et al (2002) FEBS Lett. 520 (1-3):97-101.

In one embodiment, the antibody binds to one or more of the following polypeptides: BMPR1B; E16; STEAP1; 0772P; MPF; Napi3b; Sema 5b; PSCA hlg; ETBR; MSG783; STEAP2; TrpM4; CRIPTO; CD21; CD79b; FcRH2; HER2; NCA; MDP; IL20R α ; Brevican; EphB2R; ASLG659; 35 PSCA; GEDA; BAFF-R; CD22; CD79a; CXCR5; HLA-DOB; P2X5; CD72; LY64; FcRH1; IRTA2; TENB2; PMEL17; TMEFF1; GDNF-Ra1; Ly6E; TMEM46; Ly6G6D; LGR5; RET; LY6K; GPR19; GPR54; ASPHD1; Tyrosinase; TMEM118; GPR172A; and CD33.

- In one embodiment, the antibody binds to BMPR1B;
- In one embodiment, the antibody binds to E16;
- In one embodiment, the antibody binds to STEAP1;
- In one embodiment, the antibody binds to 0772P;
- 5 In one embodiment, the antibody binds to MPF;
- In one embodiment, the antibody binds to NaPi2b;
- In one embodiment, the antibody binds to Sema 5b;
- In one embodiment, the antibody binds to PSCA hlg;
- In one embodiment, the antibody binds to ETBR;
- 10 In one embodiment, the antibody binds to MSG783;
- In one embodiment, the antibody binds to STEAP2;
- In one embodiment, the antibody binds to TrpM4;
- In one embodiment, the antibody binds to CRIPTO;
- In one embodiment, the antibody binds to CD21;
- 15 In one embodiment, the antibody binds to CD79b;
- In one embodiment, the antibody binds to FcRH2;
- In one embodiment, the antibody binds to HER2;
- In one embodiment, the antibody binds to NCA;
- In one embodiment, the antibody binds to MDP;
- 20 In one embodiment, the antibody binds to IL20R α ;
- In one embodiment, the antibody binds to Brevican;
- In one embodiment, the antibody binds to EphB2R;
- In one embodiment, the antibody binds to ASLG659;
- In one embodiment, the antibody binds to PSCA;
- 25 In one embodiment, the antibody binds to GEDA;
- In one embodiment, the antibody binds to BAFF-R;
- In one embodiment, the antibody binds to CD22;
- In one embodiment, the antibody binds to CD79a;
- In one embodiment, the antibody binds to CXCR5;
- 30 In one embodiment, the antibody binds to HLA-DOB;
- In one embodiment, the antibody binds to P2X5 ;
- In one embodiment, the antibody binds to CD72;
- In one embodiment, the antibody binds to LY64;
- In one embodiment, the antibody binds to FcRH1;
- 35 In one embodiment, the antibody binds to IRTA2;
- In one embodiment, the antibody binds to TENB2;
- In one embodiment, the antibody binds to PMEL17;

- In one embodiment, the antibody binds to TMEFF1;
 In one embodiment, the antibody binds to GDNF-Ra1;
 In one embodiment, the antibody binds to Ly6E;
 In one embodiment, the antibody binds to TMEM46;
 5 In one embodiment, the antibody binds to Ly6G6D;
 In one embodiment, the antibody binds to LGR5;
 In one embodiment, the antibody binds to RET;
 In one embodiment, the antibody binds to LY6K;
 In one embodiment, the antibody binds to GPR19;
 10 In one embodiment, the antibody binds to GPR54;
 In one embodiment, the antibody binds to ASPHD1;
 In one embodiment, the antibody binds to Tyrosinase;
 In one embodiment, the antibody binds to TMEM118;
 In one embodiment, the antibody binds to GPR172A;
 15 In one embodiment, the antibody binds to CD33.

The parent antibody may also be a fusion protein comprising an albumin-binding peptide (ABP) sequence (Dennis et al. (2002) "Albumin Binding As A General Strategy For Improving The Pharmacokinetics Of Proteins" J Biol Chem. 277:35035-35043; WO 01/45746). Antibodies of the
 20 invention include fusion proteins with ABP sequences taught by: (i) Dennis et al (2002) J Biol Chem. 277:35035-35043 at Tables III and IV, page 35038; (ii) US 20040001827 at [0076]; and (iii) WO 01/45746 at pages 12-13, and all of which are incorporated herein by reference.

Antibodies may be produced using recombinant methods and compositions, e.g., as described in US 4816567 and known in the art. In some embodiments, the antibody is produced in a eukaryotic
 25 host cell (e.g., mammalian host cell). In some embodiments, the antibody is produced in a prokaryotic host cell (e.g., E. coli).

In certain embodiments, one or more amino acid modifications may be introduced into the Fc region of an antibody provided herein, thereby generating an Fc region variant. The Fc region
 30 variant may comprise a human Fc region sequence (e.g., a human IgG1, IgG2, IgG3 or IgG4 Fc region) comprising an amino acid modification (e.g. a substitution) at one or more amino acid positions.

In certain embodiments, the invention contemplates an antibody variant that possesses some but
 35 not all effector functions, which make it a desirable candidate for applications in which the half-life of the antibody *in vivo* is important yet certain effector functions (such as complement and ADCC) are unnecessary or deleterious. *In vitro* and/or *in vivo* cytotoxicity assays can be

conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks Fc γ R binding (hence likely lacking ADCC activity), but retains FcRn binding ability.

5 DRUG LOADING OF ADC

The drug loading is the average number of drug moieties per antibody. Drug loading may range from 1 to 8 drugs (D) per antibody (Ab), i.e. where 1, 2, 3, 4, 5, 6, 7, and 8 drug moieties are covalently attached to the antibody. Compositions of ADC include collections of antibodies conjugated with a range of drugs, from 1 to 8. The average number of drugs per antibody in
10 preparations of ADC from conjugation reactions may be characterized by conventional means such as mass spectroscopy, ELISA assay, electrophoresis, and HPLC. The quantitative distribution of ADC in terms of p may also be determined. By ELISA, the averaged value of p in a particular preparation of ADC may be determined (Hamblett et al (2004) Clin. Cancer Res. 10:7063-7070; Sanderson et al (2005) Clin. Cancer Res. 11:843-852). However, the distribution of p (drug)
15 values is not discernible by the antibody-antigen binding and detection limitation of ELISA. Also, ELISA assay for detection of antibody-drug conjugates does not determine where the drug moieties are attached to the antibody, such as the heavy chain or light chain fragments, or the particular amino acid residues. In some instances, separation, purification, and characterization of homogeneous ADC where p is a certain value from ADC with other drug loadings may be
20 achieved by means such as reverse phase HPLC or electrophoresis.

For some antibody-drug conjugates, p may be limited by the number of attachment sites on the antibody. For example, an antibody may have only one or several cysteine thiol groups, or may have only one or several sufficiently reactive thiol groups through which a linker may be attached.
25 Higher drug loading, e.g. $p > 5$, may cause aggregation, insolubility, toxicity, or loss of cellular permeability of certain antibody-drug conjugates.

Typically, fewer than the theoretical maximum of drug moieties is conjugated to an antibody during a conjugation reaction. An antibody may contain, for example, many lysine residues that
30 do not react with the linker-drug intermediate (X-L-D) or linker reagent. Only the most reactive lysine groups may react with an amine-reactive linker reagent. Also, only the most reactive cysteine thiol groups may react with a thiol-reactive linker reagent or linker-drug intermediate. Generally, antibodies do not contain many, if any, free and reactive cysteine thiol groups which may be linked to a drug moiety. Most cysteine thiol residues in the antibodies of the compounds
35 exist as disulfide bridges and must be reduced with a reducing agent such as dithiothreitol (DTT) or TCEP, under partial or total reducing conditions. The loading (drug/antibody ratio, "DAR") of an ADC may be controlled in several different manners, including: (i) limiting the molar excess of

linker-drug intermediate or linker reagent relative to antibody, (ii) limiting the conjugation reaction time or temperature, and (iii) partial or limiting reductive conditions for cysteine thiol modification.

5 Where more than one nucleophilic or electrophilic group of the antibody reacts with a linker-drug intermediate, or linker reagent followed by dimer drug moiety reagent, then the resulting product is a mixture of Antibody-drug conjugate s with a distribution of drug moieties attached to an antibody, e.g. 1, 2, 3, etc. Liquid chromatography methods such as polymeric reverse phase (PLRP) and hydrophobic interaction (HIC) may separate compounds in the mixture by drug
10 loading value. Preparations of ADC with a single drug loading value (p) may be isolated, however, these single loading value ADCs may still be heterogeneous mixtures because the drug moieties may be attached, via the linker, at different sites on the antibody. Thus the antibody-drug conjugate compositions of the invention include mixtures of antibody-drug conjugate compounds where the antibody has one or more drug moieties and where the drug moieties may be attached to
15 the antibody at various amino acid residues.

EXEMPLARY DRUG MOIETIES

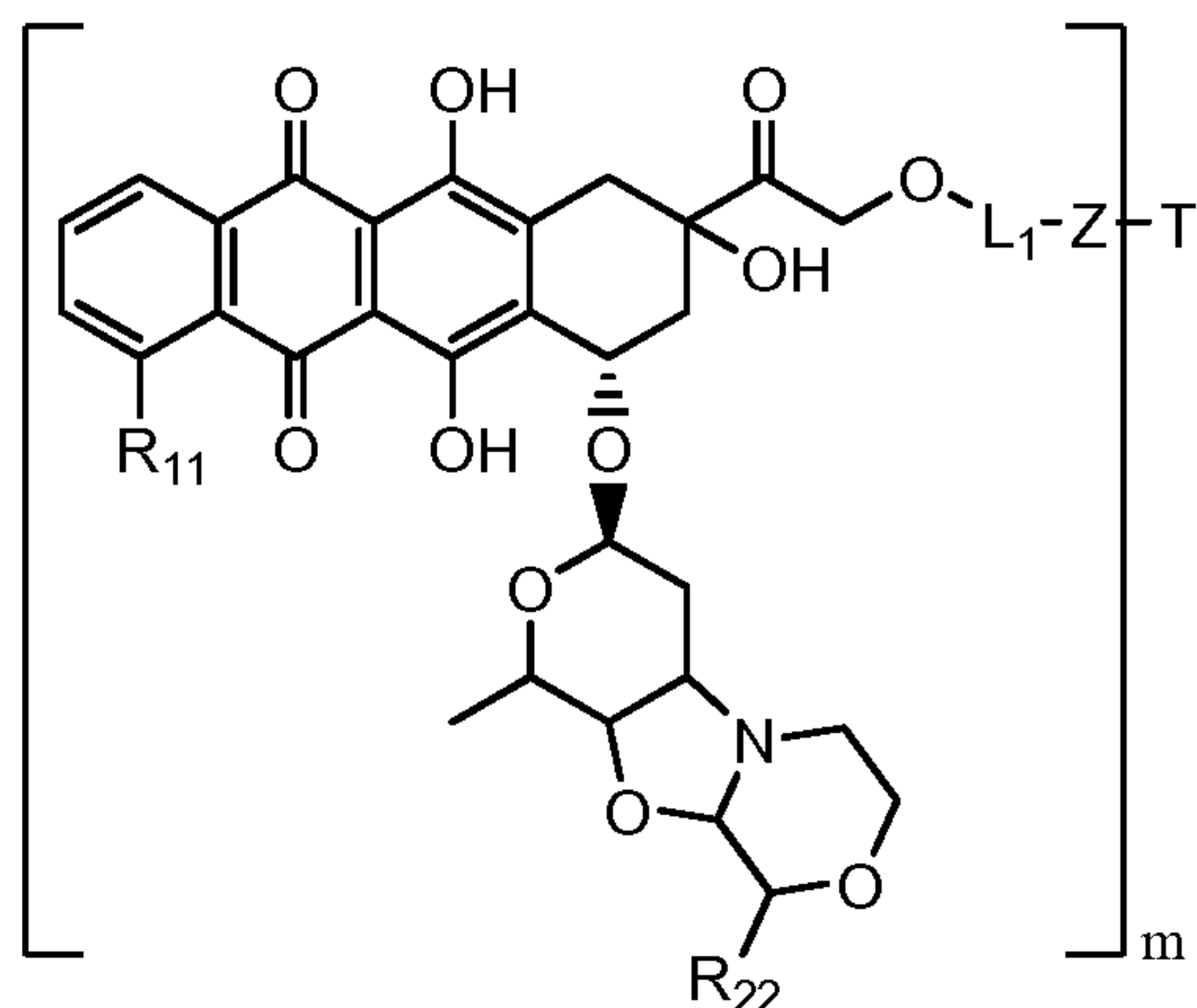
In some embodiments, an ADC comprising anthracycline. Anthracyclines are antibiotic compounds that exhibit cytotoxic activity. While not intending to be bound by any particular theory, studies have indicated that anthracyclines may operate to kill cells by a number of different
20 mechanisms, including: 1) intercalation of the drug molecules into the DNA of the cell thereby inhibiting DNA-dependent nucleic acid synthesis; 2) production by the drug of free radicals which then react with cellular macromolecules to cause damage to the cells, and/or 3) interactions of the drug molecules with the cell membrane (see, e.g., C. Peterson et al., "Transport And Storage Of Anthracycline In Experimental Systems And Human Leukemia" in Anthracycline Antibiotics In
25 Cancer Therapy; N.R. Bachur, "Free Radical Damage" id. at pp.97-102). Because of their cytotoxic potential anthracyclines have been used in the treatment of numerous cancers such as leukemia, breast carcinoma, lung carcinoma, ovarian adenocarcinoma and sarcomas (see e.g., P.H- Wiernik, in Anthracycline: Current Status And New Developments p 11).

30 Nonlimiting exemplary anthracyclines include doxorubicin, epirubicin, idarubicin, daunomycin, nemorubicin, and derivatives thereof. Immunoconjugates and prodrugs of daunorubicin and doxorubicin have been prepared and studied (Kratz et al (2006) *Current Med. Chem.* 13:477-523; Jeffrey et al (2006) *Bioorganic & Med. Chem. Letters* 16:358-362; Torgov et al (2005) *Bioconj. Chem.* 16:717-721; Nagy et al (2000) *Proc. Natl. Acad. Sci. USA* 97:829-834; Dubowchik et al
35 (2002) *Bioorg. & Med. Chem. Letters* 12:1529-1532; King et al (2002) *J. Med. Chem.* 45:4336-4343; EP 0328147; US 6630579). The antibody-drug conjugate BR96-doxorubicin reacts

specifically with the tumor-associated antigen Lewis-Y and has been evaluated in phase I and II studies (Saleh et al (2000) *J. Clin. Oncology* 18:2282-2292; Ajani et al (2000) *Cancer Jour.* 6:78-81; Tolcher et al (1999) *J. Clin. Oncology* 17:478-484).

PNU-159682 is a potent metabolite (or derivative) of nemorubicin (Quintieri, et al. (2005) *Clinical Cancer Research* 11(4):1608-1617). Nemorubicin is a semisynthetic analog of doxorubicin with a 2-methoxymorpholino group on the glycoside amino of doxorubicin and has been under clinical evaluation (Grandi et al (1990) *Cancer Treat. Rev.* 17:133; Ripamonti et al (1992) *Brit. J. Cancer* 65:703;), including phase II/III trials for hepatocellular carcinoma (Sun et al (2003) *Proceedings of the American Society for Clinical Oncology* 22, Abs1448; Quintieri (2003) *Proceedings of the American Association of Cancer Research*, 44:1st Ed, Abs 4649; Pacciarini et al (2006) *Jour. Clin. Oncology* 24:14116).

A nonlimiting exemplary ADC comprising nemorubicin or nemorubicin derivatives is shown in Formula Ia:



15 (Ia)

wherein R^{11} is hydrogen atom, hydroxy or methoxy group and R^{22} is a C_1 - C_5 alkoxy group, or a pharmaceutically acceptable salt thereof;

L_1 and Z together are a linker (L) as described herein;

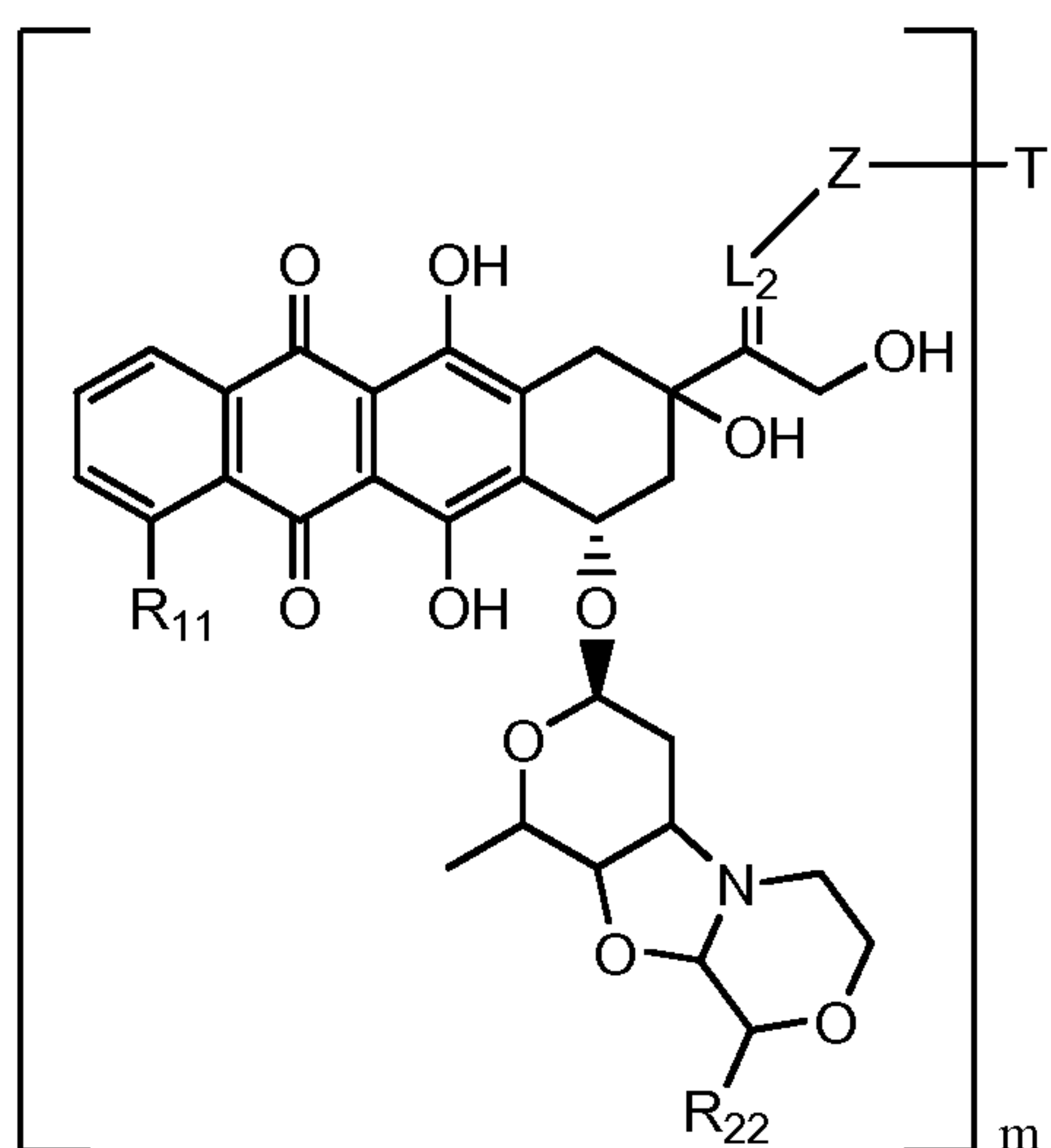
T is an antibody (Ab) as described herein; and

20 m is 1 to about 20.

In some embodiments, m is 1 to 10, 1 to 7, 1 to 5, or 1 to 4.

In some embodiments, R^{11} and R^{22} are both methoxy (-OMe).

A further non-limiting exemplary ADC comprising nemorubicin or nemorubicin derivatives is shown in Formula Ib:



(Ic)

wherein R^{11} is hydrogen atom, hydroxy or methoxy group and R^{22} is a C_1 - C_5 alkoxy group, or a pharmaceutically acceptable salt thereof;

5 L_2 and Z together are a linker (L) as described herein;

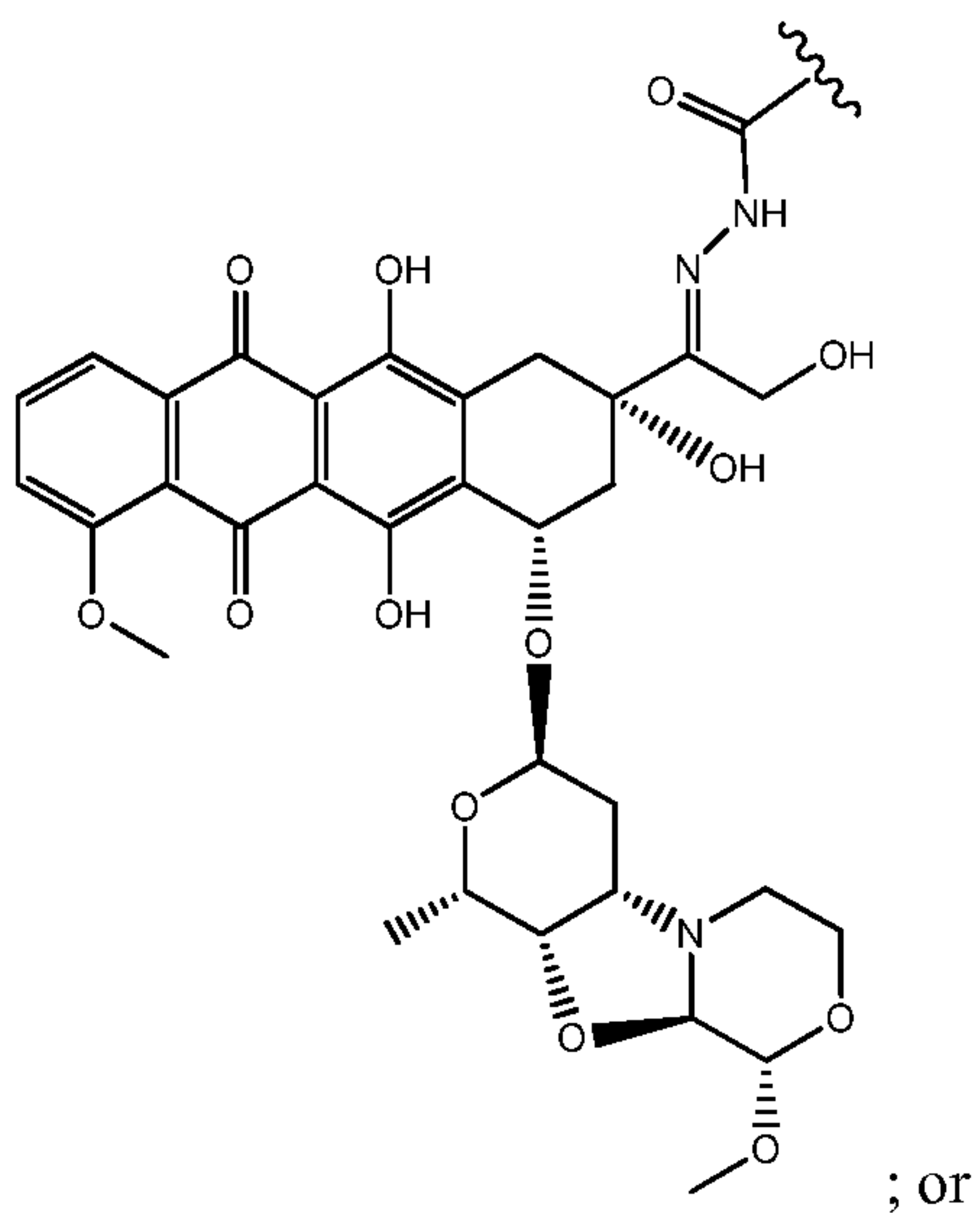
T is an antibody (Ab) as described herein; and

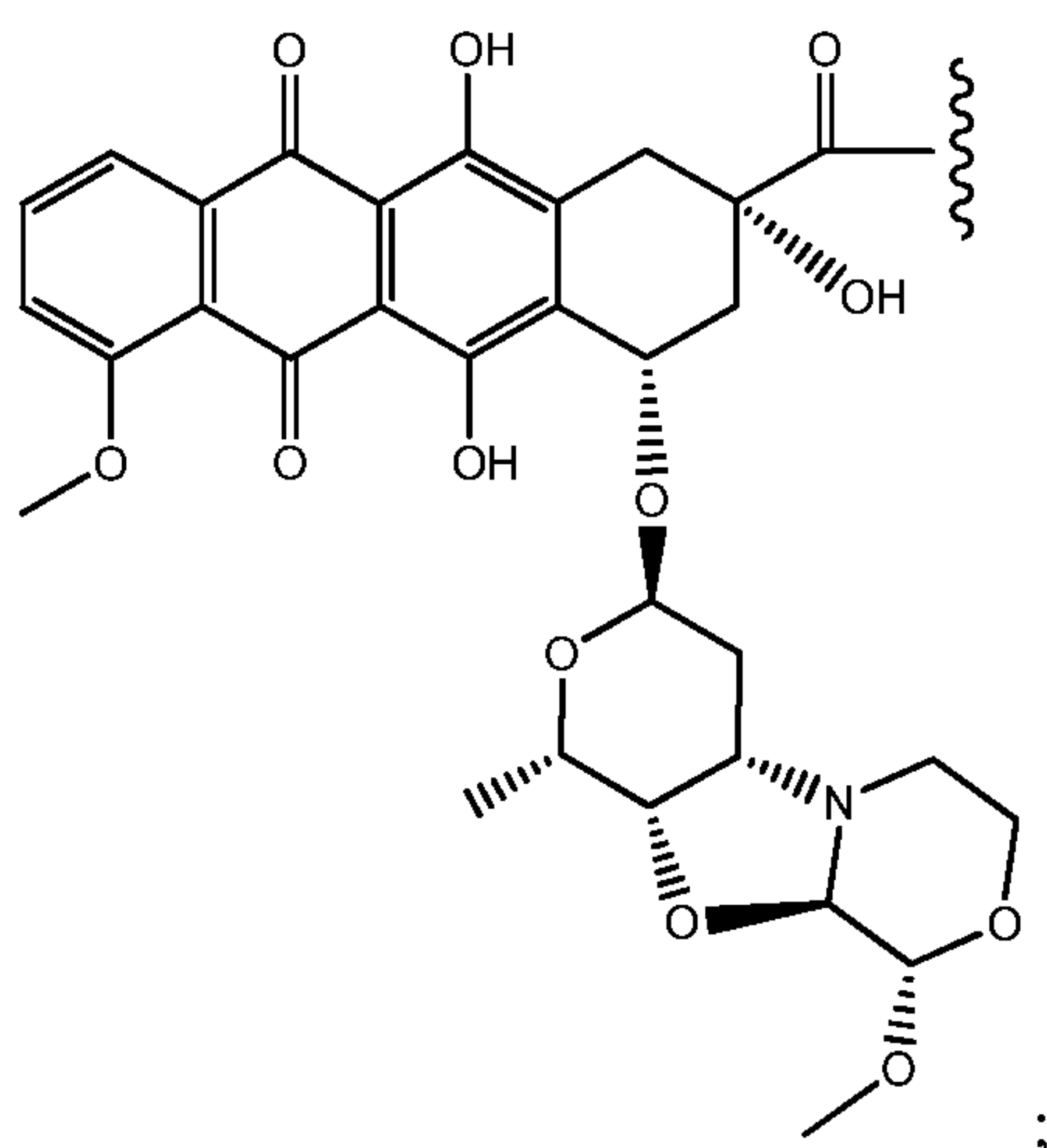
m is 1 to about 20. In some embodiments, m is 1 to 10, 1 to 7, 1 to 5, or 1 to 4.

In some embodiments, R^{11} and R^{22} are both methoxy (-OMe).

10 In some embodiments, the nemorubicin component of a nemorubicin-containing ADC is PNU-159682.

In some such embodiments, the drug portion of the ADC may have one of the following structures:

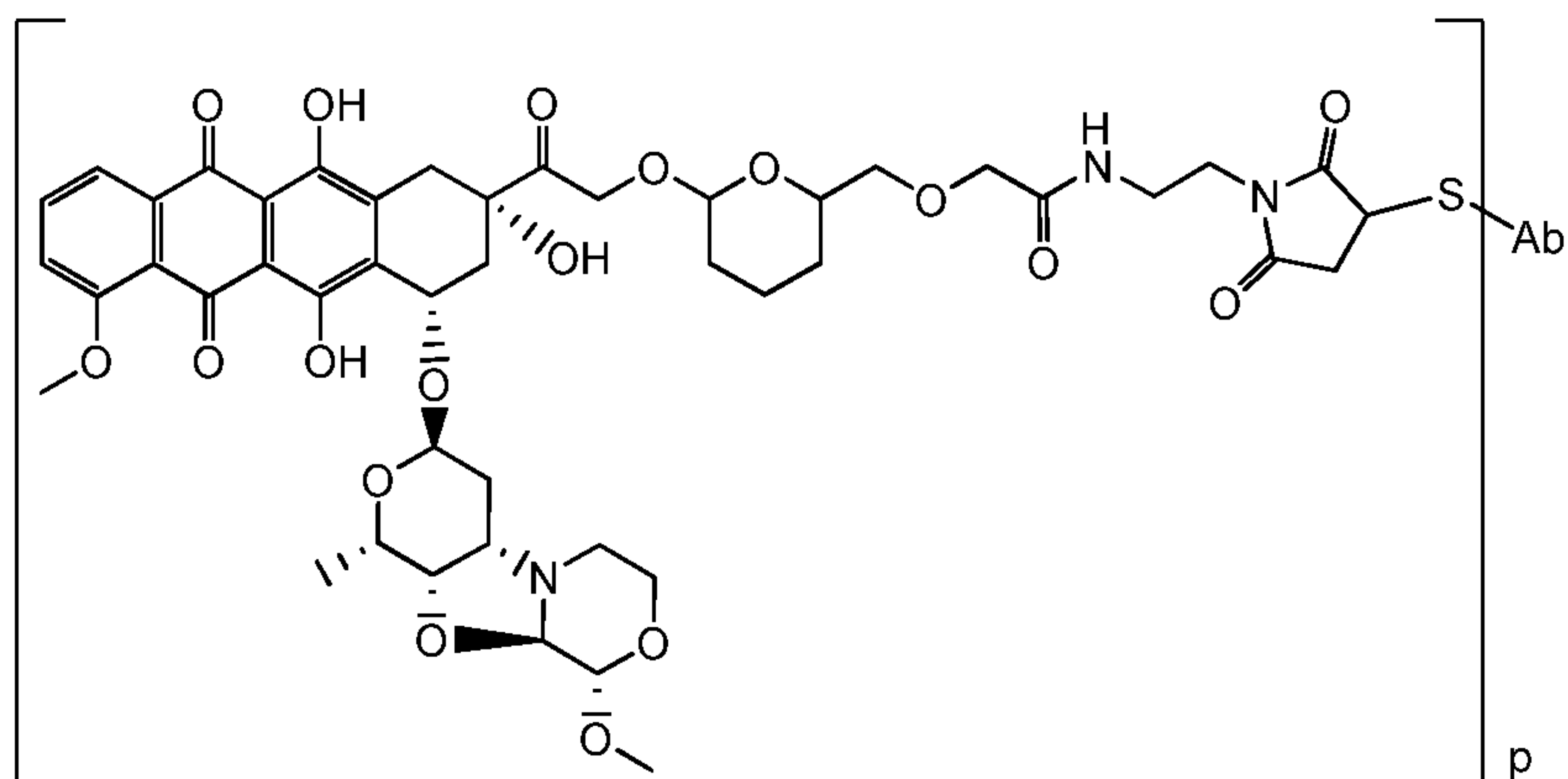




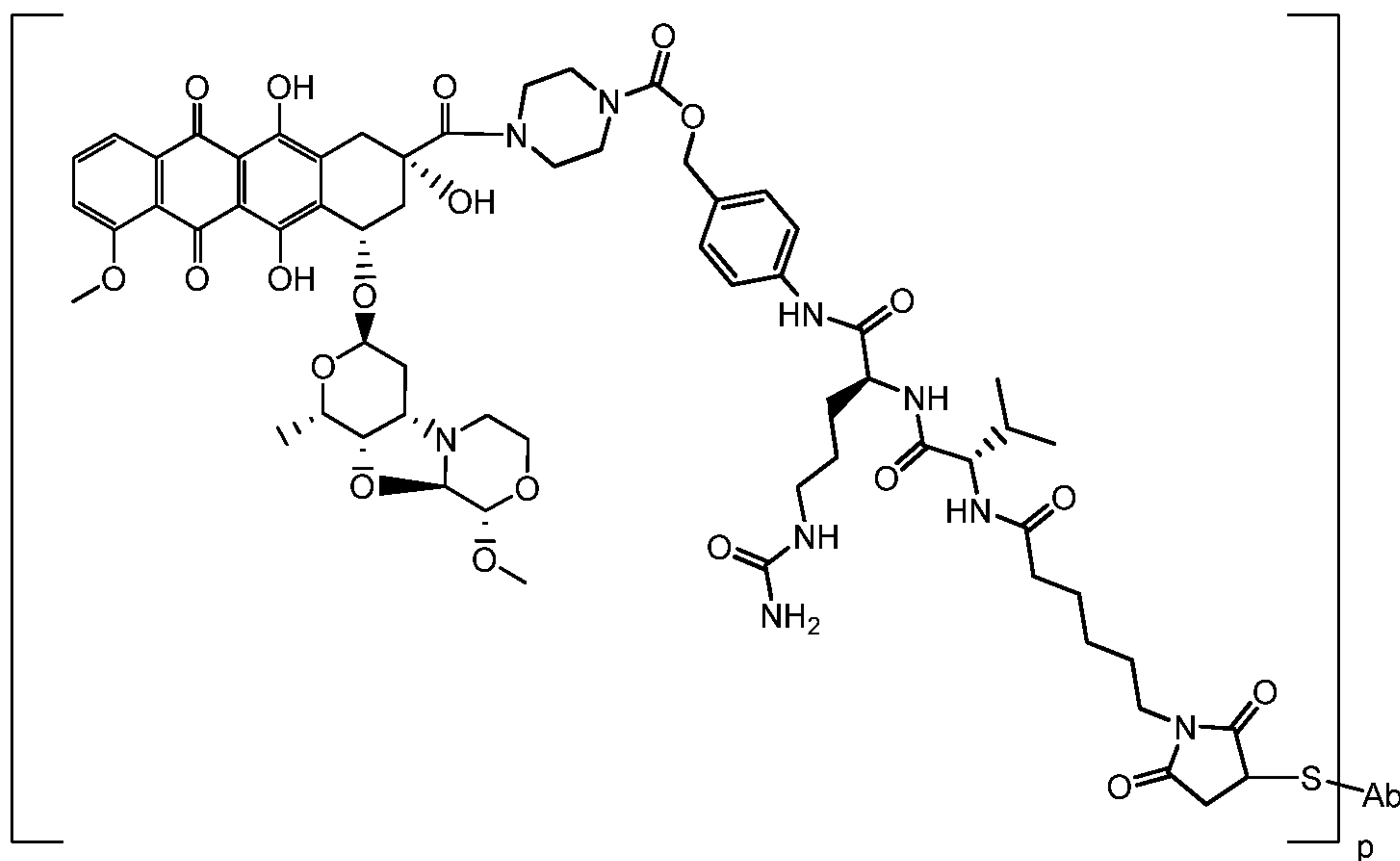
wherein the wavy line indicates the attachment to the linker (L).

Anthracyclines, including PNU-159682, may be conjugated to antibodies through several linkage sites and a variety of linkers (US 2011/0076287; WO2009/099741; US 2010/0034837; WO 2010/009124), including the linkers described herein.

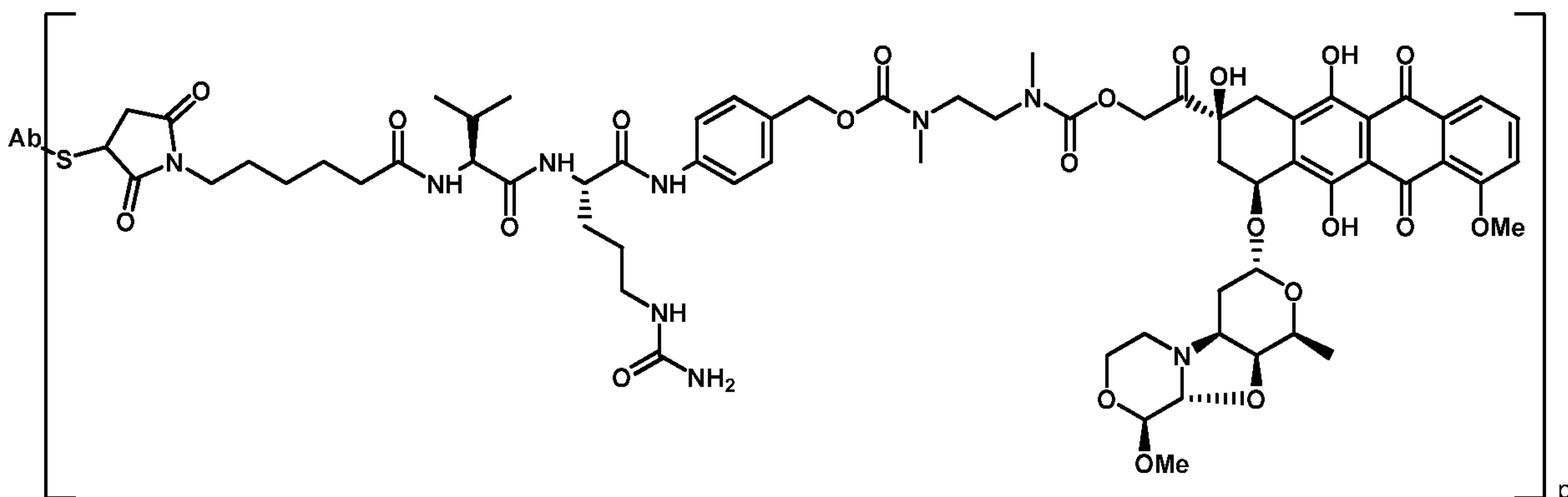
Exemplary ADCs comprising a nemorubicin and linker include, but are not limited to:



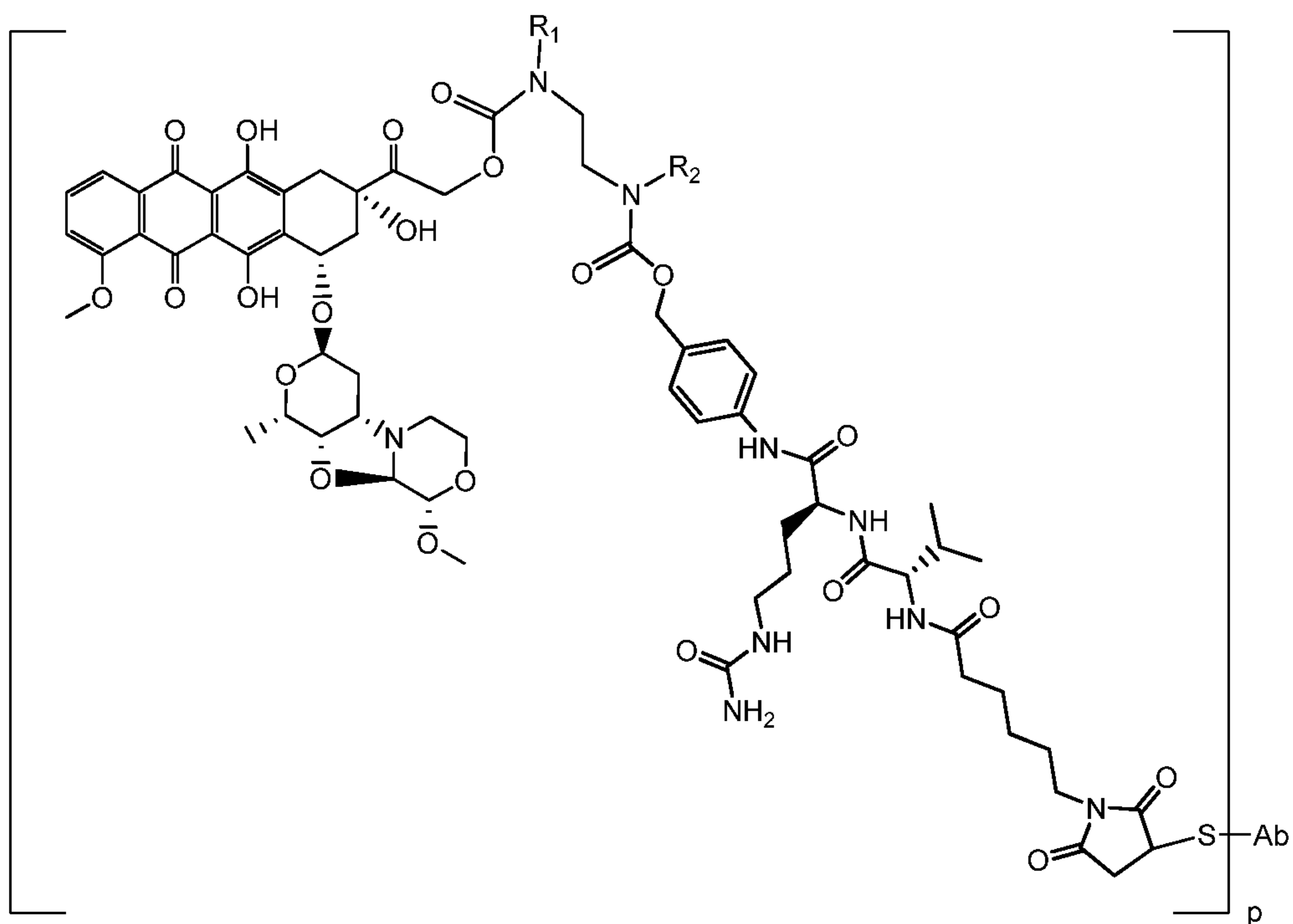
PNU-159682 maleimide acetal-Ab;



PNU-159682-val-cit-PAB-Ab;

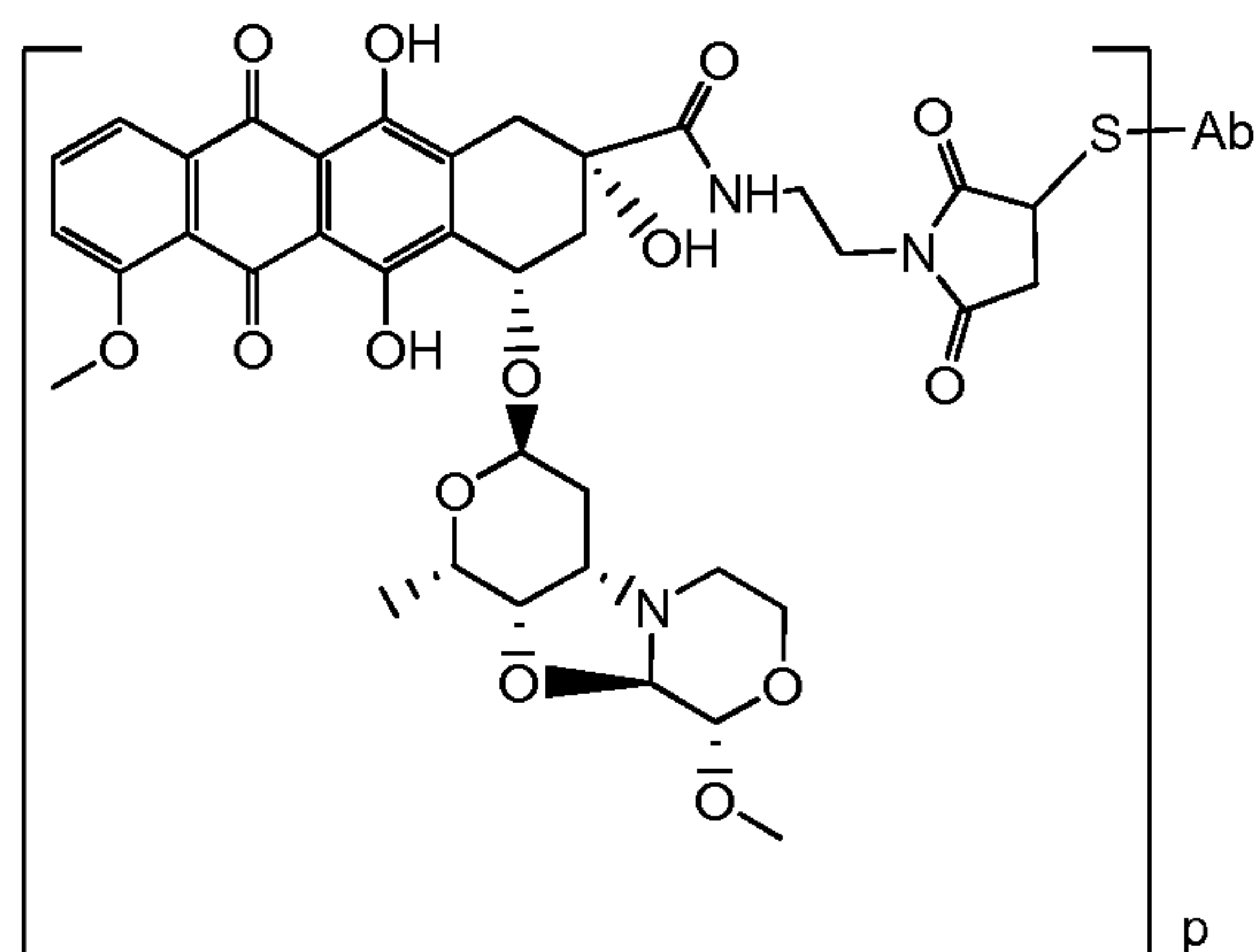


PNU-159682-val-cit-PAB-spacer-Ab;



PNU-159682-val-cit-PAB-spacer(R^1R^2)-Ab, wherein:

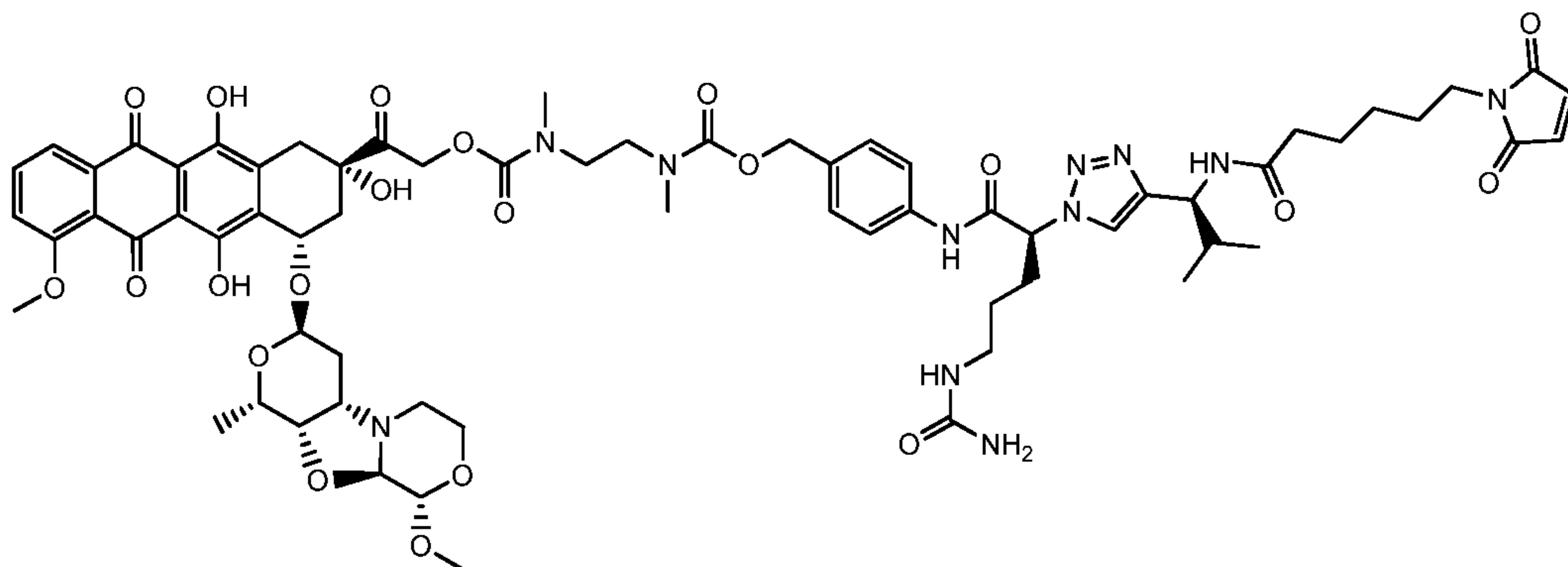
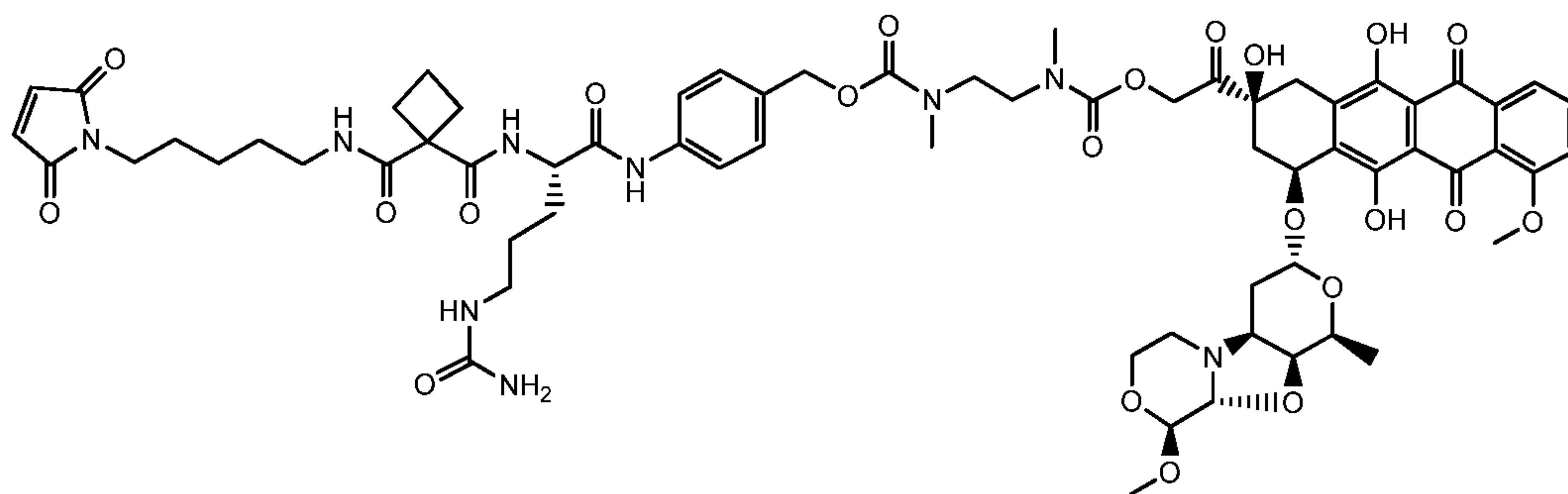
R_1 and R_2 are independently selected from H and C_1 - C_6 alkyl; and



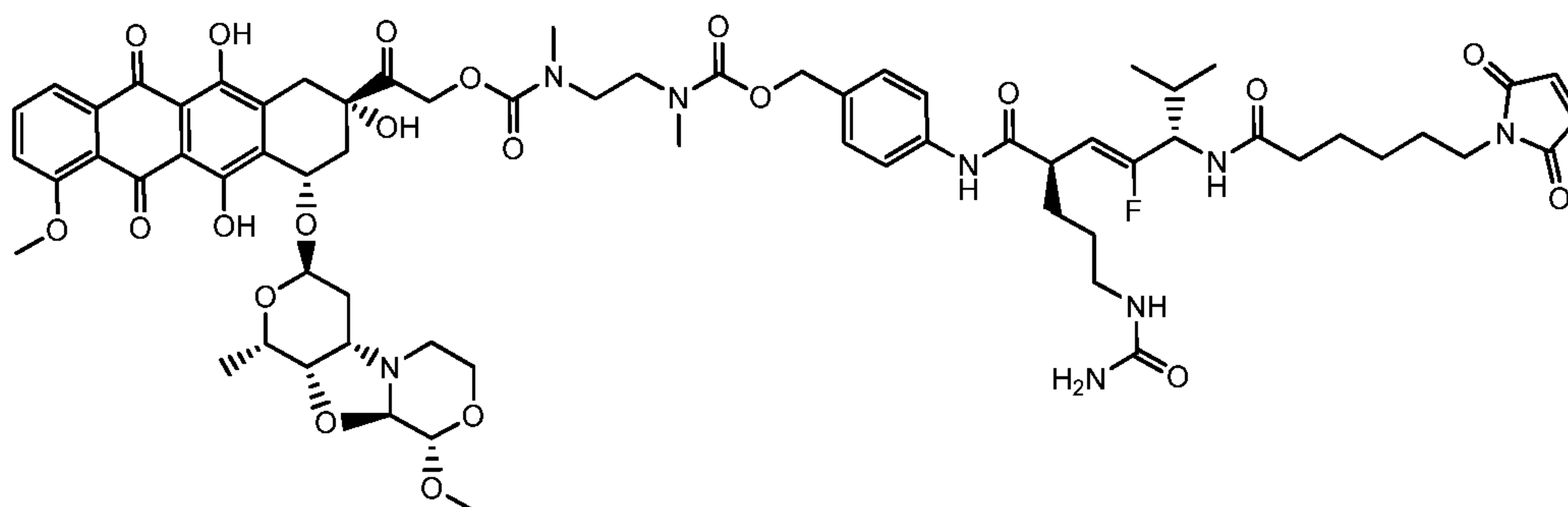
5 PNU-159682-maleimide-Ab.

The linker of PNU-159682 maleimide acetal-Ab is acid-labile, while the linkers of PNU-159682-val-cit-PAB-Ab, PNU-159682-val-cit-PAB-spacer-Ab, and PNU-159682-val-cit-PAB-spacer(R^1R^2)-Ab are protease cleavable.

10 Exemplary ADCs comprising an anthracycline derivative and peptidomimetic linker include, but are not limited to:



5



15

INDICATIONS AND METHODS OF TREATMENT

It is contemplated that the antibody-drug conjugates (ADC) of the present invention may be used to treat various diseases or disorders, e.g. characterized by the overexpression of a tumor antigen. Exemplary conditions or hyperproliferative disorders include benign or malignant solid tumors and hematological disorders such as leukemia and lymphoid malignancies. Others include neuronal, glial, astrocytal, hypothalamic, glandular, macrophagal, epithelial, stromal, blastocoelic, inflammatory, angiogenic and immunologic, including autoimmune, disorders.

In certain embodiments, an ADC of the invention comprising an anti-NaPi2b antibody, such as those described above, is used in a method of treating solid tumor, e.g., ovarian,

In another embodiment, an ADC of the invention comprising an anti-CD33 antibody, such as those described herein, is used in a method of treating hematological malignancies such as non-

Hodgkin's lymphoma (NHL), diffuse large hematopoietic lymphoma, follicular lymphoma, mantle cell lymphoma, chronic lymphocytic leukemia, multiple myeloma, acute myeloid leukemia (AML), and myeloid cell leukemia (MCL), and including B-cell related cancers and proliferative disorders. See: US 8226945; Li et al (2013) Mol. Cancer. Ther. 12(7):1255-1265; Polson et al
5 (2010) Leukemia 24:1566-1573; Polson et al (2011) Expert Opin. Investig. Drugs 20(1):75-85, the contents of which are incorporated by reference.

In another embodiment, an ADC of the invention comprising an anti-MUC16 antibody, such as those described herein, is used in a method of treating ovarian, breast and pancreatic cancers. The
10 cancer may be associated with the expression or activity of a MUC16/CA125/O772P polypeptide. See: WO 2007/001851; US 7989595; US 8449883; US 7723485; Chen et al (2007) Cancer Res. 67(10): 4924-4932; Junutula, et al., (2008) Nature Biotech., 26(8):925-932, the contents of which are incorporated by reference.

15 In certain embodiments, an ADC of the invention comprising an anti-HER2 antibody, such as those described above, is used in a method of treating cancer, e.g., breast or gastric cancer, more specifically HER2+ breast or gastric cancer, wherein the method comprises administering such ADC to a patient in need of such treatment. In one such embodiment, the ADC comprises the anti-HER2 antibody trastuzumab or pertuzumab.

20 Generally, the disease or disorder to be treated is a hyperproliferative disease such as cancer. Examples of cancer to be treated herein include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia or lymphoid malignancies. More particular examples of such cancers include squamous cell cancer (e.g. epithelial squamous cell cancer), lung cancer including
25 small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer,
30 prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, as well as head and neck cancer.

Autoimmune diseases for which the Antibody-drug conjugate s may be used in treatment include
35 rheumatologic disorders (such as, for example, rheumatoid arthritis, Sjögren's syndrome, scleroderma, lupus such as systemic lupus erythematosus (SLE) and lupus nephritis, polymyositis/dermatomyositis, cryoglobulinemia, anti-phospholipid antibody syndrome, and psoriatic arthritis), osteoarthritis, autoimmune gastrointestinal and liver disorders (such as, for

example, inflammatory bowel diseases (e.g., ulcerative colitis and Crohn's disease), autoimmune gastritis and pernicious anemia, autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, and celiac disease), vasculitis (such as, for example, ANCA-associated vasculitis, including Churg-Strauss vasculitis, Wegener's granulomatosis, and

5 polyarteritis), autoimmune neurological disorders (such as, for example, multiple sclerosis, opsoclonus myoclonus syndrome, myasthenia gravis, neuromyelitis optica, Parkinson's disease, Alzheimer's disease, and autoimmune polyneuropathies), renal disorders (such as, for example, glomerulonephritis, Goodpasture's syndrome, and Berger's disease), autoimmune dermatologic disorders (such as, for example, psoriasis, urticaria, hives, pemphigus vulgaris, bullous

10 pemphigoid, and cutaneous lupus erythematosus), hematologic disorders (such as, for example, thrombocytopenic purpura, thrombotic thrombocytopenic purpura, post-transfusion purpura, and autoimmune hemolytic anemia), atherosclerosis, uveitis, autoimmune hearing diseases (such as, for example, inner ear disease and hearing loss), Behcet's disease, Raynaud's syndrome, organ transplant, and autoimmune endocrine disorders (such as, for example, diabetic-related

15 autoimmune diseases such as insulin-dependent diabetes mellitus (IDDM), Addison's disease, and autoimmune thyroid disease (e.g., Graves' disease and thyroiditis)). More preferred such diseases include, for example, rheumatoid arthritis, ulcerative colitis, ANCA-associated vasculitis, lupus, multiple sclerosis, Sjögren's syndrome, Graves' disease, IDDM, pernicious anemia, thyroiditis, and glomerulonephritis.

20

For the prevention or treatment of disease, the appropriate dosage of an ADC will depend on the type of disease to be treated, as defined above, the severity and course of the disease, whether the molecule is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antibody, and the discretion of the attending physician. The

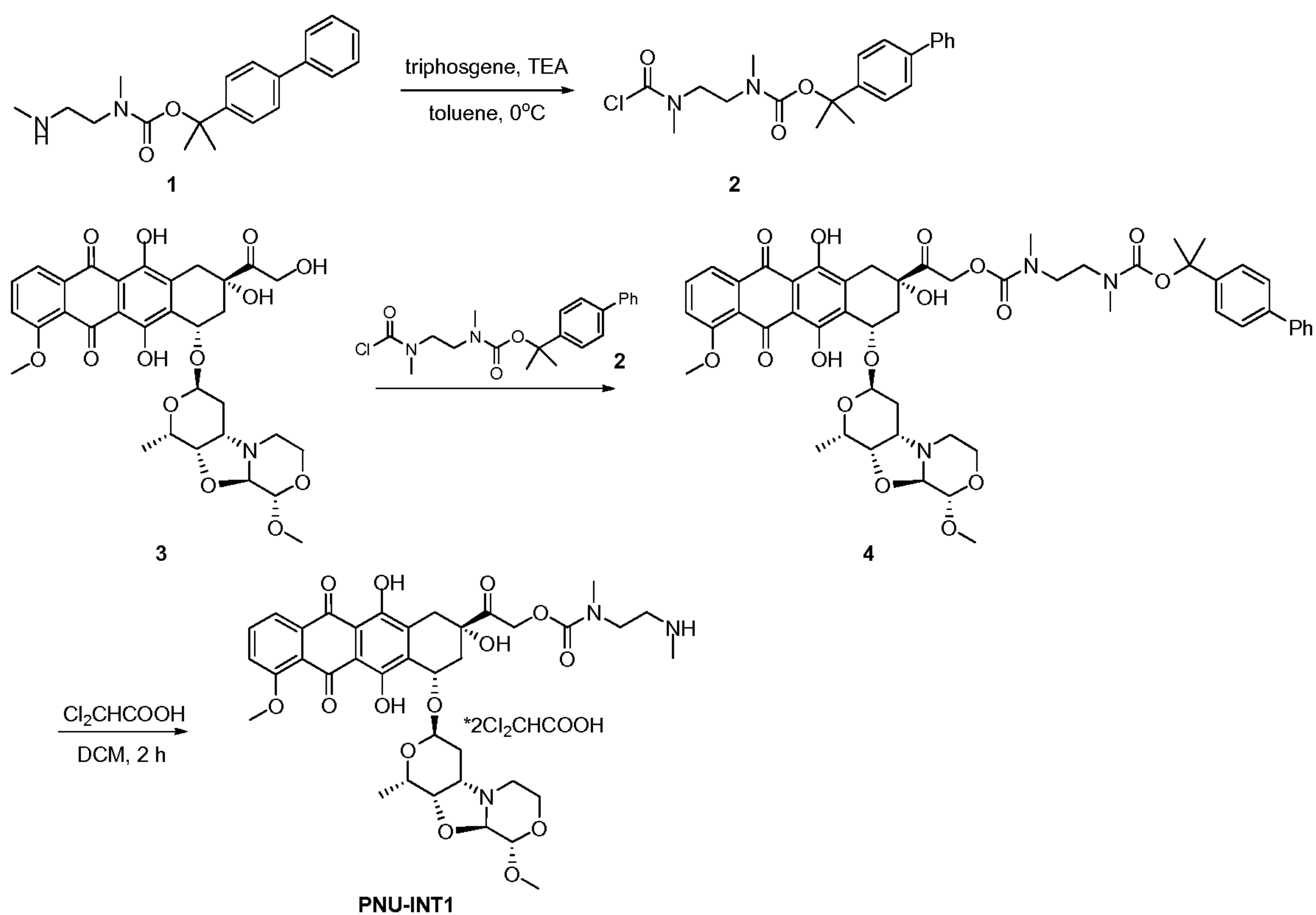
25 molecule is suitably administered to the patient at one time or over a series of treatments.

Depending on the type and severity of the disease, about 1 $\mu\text{g}/\text{kg}$ to 15 mg/kg (e.g. 0.1-20 mg/kg) of molecule is an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. A typical daily dosage might range from about 1 $\mu\text{g}/\text{kg}$ to 100 mg/kg or more, depending on the factors mentioned above. An

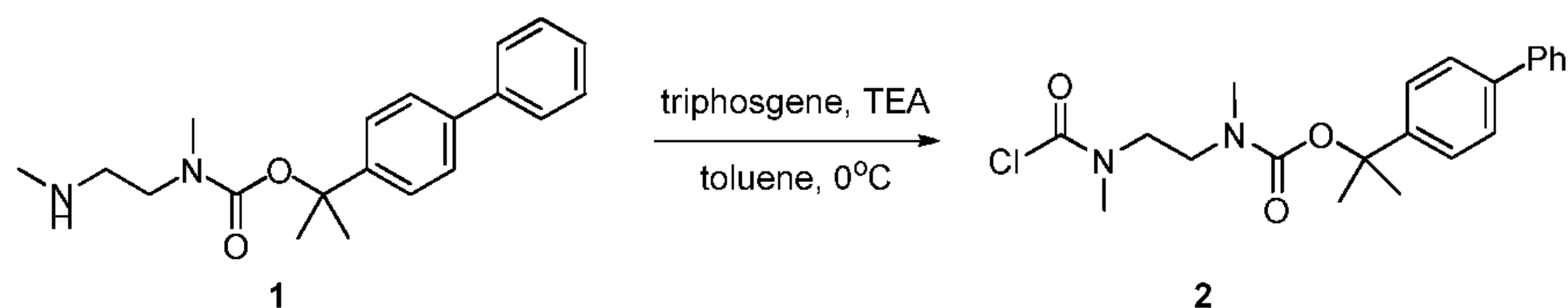
30 exemplary dosage of ADC to be administered to a patient is in the range of about 0.1 to about 10 mg/kg of patient weight.

EXPERIMENTALS

35 **Scheme 1. Synthesis of common intermediate PNU-INT1**

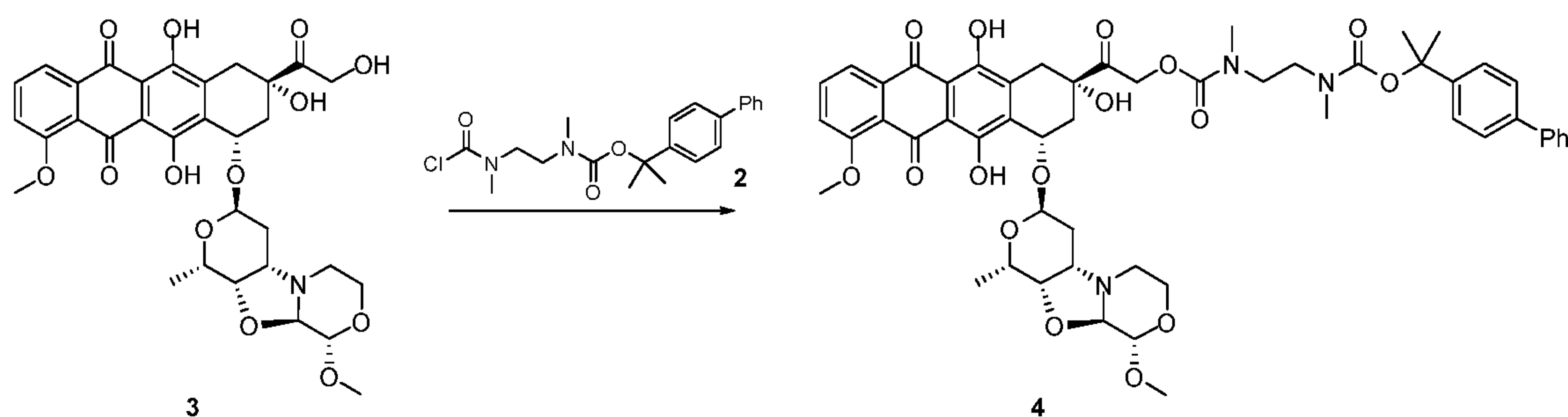


5 Step 1:



After triphosgene (218.2 mg, 0.735 mmol) in toluene (6 mL) was cooled to 0°C, a solution of compound **1** (600 mg, 1.84 mmol) and triethylamine (372 mg, 3.68 mmol) in toluene (4 mL) were added dropwise. After the reaction mixture was warmed to r.t. over 1 h, the solution was filtered and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (EtOAc/Hex 3:7) to give the desired product **2** as white solid (600 mg, 83.9%) MS (ESI): 405.59 [M+NH₄]⁺.

Step 2:

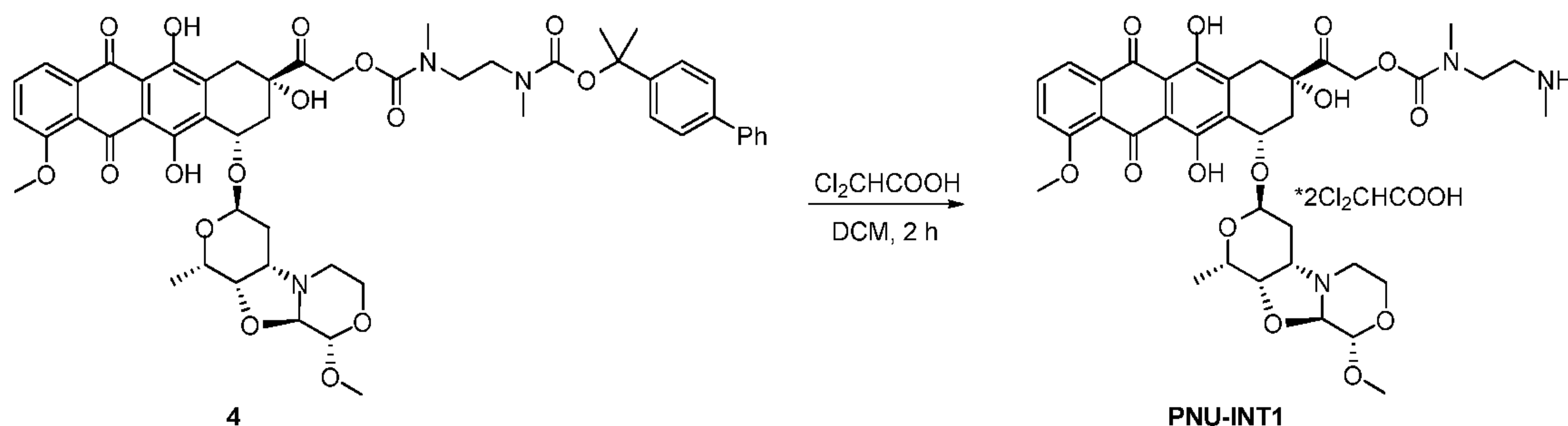


To a solution of compound **3** (150 mg, 0.234 mmol) in anhydrous DCM (2.5 mL), molecular sieves (powder-4 Å, 100 mg), 4-dimethylaminopyridine (142.8 mg, 1.17 mmol) and a solution of compound **2** (272.75 mg, 0.701 mmol) in anhydrous DCM (0.5 mL) were added. The solution was stirred in the dark at 25°C for 5 days. The crude product was purified by prep-TLC (MeOH:CH₂Cl₂ = 1:40) to give the product **4** (140 mg, 60.2%).

LCMS: (5-95, AB, 1.5 min), 0.983 min, MS = 994.4 [M+H]⁺;

Step 3:

10



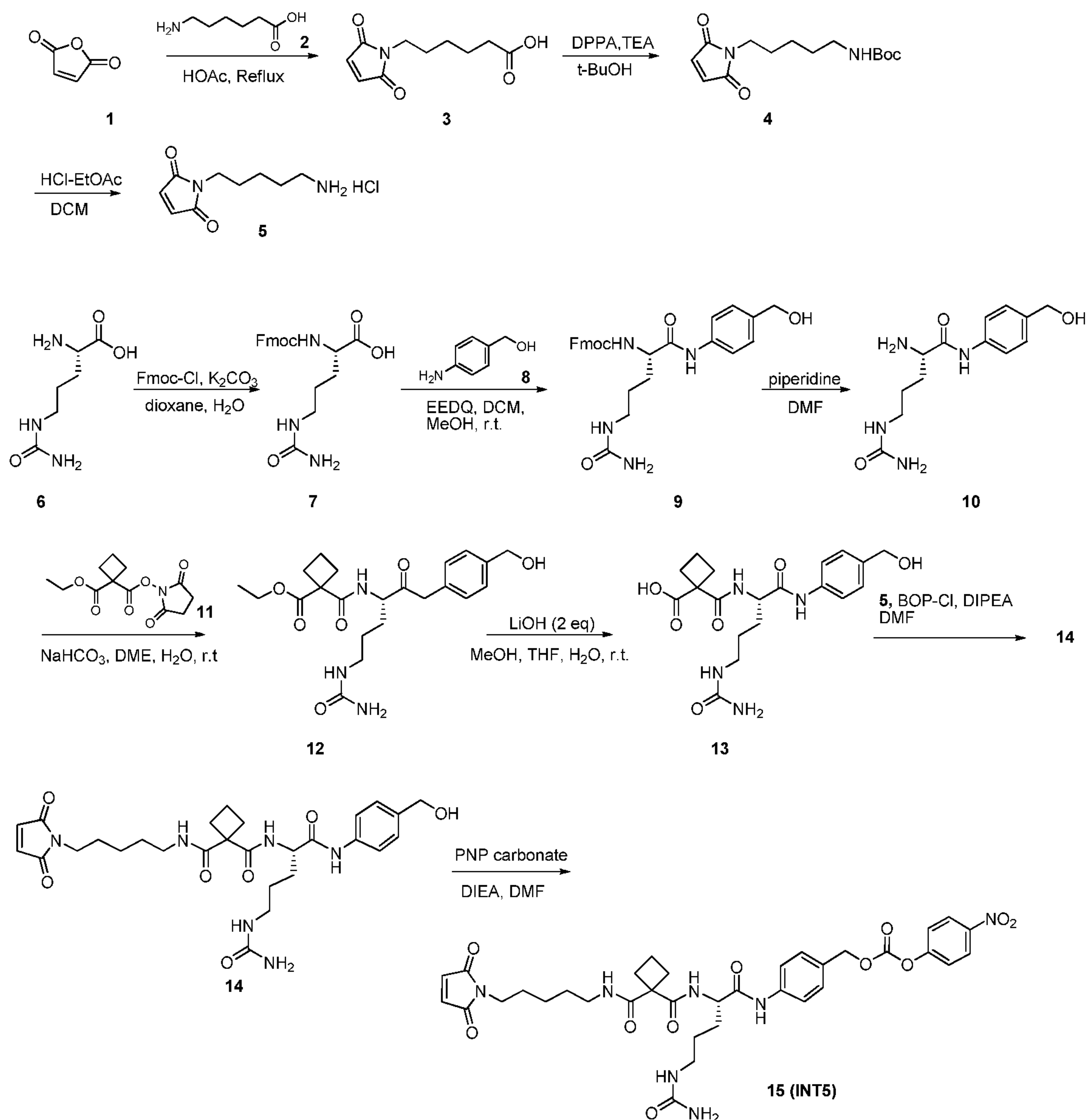
15

To a solution of compound **4** (80.0 mg, 0.080 mmol) in DCM (1 mL) in ice bath, a solution of dichloroacetic acid (1.61 mmol) in DCM (0.4 mL) was added. The solution was stirred at r.t. for 2 h. A mixture of diethyl ether and hexane was added. The crude red solid was used in the next step without further purification (52 mg, 85 %).

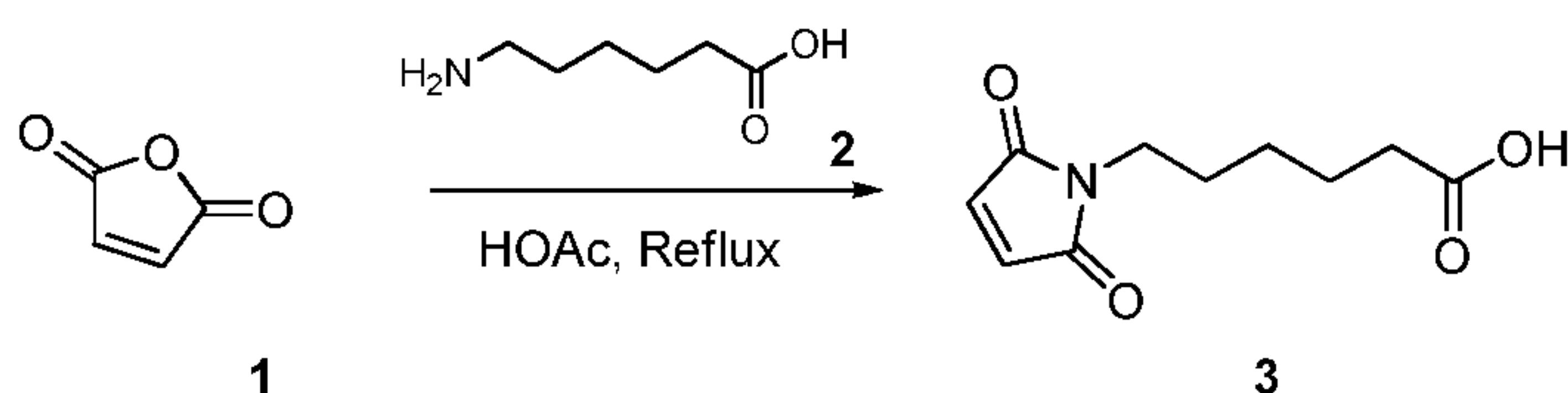
Synthesis of INT5

20 (S)-4-(2-(1-(5-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)pentylcarbamoyl)cyclobutanecarboxamido)-5-ureidopentanamido)benzyl 4-nitrophenyl carbonate

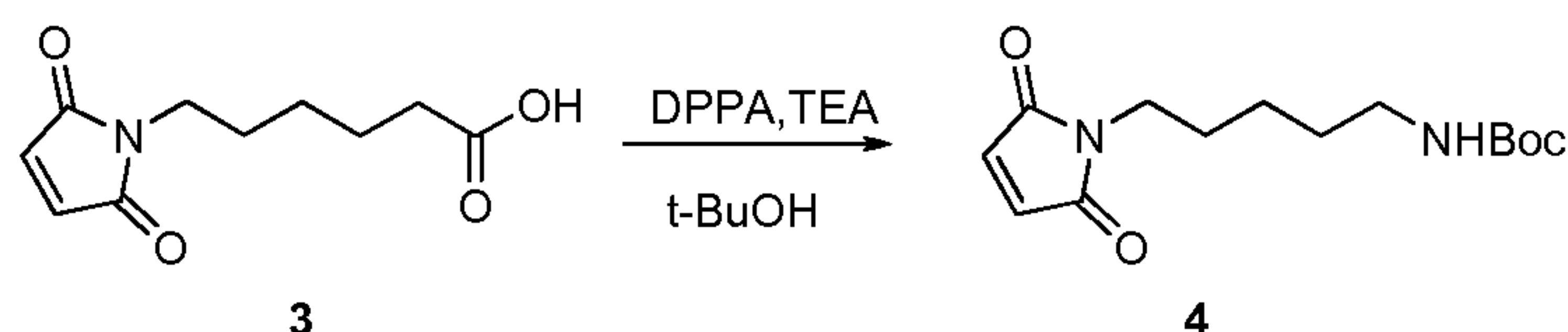
Scheme 1



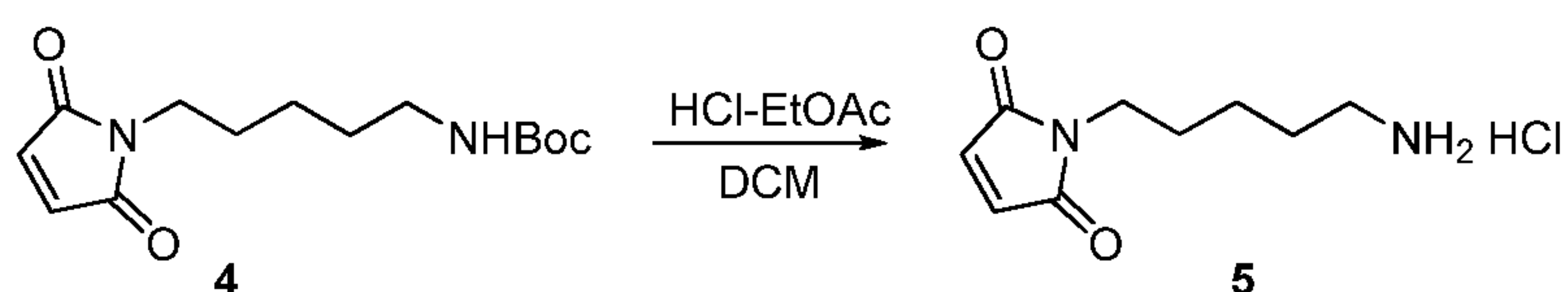
5 Procedure



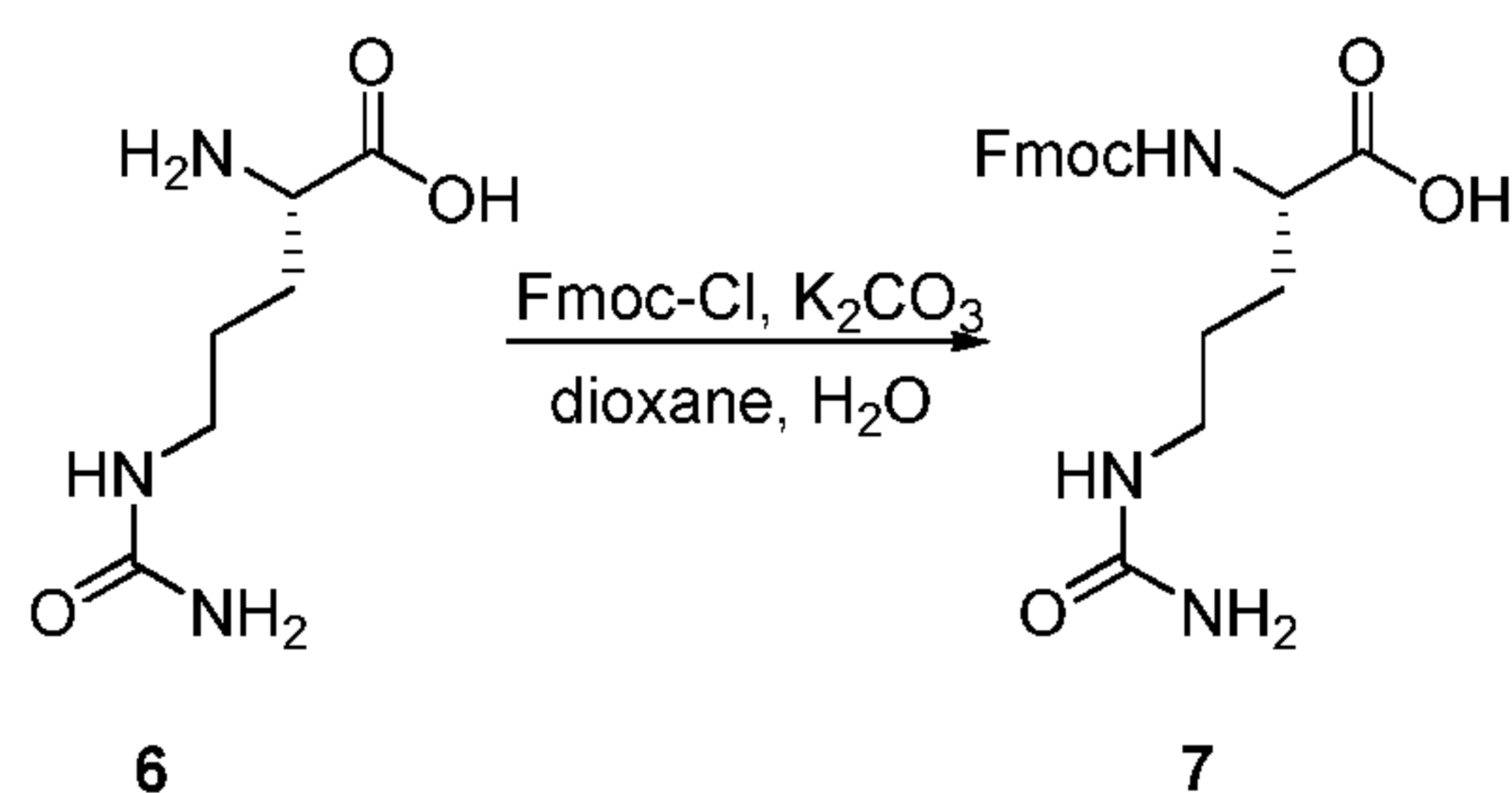
Compound **1** (150 g, 1.53 mol) was added to a stirred solution of Compound **2** (201 g, 1.53 mol) in HOAc (1000 mL). After the mixture was stirred at r.t. for 2 h, it was heated at reflux for 8 h. The organic solvents were removed under reduced pressure and the residue was extracted with EtOAc (500 mL \times 3), washed with H₂O. The combined organic layers was dried over Na₂SO₄ and concentrated to give the crude product. It was washed with petroleum ether to give compound **3** as white solid (250 g, 77.4 %).



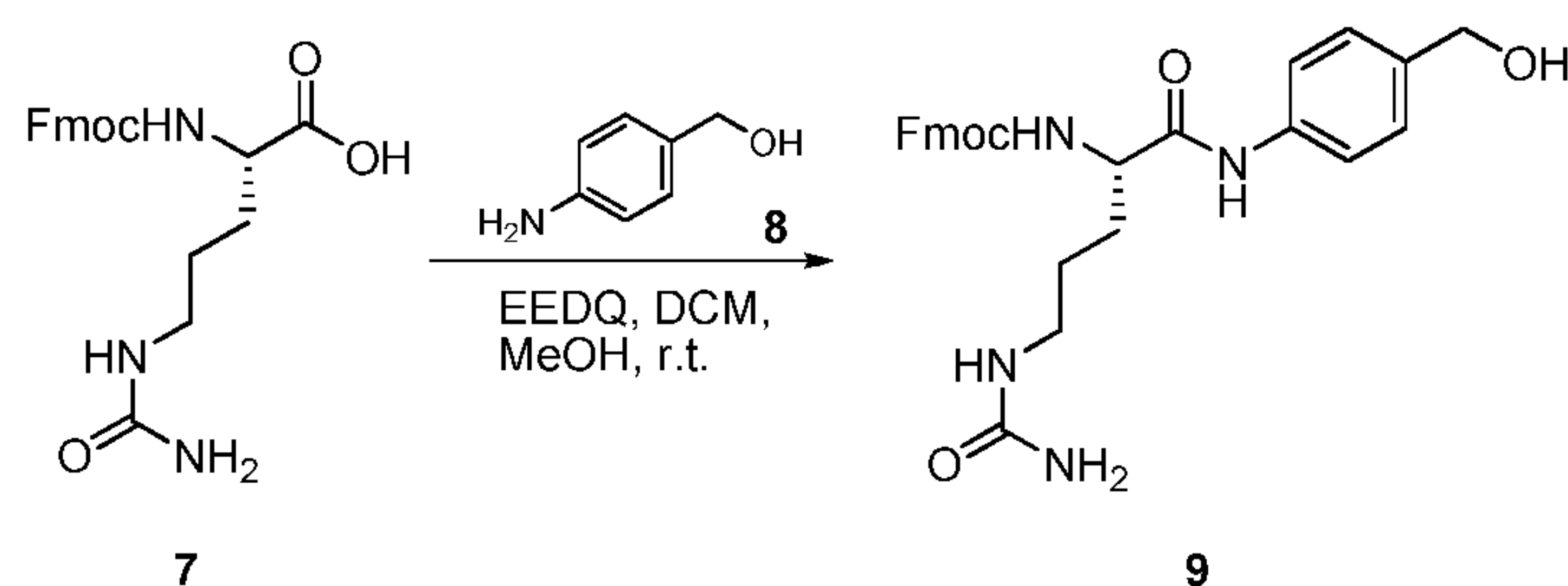
DPPA (130 g, 473 mmol) and TEA (47.9 g, 473 mmol) was added to a solution of compound **3** (100 g, 473 mmol) in t-BuOH (200 mL). The mixture was heated at reflux for 8 h under N₂. The mixture was concentrated, and the residue was purified by column chromatography on silica gel (PE:EtOAc= 3:1) to give compound **4** (13 g, 10%).



To a solution of compound **4** (28 g, 992 mmol) in anhydrous EtOAc (30 mL) was added HCl/EtOAc (50 mL) dropwise. After the mixture was stirred at r.t. for 5 h, it was filtered and the solid was dried to give compound **5** (16 g, 73.7%). ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.02 (s, 2H), 6.99 (s, 2H), 3.37-3.34 (m, 2H), 2.71-2.64 (m, 2H), 1.56-1.43 (m, 4H), 1.23-1.20 (m, 2H).



To a mixture of compound **6** (17.50 g, 0.10 mol) in a mixture of dioxane and H₂O (50 mL / 75 mL) was added K₂CO₃ (34.55 g, 0.25 mol). Fmoc-Cl (30.96 g, 0.12 mol) was added slowly at 0 °C. The reaction mixture was warmed to r.t. over 2 h. Organic solvent was removed under reduced pressure, and the water slurry was adjusted to pH = 3 with 6 M HCl solution, and extracted with EtOAc (100 mL × 3). The organic layer was dried over Na₂SO₄, filtered, and concentrated under reduced pressure to give the desired product **7** (38.0 g, 95.6%).



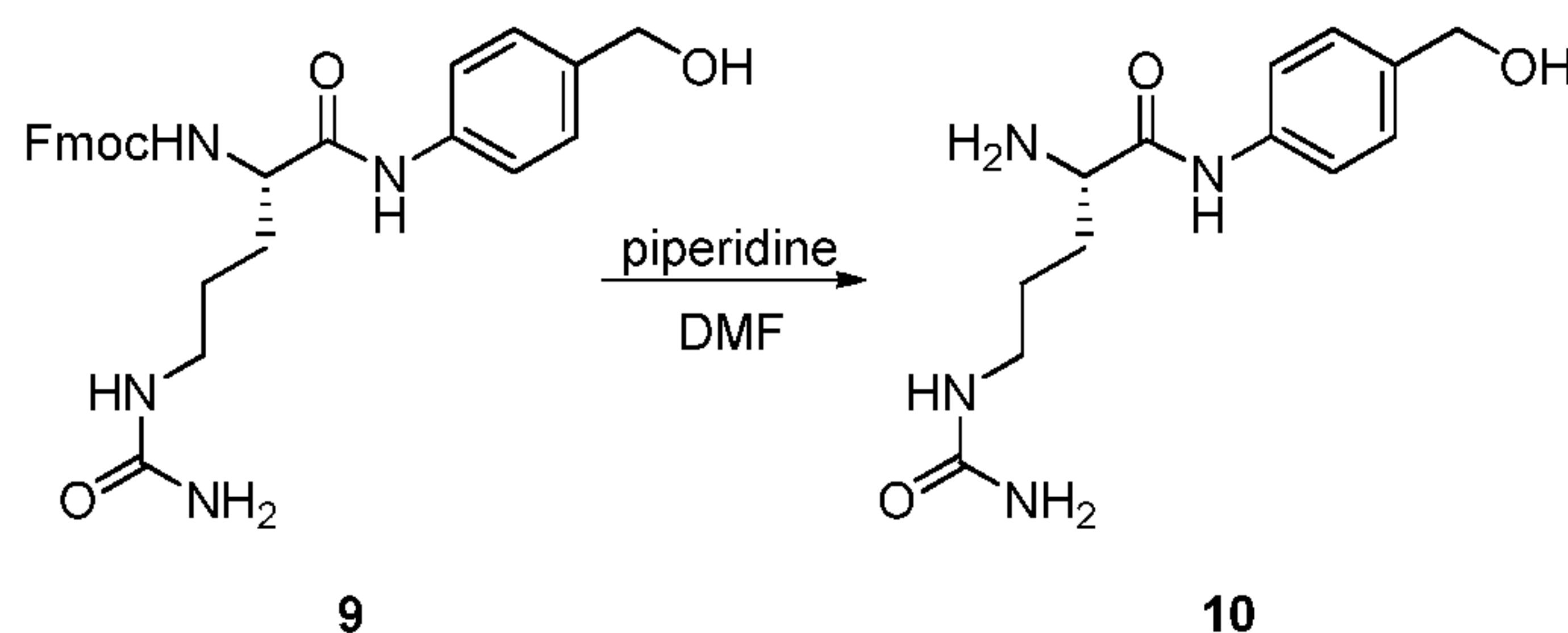
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To a solution of compound **7** (4.0 g, 10 mmol) in a mixture of DCM and MeOH (100 mL / 50 mL) were added 4-amino-phenyl-methanol (**8**) (1.6 g, 13 mmol, 1.3 eq) and EEDQ (3.2 g, 13 mmol, 1.3 eq). After the mixture was stirred at r.t. for 16 h under N₂, it was concentrated to give a brown

solid. MTBE (200 mL) was added and it was stirred at 15°C for 2 h. The solid was collected by filtration, washed with MTBE (50 mL × 2) to give the crude product **9** as an orange solid (4.2 g, 84%).

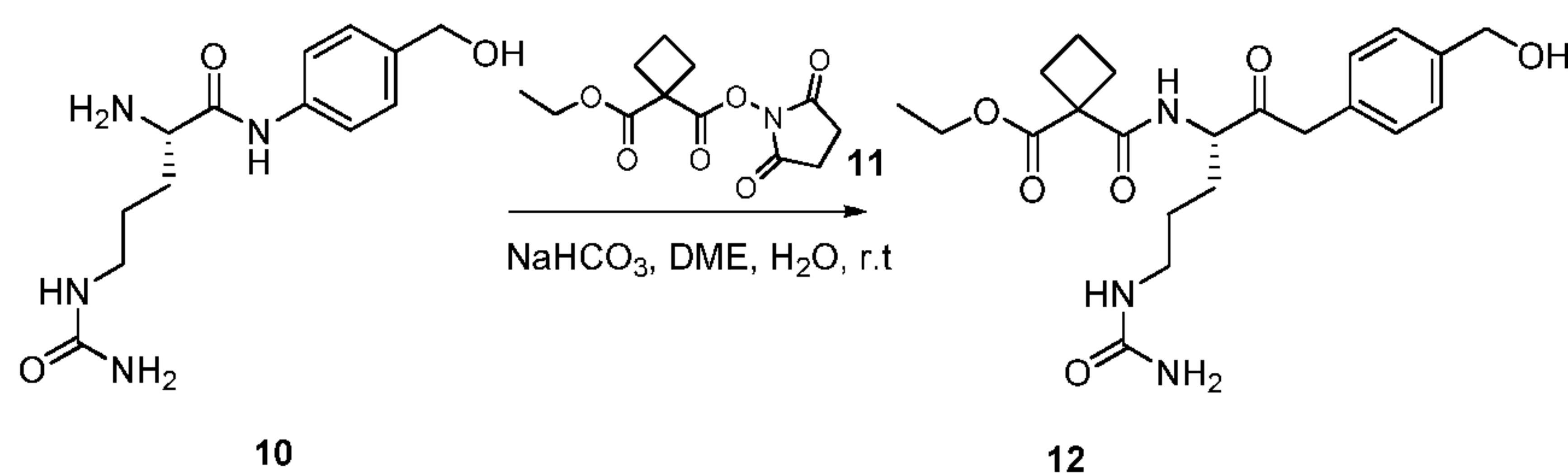
LCMS (ESI): m/z 503.0 [M+1].

5



To a stirred solution of compound **9** (4.2 g, 8.3 mmol) in dry DMF (20 mL) was added piperidine (1.65 mL, 17 mmol, 2.0 eq) dropwise at r.t. The mixture was stirred at r.t. for 30 min, and solid precipitate formed. Dry DCM (50 mL) was added, and the mixture became transparent immediately. The mixture was stirred at r.t. for another 30 min, and LCMS showed compound **9** was consumed. It was concentrated to dryness under reduced pressure (make sure no piperidine remained), and the residue was partitioned between EtOAc and H₂O (50 mL / 20 mL). Aqueous phase was washed with EtOAc (50 mL × 2) and concentrated to give **10** as an oily residual (2.2 g, 94%) (contained small amount of DMF).

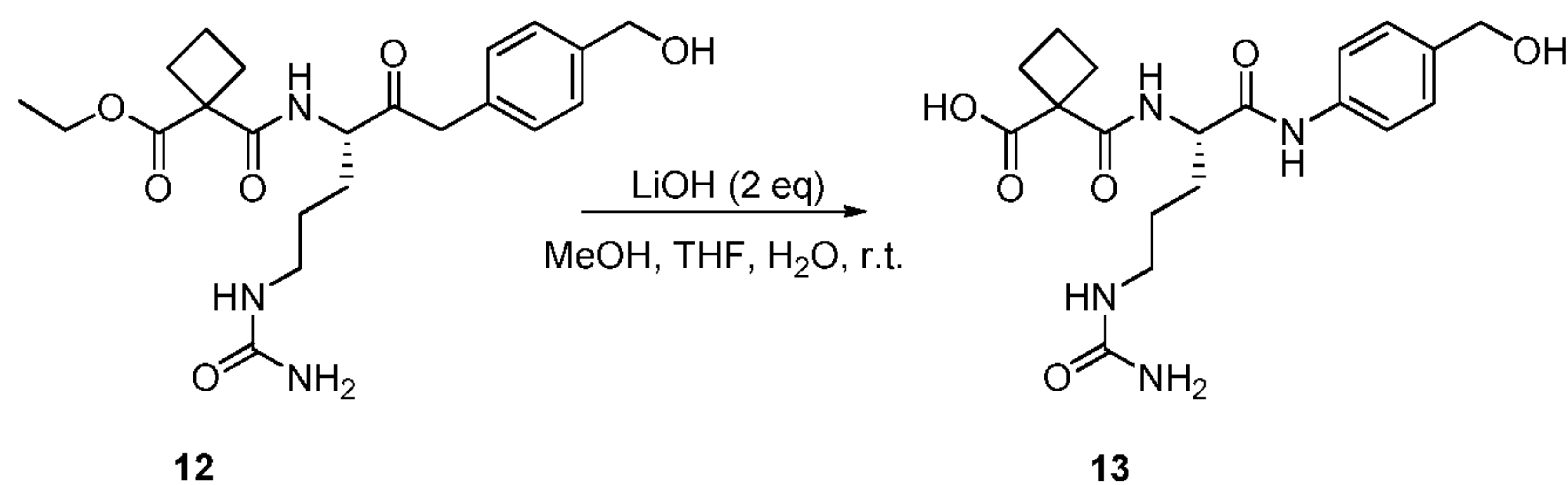
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To a solution of compound **11** (8.0 g, 29.7 mmol) in DME (50 mL) was added a solution of compound **10** (6.0 g, 21.4 mmol) and NaHCO₃ (7.48 g, 89.0 mmol) in water (30 mL). After the mixture was stirred at r.t. for 16 h, it was concentrated to dryness under reduced pressure and the residue was purified by column chromatography (DCM:MeOH = 10:1) to give crude compound **12** as white solid (6.4 g, 68.7%).

20

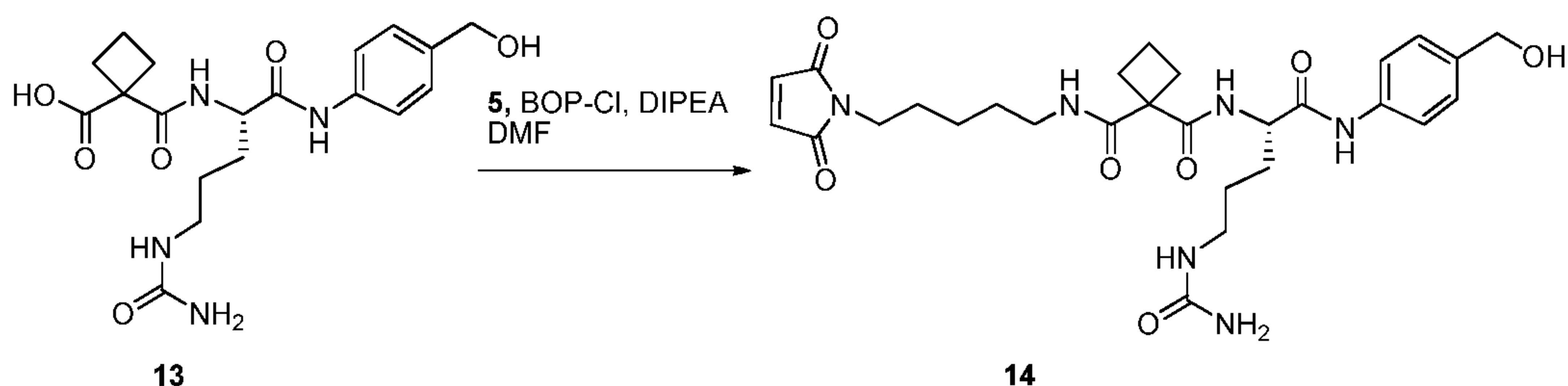
LCMS (ESI): m/z 435.0 [M+1].



To a stirred solution of compound 12 (6.4 g, 14.7 mmol) in a mixture of THF and MeOH (20 mL/10 mL) was added a solution of LiOH.H₂O (1.2 g, 28.6 mmol) in H₂O (20 mL) at r.t. After the reaction mixture was stirred at r.t. for 16 h, solvent was removed under reduced pressure, the residue obtained was purified by prep-HPLC to give compound 13 (3.5 g, yield: 58.5%).

LCMS (ESI): m/z 406.9 [M+1].

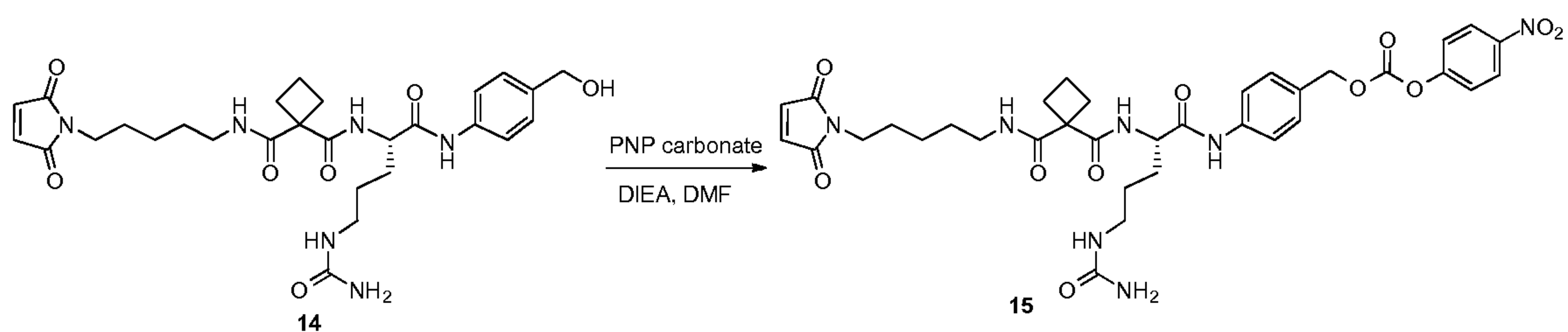
¹H NMR (400 MHz, Methanol-*d*₄) δ 8.86 (d, $J = 8.4$ Hz, 2 H), 8.51 (d, $J = 8.4$ Hz, 2 H), 5.88 - 5.85 (m, 1 H), 5.78 (s, 2 H), 4.54 - 4.49 (m, 3 H), 4.38 - 4.32 (m, 1 H), 3.86 - 3.75 (m, 1 H), 3.84 - 3.80 (m, 2 H), 3.28 - 3.21 (m, 1 H), 3.30 - 3.24 (m, 1 H), 3.00 - 2.80 (m, 1 H), 2.37 - 2.28 (m, 2 H).



DIPEA (1.59 g, 12.3 mmol) and BOP-Cl (692 mg, 2.71 mmol) was added to a solution of compound 13 (1.0 g, 2.46 mmol) in DMF (10 mL) at 0°C, followed by compound 5 (592 mg, 2.71 mmol). The mixture was stirred at 0°C for 0.5 h. The reaction mixture was quenched with a citric acid solution (10 mL), extracted with DCM/MeOH (10:1). The organic layer was dried and concentrated, and the residue was purified by column chromatography on silica gel (DCM:MeOH = 10:1) to give compound 14 (1.0 g, 71 %).

¹H NMR (400 MHz, DMSO-*d*₆): δ 10.00 (s, 1H), 7.82-7.77 (m, 2H), 7.53 (d, $J = 8.4$ Hz, 2 H), 7.19 (d, $J = 8.4$ Hz, 2 H), 6.96 (s, 2H), 5.95 (t, $J = 6.4$ Hz, 1H), 5.39 (s, 2H), 5.08 (t, $J = 5.6$ Hz, 1H), 4.40-4.35 (m, 3H), 4.09 (d, $J = 4.8$ Hz, 1 H), 3.01 (d, $J = 3.2$ Hz, 2 H), 3.05-2.72 (m, 4H), 2.68-2.58 (m, 3H), 2.40-2.36 (m, 4H), 1.72-1.70 (m, 3H), 1.44-1.42 (m, 1H), 1.40-1.23 (m, 6H), 1.21-1.16 (m, 4H).

25

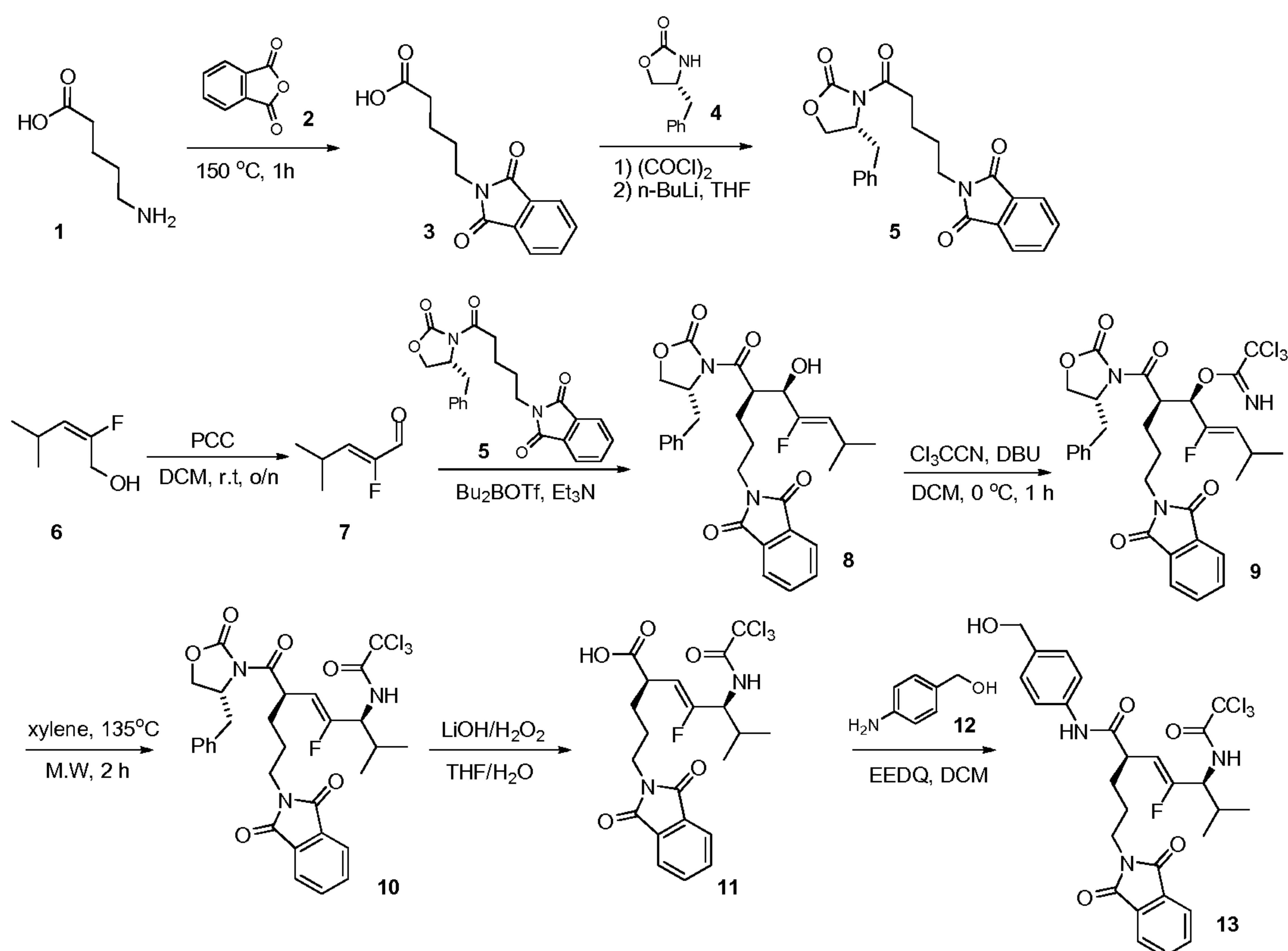


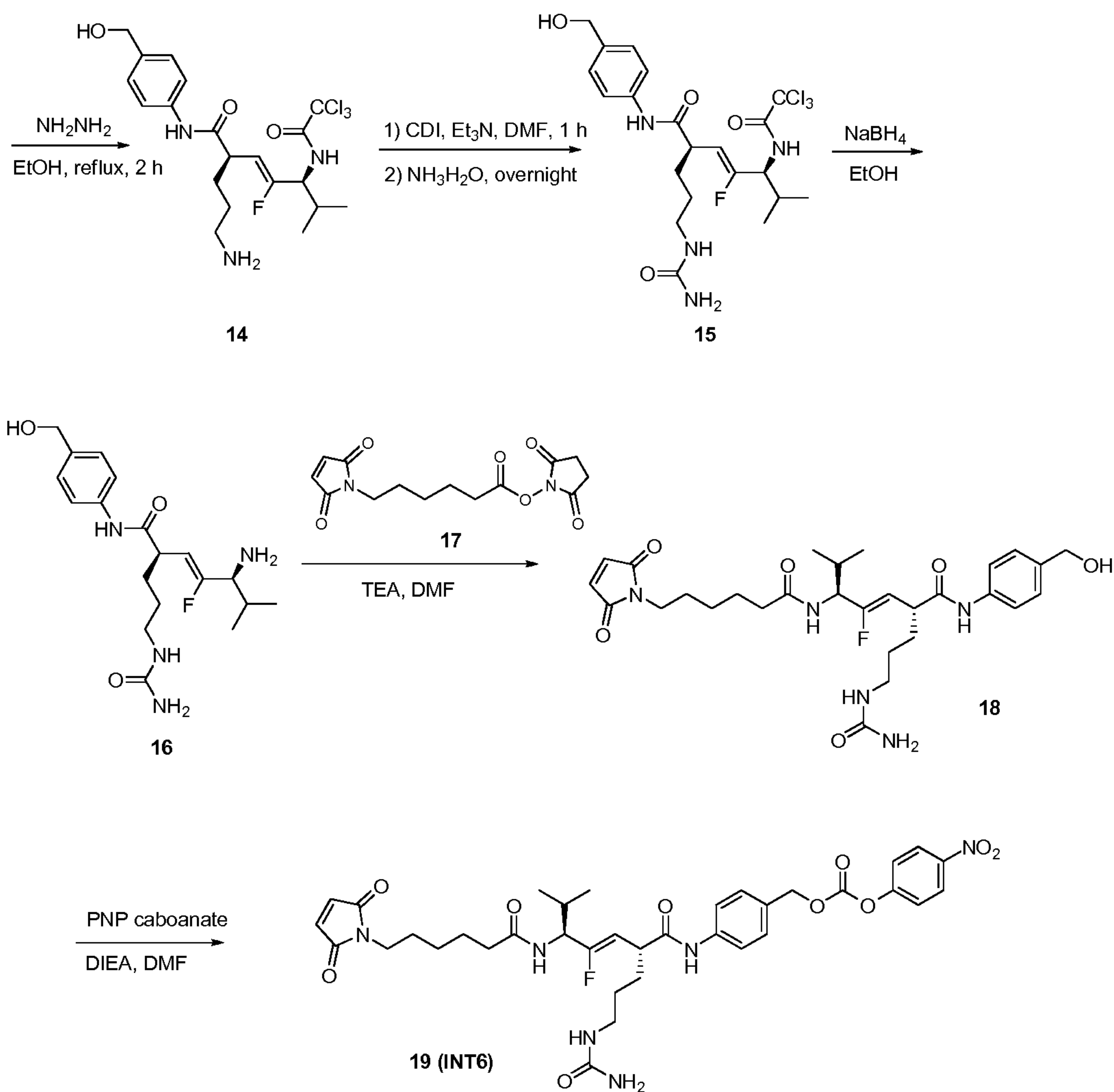
To a solution of compound **14** (500 mg, 0.035 mmol) in dry DMF (20 ml) was added compound **PNP** (533 mg, 1.75 mmol) and DIPEA (340 mg, 2.63 mmol) at 20°C, and the mixture was allowed to stir at 16°C for 2h under N₂ atmosphere. The mixture was concentrated and purified by pre-TLC (DCM/MeOH=10/1) to give the product **INT5** (250 mg, 39%) LCMS (ESI, 5-95AB, 1.5 min): 0.842 min, m/z 736.4 [M+1].

Synthesis of INT6

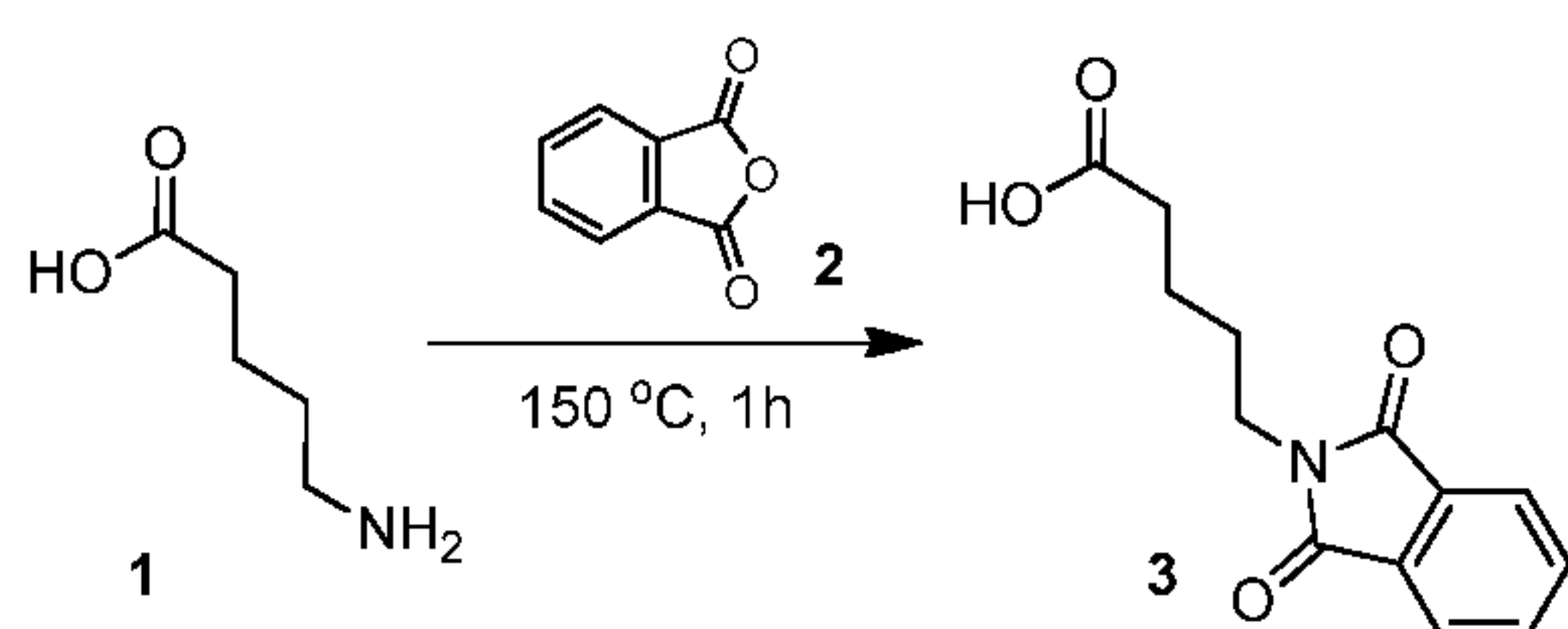
4-((2R,5S,Z)-5-(6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamido)-4-fluoro-6-methyl-2-(3-ureidopropyl)hept-3-enamido)benzyl 4-nitrophenyl carbonate

Scheme 2



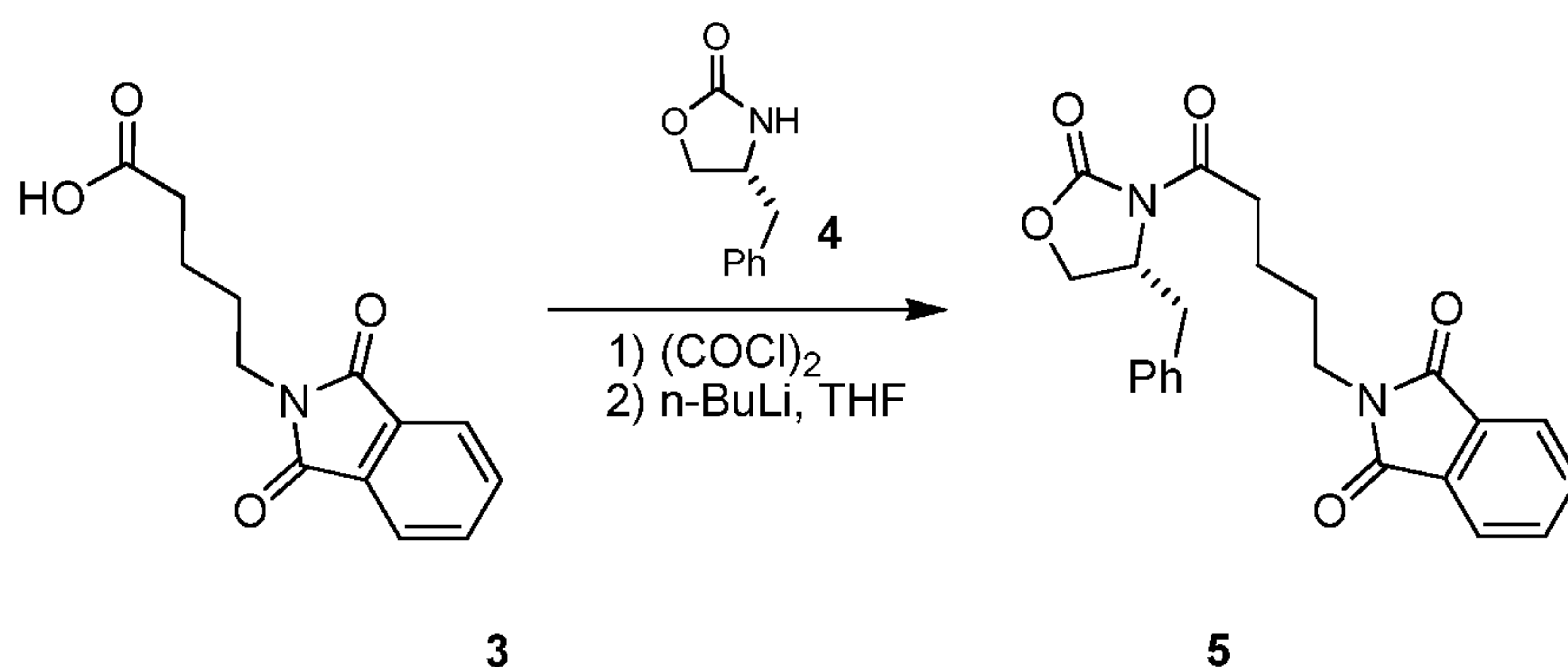


Experimental



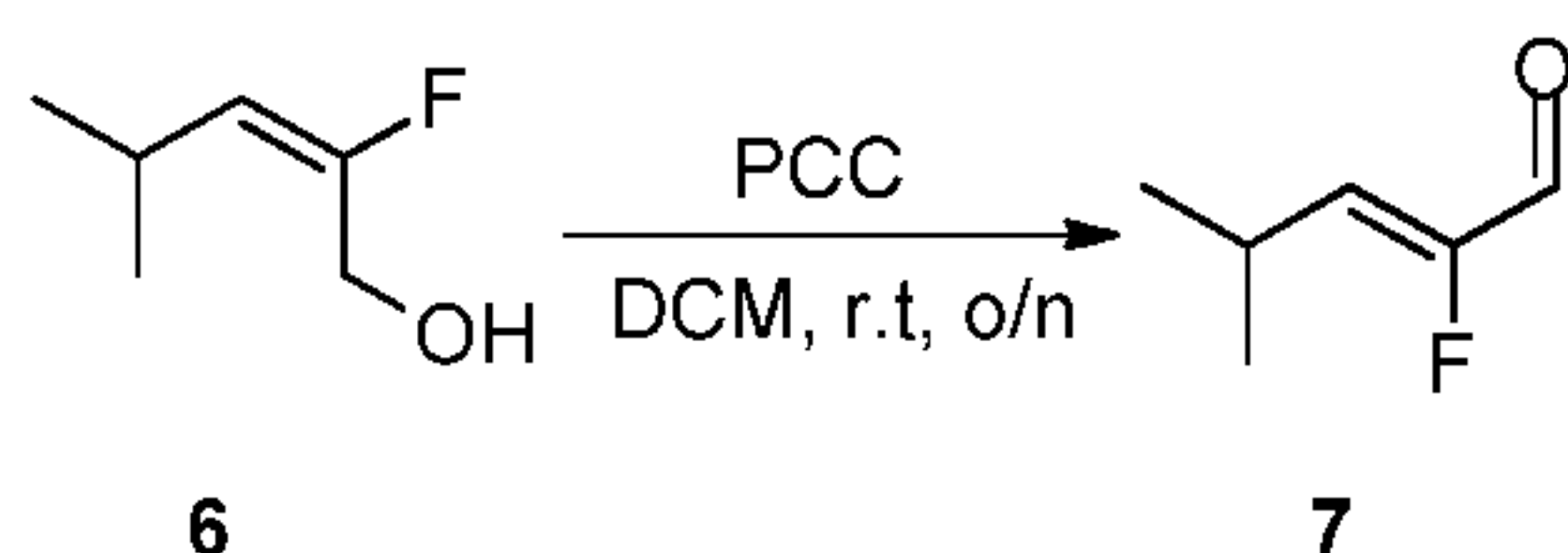
A mixture of compound **1** (10.0 g, 85.36 mmol), compound **2** (13.3 g, 89.79 mmol) was stirred at 150 °C for 1 h. The mixture was cooled to 25 °C and solid was dissolved in hot water. The mixture was further cooled in an ice bath. The precipitate was collected by filtration and washed with water. The filter cake was dried to give compound **3** as white solid (19.0 g, 90.0 %).

¹H NMR (400 MHz, DMSO-*d*₆) δ 11.96 (br, 1H), 7.78 - 7.77 (m, 4H), 3.52 (t, *J* = 6.8 Hz, 2H), 2.18 (t, *J* = 7.2 Hz, 2H), 1.59 - 1.51 (m, 2H), 1.47 - 1.41 (m, 2H).



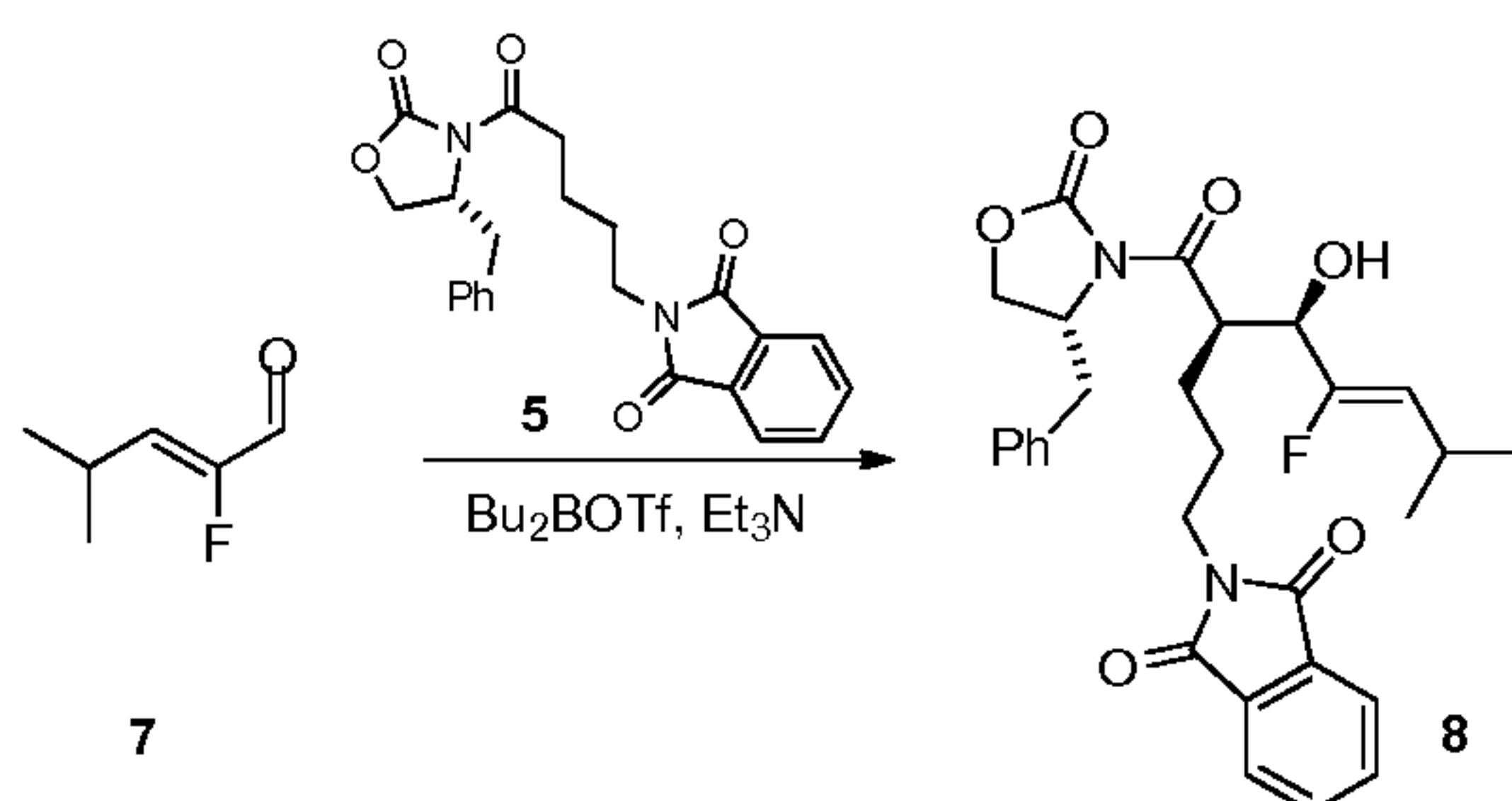
To a mixture of compound **3** (9.0 g, 36.40 mmol) in anhydrous DCM (100 mL) were added (COCl)₂ (15.0 mL, 157.76 mmol), DMF (1 mL) dropwise at r.t. The reaction mixture was stirred at r.t. for 0.5 h. The mixture was concentrated under reduced pressure, and the residue was diluted in anhydrous THF (60 mL), and concentrated again to give the acyl chloride as yellow solid. To a mixture of compound **4** (6.6 g, 37.25 mmol) in anhydrous THF (60 mL) was added *n*-BuLi (15.0 mL, 2.5 M, 37.5 mmol) dropwise at -78 °C under N₂. The above acyl chloride in THF (40 mL) was added slowly into the mixture at -78 °C. The reaction mixture was stirred at -78 °C for 15 min, then quenched with aq. NH₄Cl solution (30 mL). The mixture was extracted with EtOAc washed with water. The combined organic layers was dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (PE/EtOAc 3:1) to give crude compound **5** as white solid (13.0 g, 87.9%).

¹H NMR (400 MHz, DMSO-*d*₆) δ 7.89 - 7.83 (m, 4H), 7.32 - 7.28 (m, 2H), 7.25 - 7.22 (m, 1H), 7.19 - 7.17 (m, 2H), 4.66 - 4.60 (m, 1H), 4.30 (t, *J* = 8.4 Hz, 1H), 4.17 (dd, *J* = 9.2, 2.8 Hz, 1H), 3.61 (t, *J* = 6.4 Hz, 2H), 3.00 - 2.78 (m, 4H), 1.70 - 1.60 (m, 4H).



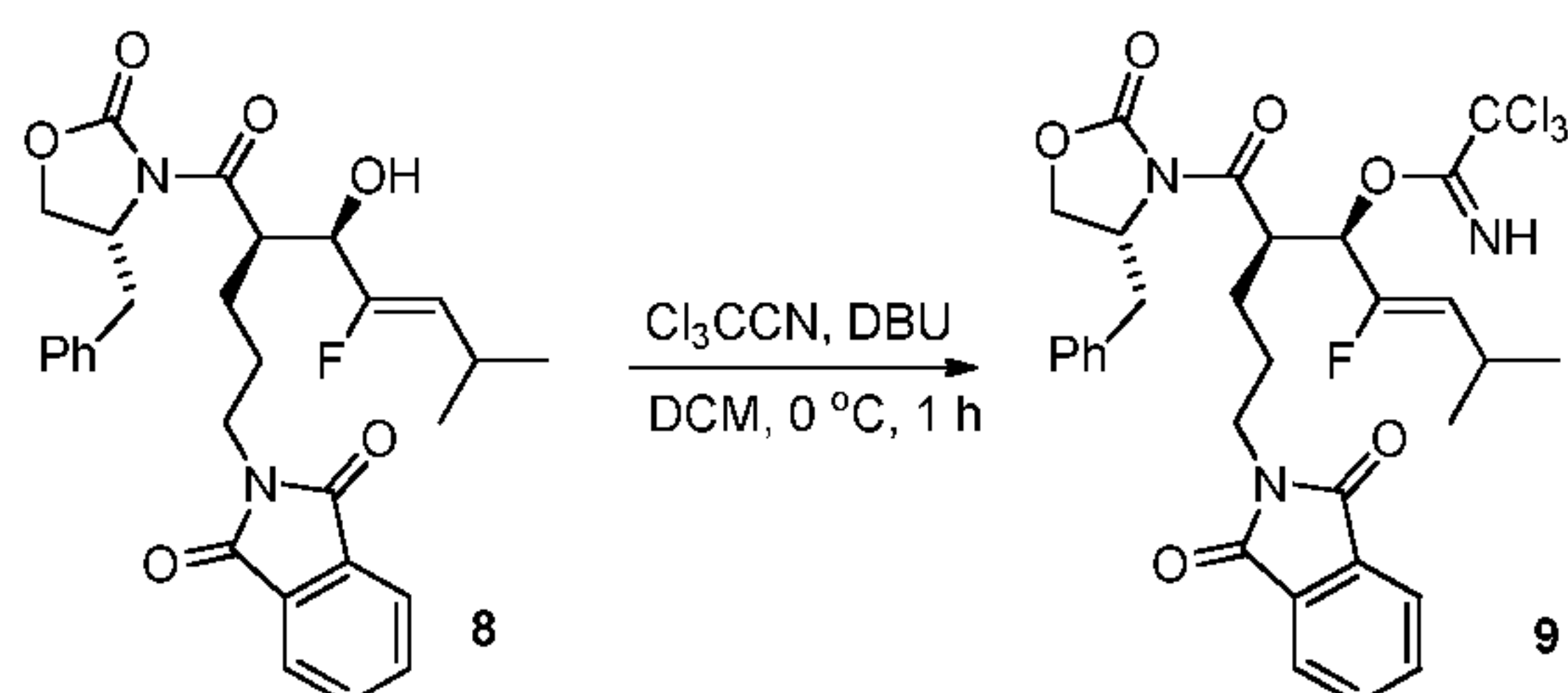
To a solution of compound **6** (3.0 g, 25.39 mmol) in DCM (100 mL) was added PCC (10.9 g, 50.78 mmol). The mixture was stirred at 25 °C for 16 h under N₂. The mixture was filtered through a silica gel plug. The filtrate was concentrated under reduced pressure at a bath temperature of 25 °C to give compound **7** as an oil (1.8 g, 61.0 %).

¹H NMR (400 MHz, CDCl₃) δ 9.18 (d, *J* = 18.4 Hz, 1 H), 5.79 (dd, *J* = 32.8, 9.2 Hz, 1 H), 3.02 - 2.93 (m, 1 H), 1.13 (d, *J* = 6.8 Hz, 6 H).



A solution of compound **5** (6.0 g, 14.7 mmol) in DCM (20 mL) was cooled to 0°C with an ice bath. Bu_2BOTf in DCM (1.0 M, 15 mL, 15 mmol) was added dropwise followed by Et_3N (3.03 g, 30 mmol) at a rate to keep the internal temperature below 3°C . The ice bath was replaced by a dry ice-acetone bath. When the internal temperature dropped below -65°C , compound **7** (1.5 g, 12.9 mmol) in DCM (10 mL) was added dropwise. The solution was stirred for 20 min in the dry ice-acetone bath, then for 1 h in ice bath. The reaction mixture was quenched with aqueous phosphate buffer (pH = 7.0, 20 mL) and MeOH (10 mL). To this cloudy solution was added a mixture of MeOH/30% H_2O_2 (2:1, 20 mL) at such a rate as keep the internal temperature below 10°C . After the solution was stirred for an additional 1 h, the volatile was removed on a rotary evaporator at a bath temperature of $25\text{-}30^\circ\text{C}$. The slurry was extracted with EtOAc ($50\text{ mL} \times 3$). The combined organic layer was washed with saturated Na_2SO_3 solution (15 mL), 5% NaHCO_3 solution (30 mL) and brine (25 mL). It was dried over Na_2SO_4 , filtered and concentrated. The residue was purified by column chromatography on silica gel (PE/EtOAc 3:1) to give crude compound **8** as oil (4.0 g, 59.7%).

LCMS (ESI): m/z 505.0 [M-17].

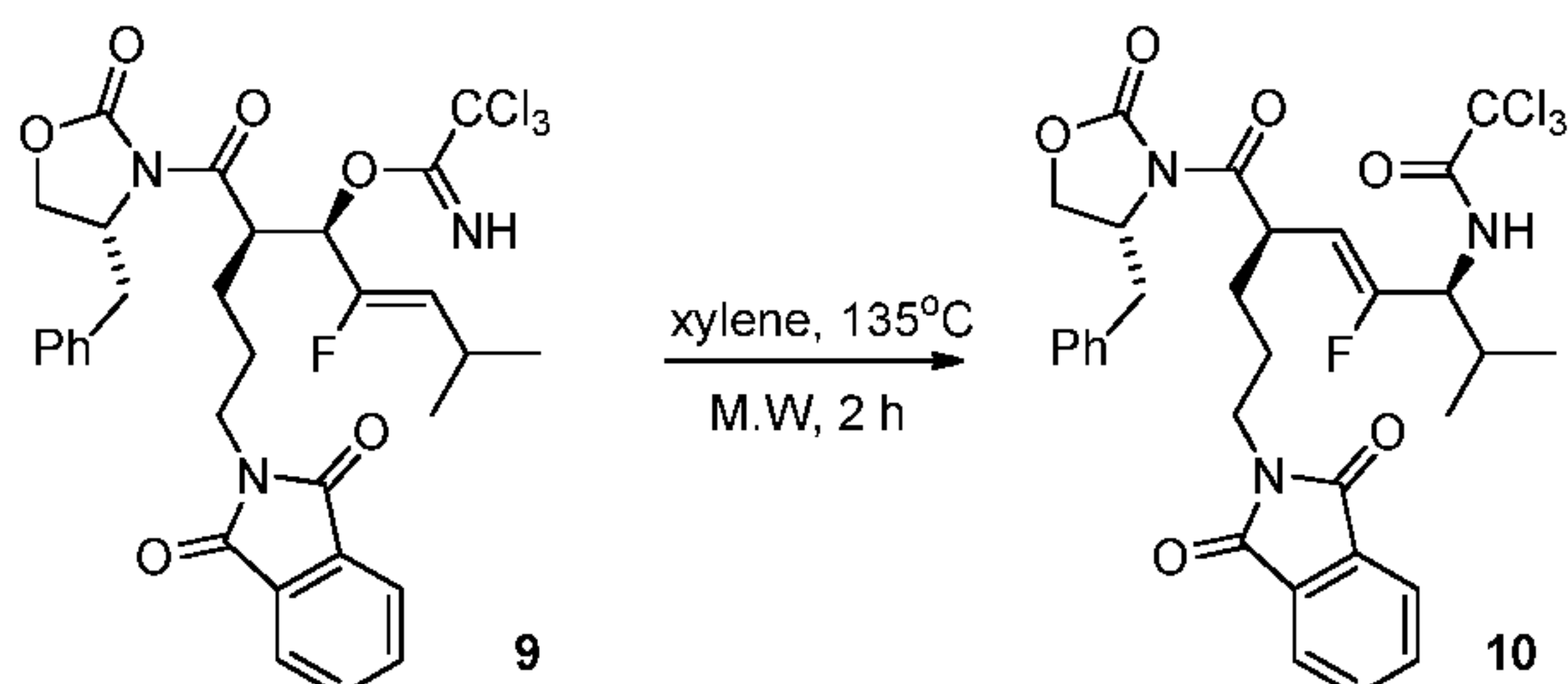


To a solution of compound **8** (4.0 g, 7.65 mmol) and Cl_3CCN (1.67 g, 11.48 mmol) in DCM (20 mL) was added DBU (234 mg, 1.53 mmol) at 0°C under N_2 . The mixture was stirred at 0°C for 1 h. After the solvent was removed, the residue was purified by column chromatography on silica gel (5%-20% petroleum in EtOAc) to give compound **9** (3.0 g, 58.8%).

LCMS (ESI): m/z 505.1 [M-160].

^1H NMR (400 MHz, CDCl_3) δ 8.47 (s, 1H), 7.83 - 7.80 (m, 2H), 7.72 - 7.69 (m, 2H), 7.36 - 7.28 (m, 2H), 7.28 - 7.22 (m, 3H), 5.69 - 5.63 (q, 1H), 4.89 (dd, $J = 37.6, 9.6$ Hz, 1H), 4.63 - 4.58 (m,

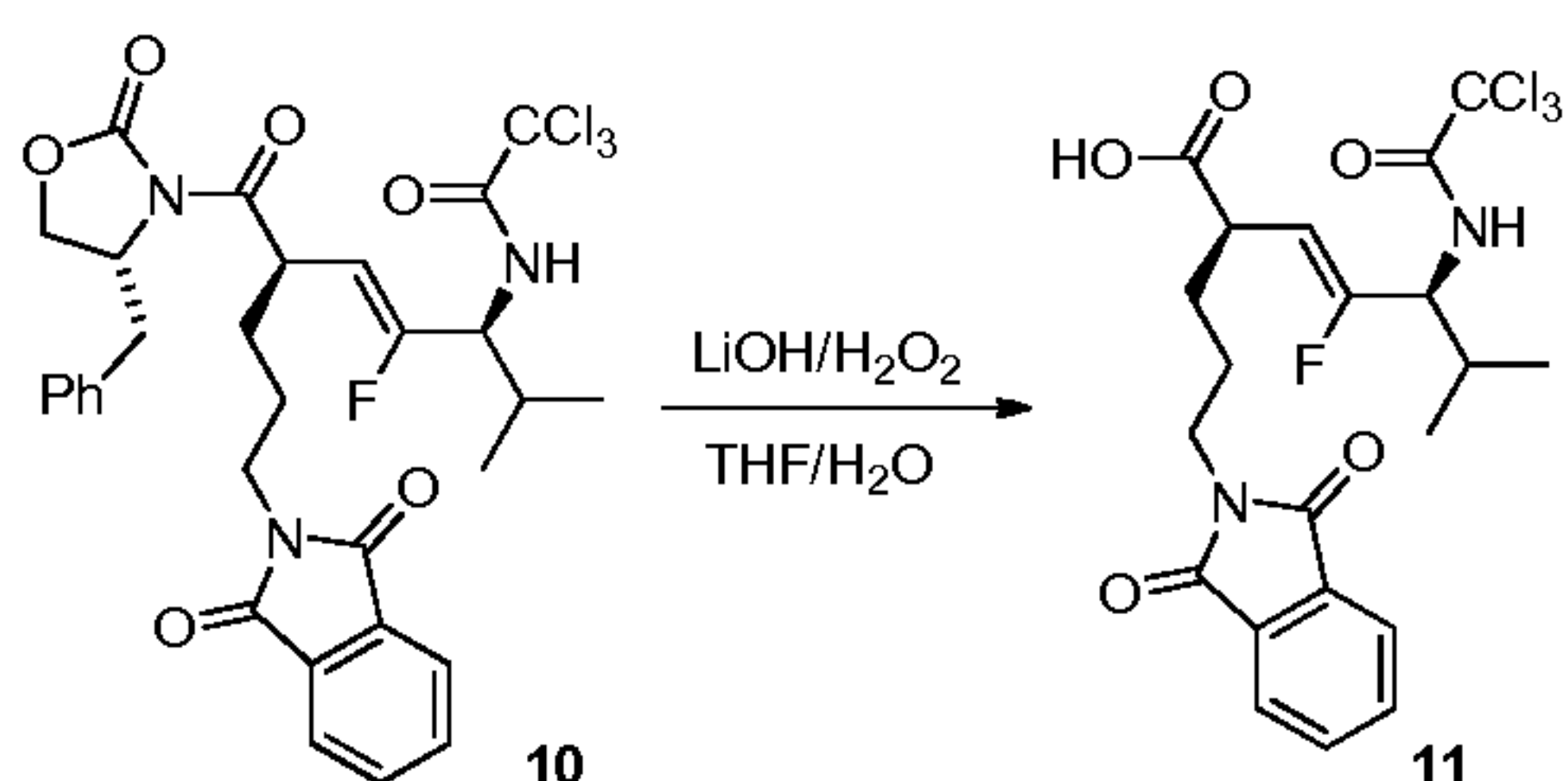
2H), 4.20 - 4.11 (m, 2H), 3.74 - 3.69 (m, 2H), 3.35 (dd, $J = 13.2, 3.2$ Hz, 1H), 2.78 - 2.69 (m, 2H), 1.99 - 1.85 (m, 2H), 1.80 - 1.76 (m, 2H), 0.96 - 0.92 (q, 6H).



- 5 A solution of compound **9** (3.0 g, 4.50 mmol) in xylene (5 mL) was heated in microwave for 2 h at 135°C. The mixture was cooled to 25 °C and purified by column chromatography on silica gel (5%-10%-50% of petroleum in EtOAc) to give compound **10** (1.4 g, 46.7%).

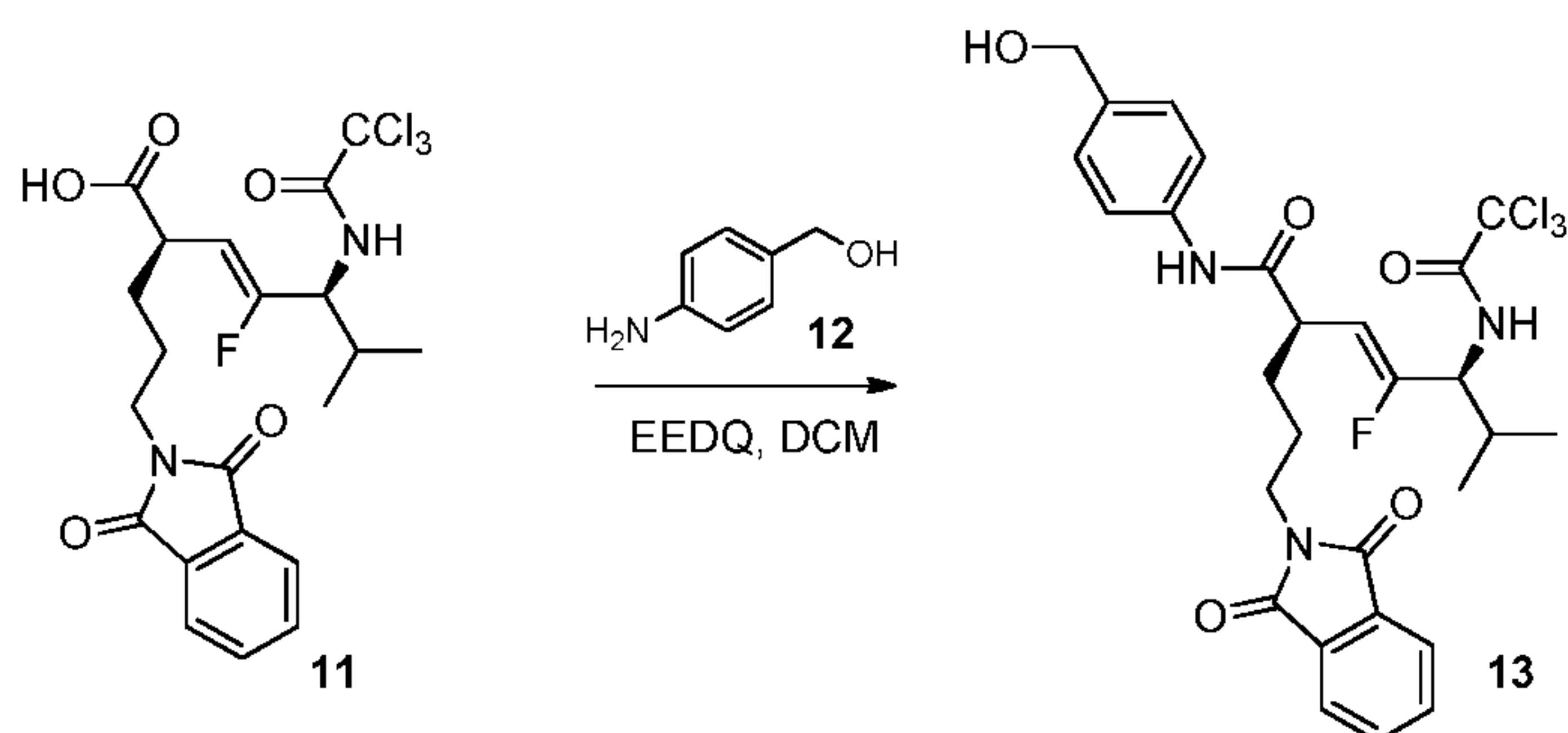
LCMS (ESI): m/z 685.0 [M+H₂O].

- 10 ¹H NMR (400 MHz, CDCl₃) δ 7.83 - 7.81 (m, 2H), 7.71 - 7.69 (m, 2H), 7.36 - 7.32 (m, 2H), 7.29 - 7.25 (m, 1H), 7.21 - 7.19 (m, 2H), 6.90 (d, $J = 8.8$ Hz, 1H), 5.11 (dd, $J = 36.4, 9.6$ Hz, 1H), 4.81 - 4.76 (m, 1H), 4.68 - 4.64 (m, 1H), 4.30 - 4.16 (m, 3H), 3.75 - 3.68 (m, 2H), 3.27 (dd, $J = 13.2, 3.2$ Hz, 1H), 2.80 - 2.74 (q, 1H), 2.08-2.05 (m, 1H), 1.93 - 1.90 (m, 1H), 1.76 - 1.70 (m, 2H), 1.65 - 1.62 (m, 1H), 1.00 (dd, $J = 6.8, 3.2$ Hz, 6H).



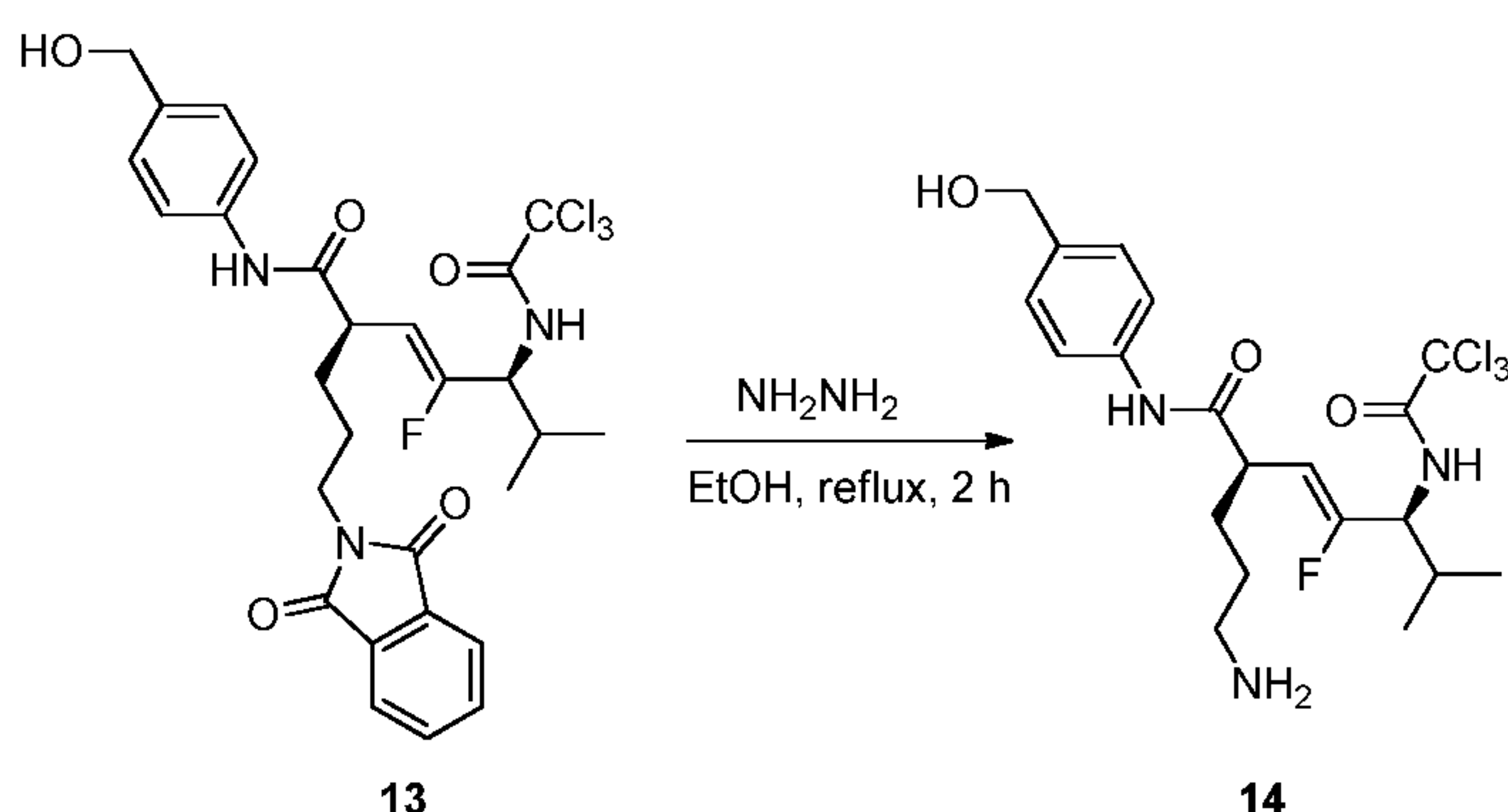
- 15 To a solution of compound **10** (1.4 g, 2.1 mmol) in THF/H₂O (v/v 4:1, 10 mL) was added H₂O₂ (1.43 g, 30 % in water, 12.6 mmol), followed by LiOH·H₂O (264.6 mg, 6.3 mmol). After the solution was stirred for 1.5 h at 25 °C, saturated Na₂SO₃ solution (8 mL) was added. After removal of the solvent, the residue was extracted with DCM (20 mL × 2). The aqueous solution was
- 20 acidified to pH = 1.0 with 1M HCl, and extracted with EtOAc/MeOH (10/1, 25 mL × 3). The combined organic layer was dried over Na₂SO₄, filtered, and concentrated to give compound **11** (1.0 g, 93.4%).

LCMS (ESI): m/z 527.0 [M+Na⁺].

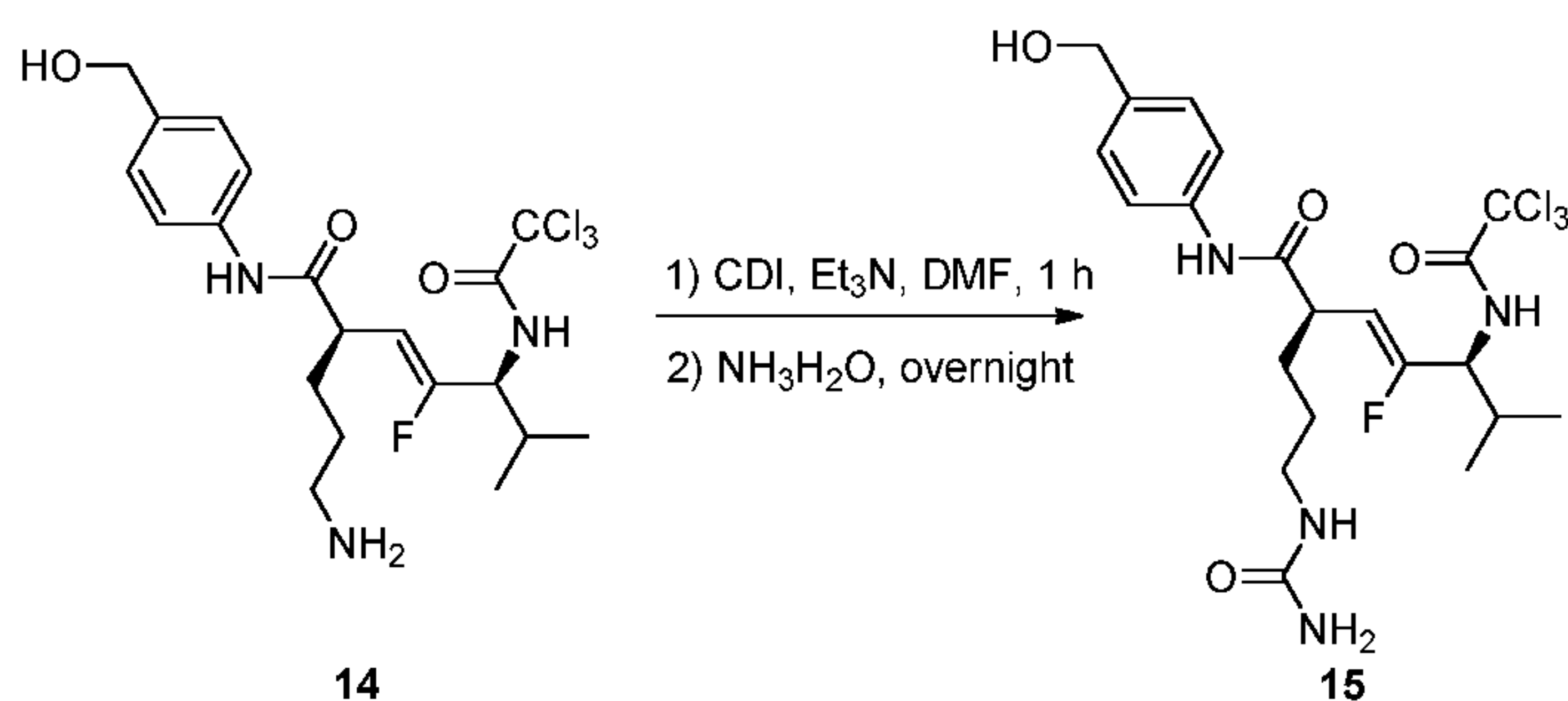


To a solution of compound **11** (1.0 g, 1.97 mmol) and (4-aminophenyl) methanol (364 mg, 2.96 mmol) in DCM/MeOH (v/v 2:1, 7.5 mL) was added EEDQ (732 mg, 2.96 mmol) at 0°C under N₂. The mixture was stirred at 25°C for 16 h. The solvent was removed, and the residue was purified by column chromatography on silica gel (30% petroleum in EtOAc) to give crude compound **13** (1.0 g, 82.8 %).

LCMS (ESI): m/z 614.0 [M+H⁺].



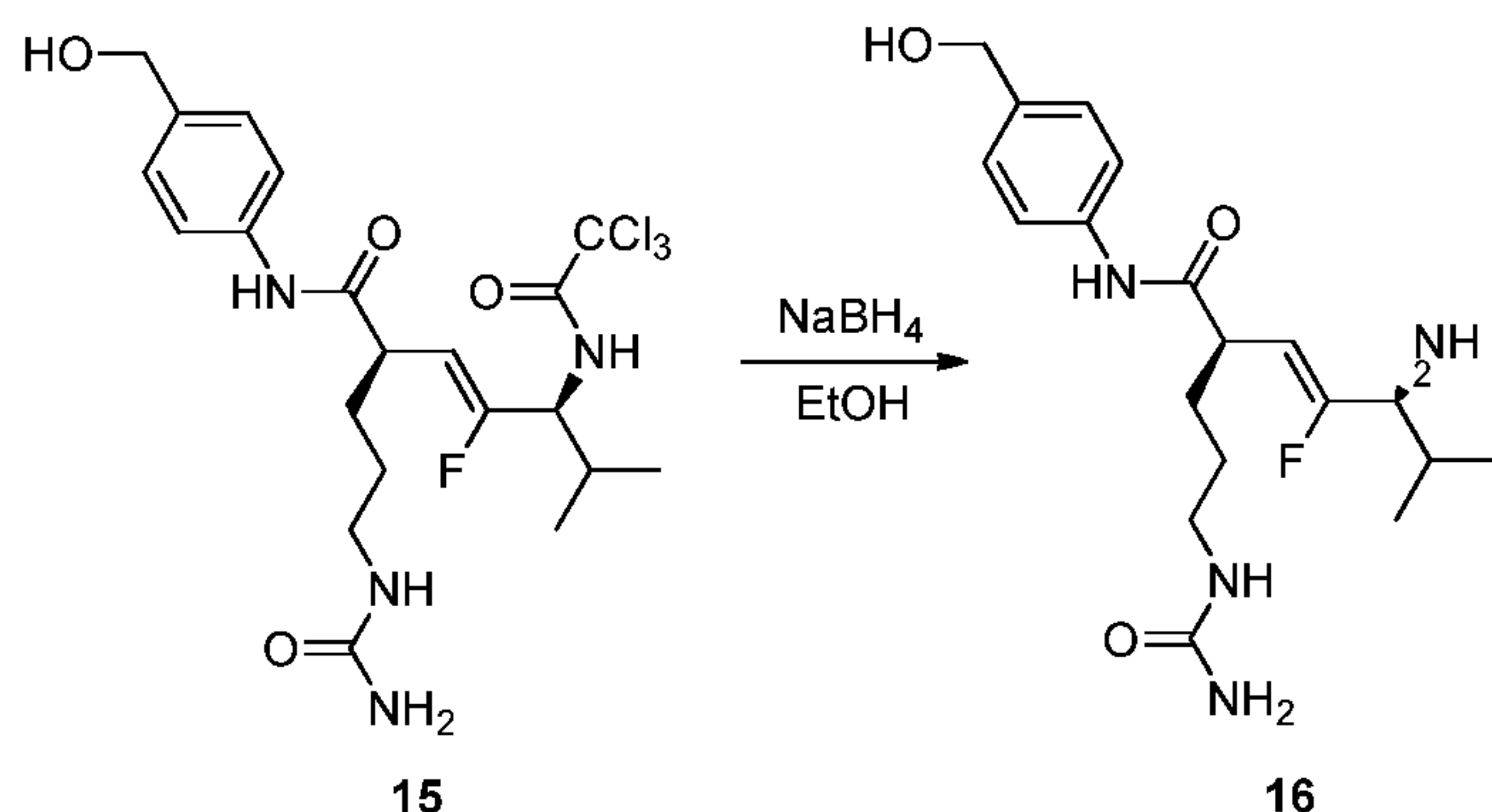
To a solution of compound **13** (1.5 g, 2.45 mmol) in EtOH (20 mL) was added NH₂NH₂·xH₂O (471 mg, c = 50 %, 7.35 mmol). The reaction mixture was stirred at 100 °C for 2 h. The mixture was concentrated under reduced pressure to afford compound **14** (1.18 g, 100 %) as crude product.



To a mixture of compound **14** (1.18 g, 2.45 mmol) in DMF (10 mL) was added TEA (496 mg, 4.90 mmol), followed by CDI (795 mg, 4.90 mmol). The mixture was stirred at r.t. for 1 h, then NH₃·H₂O (5 mL) was added. The reaction mixture was stirred at r.t. overnight. After removal of the solvent, the residue was purified by prep-HPLC (FA) to afford compound **15** (350 mg, 27.1 %, 2 steps) as a solid.

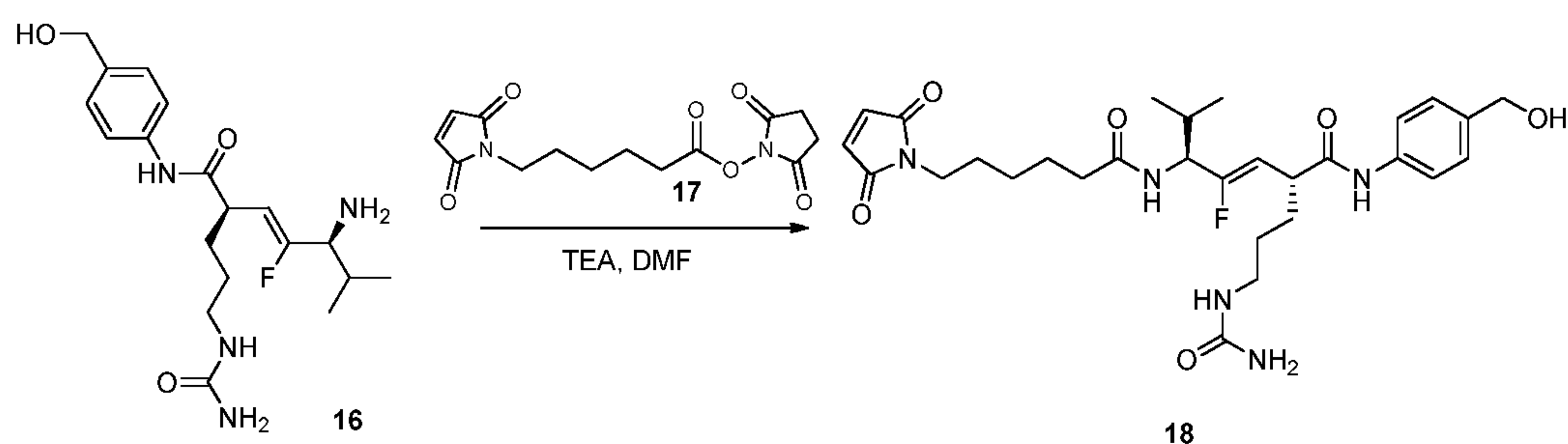
^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 9.96 (s, 1H), 9.24 (d, $J = 8.4$ Hz, 1H), 7.53 (d, $J = 8.4$ Hz, 2H), 7.22 (d, $J = 8.4$ Hz, 2H), 5.94 (s, 1H), 5.38 (br, 2H), 5.09 (dd, $J = 38.4, 9.6$ Hz, 1H), 4.42 (s, 2H), 4.07 - 3.97 (m, 1H), 3.50 - 3.40 (m, 2H), 2.95 (dd, $J = 15.2, 5.2$ Hz, 2H), 2.18 - 2.14 (m, 1H), 1.70 - 1.65 (m, 1H), 1.42 - 1.30 (m, 3H), 0.94 - 0.89 (m, 6H).

5



To a solution of compound **15** (120 mg, 0.23 mmol) in anhydrous EtOH (10 mL) was added NaBH_4 (104 mg, 2.74 mmol) at 0°C . The reaction mixture was stirred at r.t. for 4 h. H_2O (1 mL) was added to quench the reaction. The mixture was concentrated under reduced pressure, and the residue was purified by prep-TLC (DCM/MeOH = 4:1) to afford crude compound **16** (50 mg) which contained an unknown impurity.

10

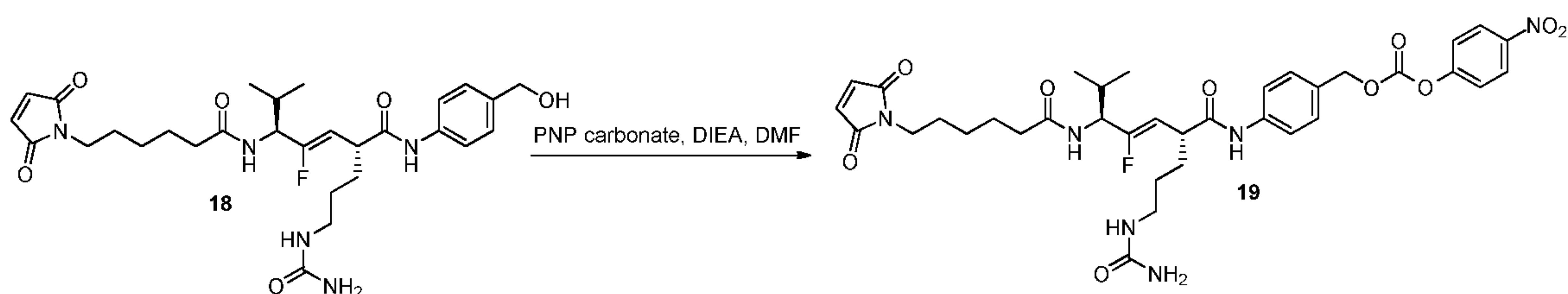


15

To a mixture of compound **16** (50 mg, 0.13 mmol) in DMF (4 mL) was added TEA (39 mg, 0.39 mmol), followed by compound **17** (61 mg, 0.20 mmol). The reaction mixture was stirred at r.t. for 3 h. The mixture was purified by prep-HPLC (FA) to afford compound **18** (30 mg, 40 %) as white solid.

20

^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 9.93 (s, 1H), 8.00 (d, $J = 8.4$ Hz, 1H), 7.53 (d, $J = 8.4$ Hz, 2H), 7.21 (d, $J = 8.4$ Hz, 2H), 7.00 (s, 2H), 5.94 (s, 1H), 5.37 (br, 2H), 4.95 (dd, $J = 38.8, 9.6$ Hz, 1H), 4.42 (s, 2H), 4.24 - 4.15 (m, 1H), 3.47 - 3.35 (m, 2H), 2.95 (dd, $J = 10.0, 5.2$ Hz, 2H), 2.13 - 2.09 (m, 2H), 1.90 - 1.85 (m, 1H), 1.20 - 1.15 (m, 1H), 1.49 - 1.43 (m, 6H), 1.28 - 1.25 (m, 1H), 1.19 - 1.15 (m, 2H), 0.84 (dd, $J = 6.4, 2.8$ Hz, 6H).



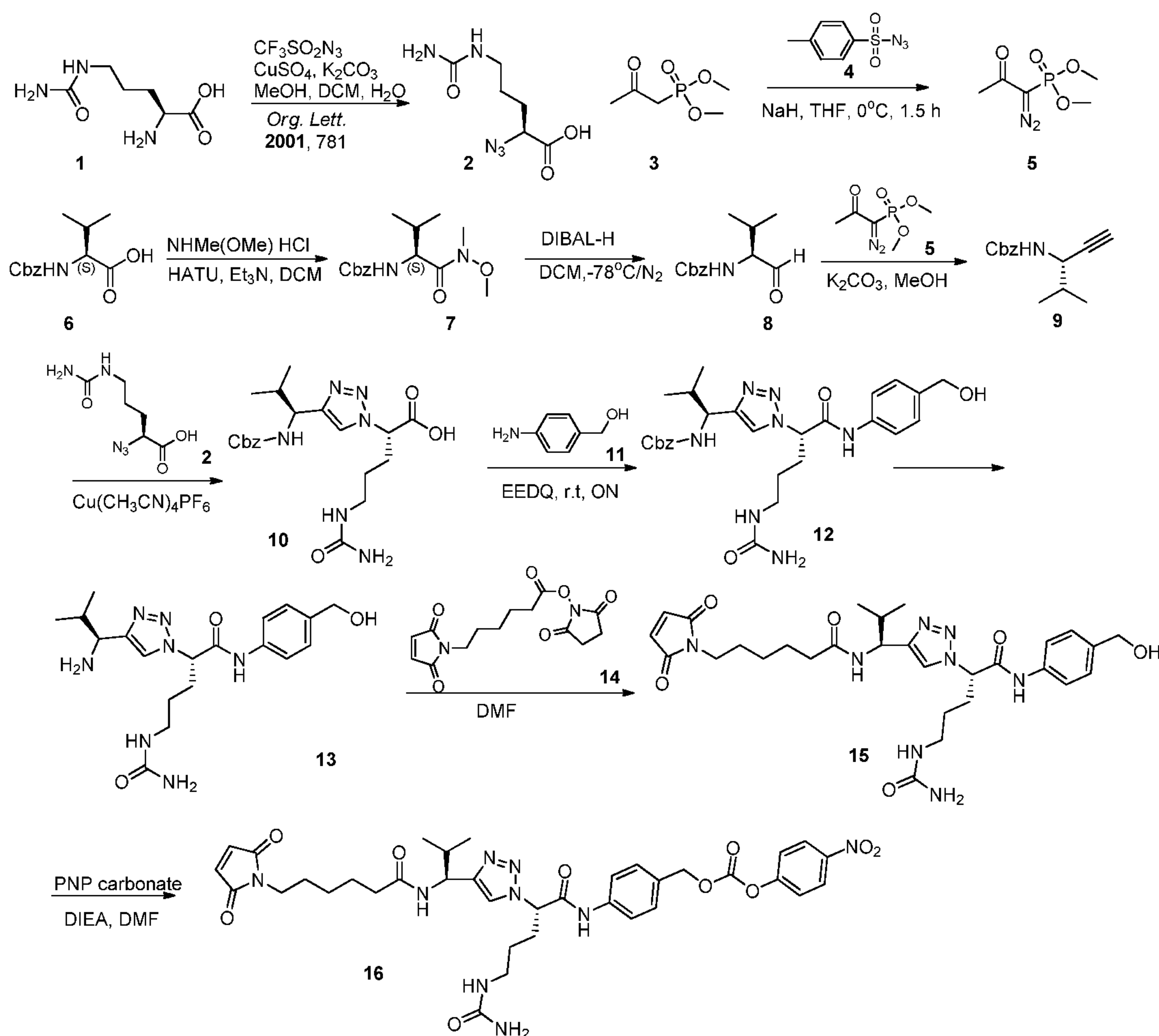
To a solution of compound **18** (20 mg, 0.035 mmol) in dry DMF (2 mL) was added PNP carbonate (32 mg, 0.105 mmol) and DIPEA (9 mg, 0.07 mmol) at 20°C. After the mixture was stirred at 16°C for 16 h under N₂, it was filtered and purified by prep-TLC (DCM/MeOH=10/1), to give compound **19** (INT6) (18 mg, yield: 69%).

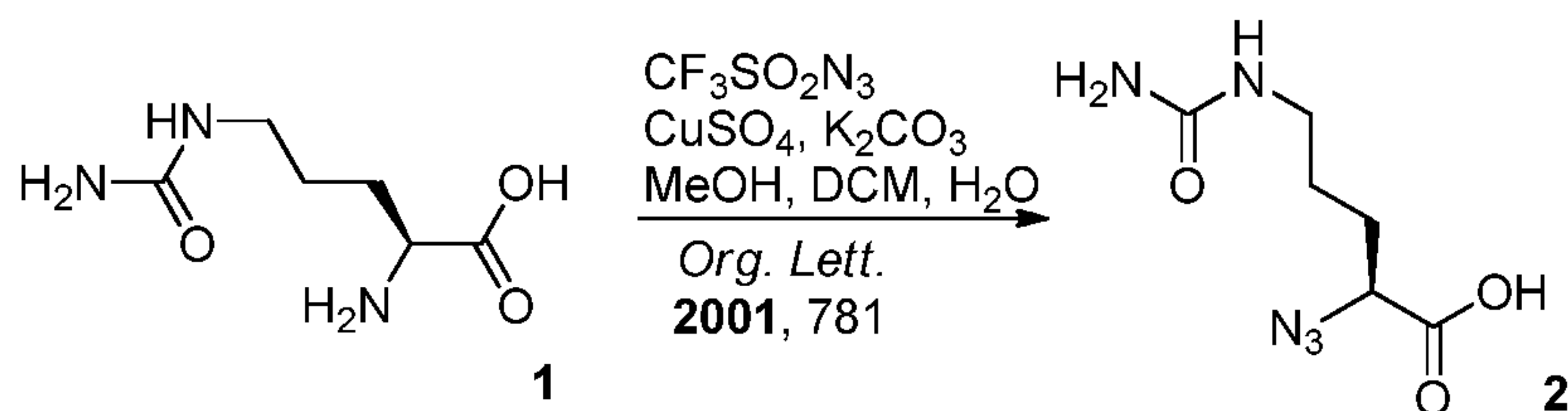
Synthesis of INT7

4-((S)-2-(4-((S)-1-(6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamido)-2-methylpropyl)-1H-1,2,3-triazol-1-yl)-5-ureidopentanamido)benzyl 4-nitrophenyl carbonate

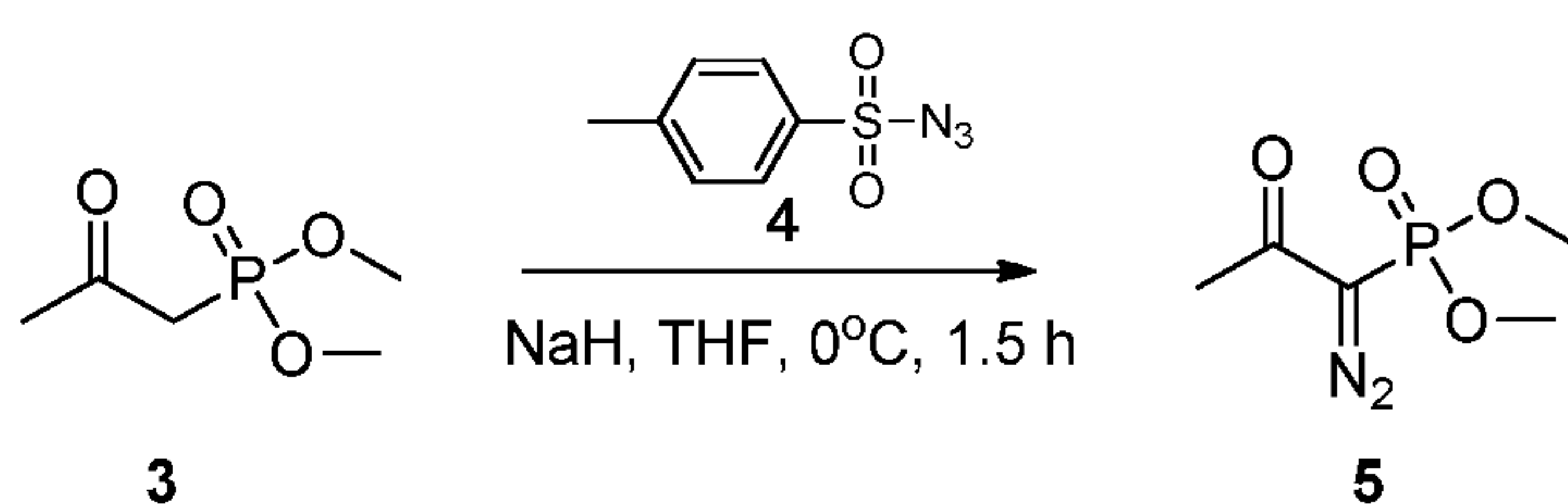
10

Scheme 3



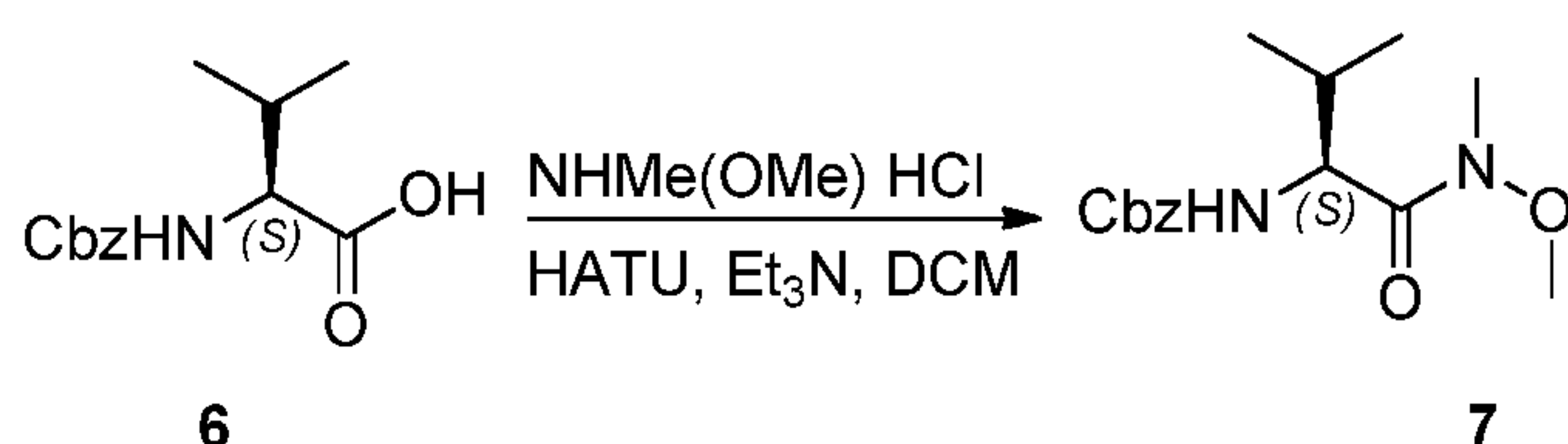
Experimental

A solution of NaN_3 (20 g, 285.7 mmol) was dissolved in distilled H_2O (75 mL) and DCM (100 mL) was added. It was cooled in an ice bath and Tf_2O (19.2 mL, 114.28 mmol) was added slowly over 30 min while stirring continued for 3 h. The mixture was placed in a separation funnel and the CH_2Cl_2 phase collected. The aqueous portion was extracted with CH_2Cl_2 (50 mL \times 2). The organic fractions, containing the triflyl azide were pooled and washed once with saturated Na_2CO_3 (150 mL) and used without further purification. Compound **1** (10 g, 57.14 mmol) was combined with K_2CO_3 (11.83 g, 85.7 mmol) and $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (1.43 g, 5.71 mmol) distilled H_2O (50 mL) and MeOH (100 mL). The triflyl azide in CH_2Cl_2 (120 mL) generated above was added and the mixture was stirred at r.t. overnight. Subsequently, the organic solvents were removed under reduced pressure and the aqueous slurry was diluted with H_2O (100 mL). It was acidified to pH 6 with conc. HCl and diluted with 0.2 M pH 6.2 phosphate buffer (150 mL) and washed with EtOAc (100 mL \times 3) to remove sulfonamide byproduct. The aqueous phase was then acidified to pH 2 with conc. HCl . It was extracted with EtOAc/MeOH (20:1) (100 mL \times 4). The EtOAc/MeOH extractions were combined, dried over Na_2SO_4 and evaporated to give compound **2** without further purification (10 g, 87 %).



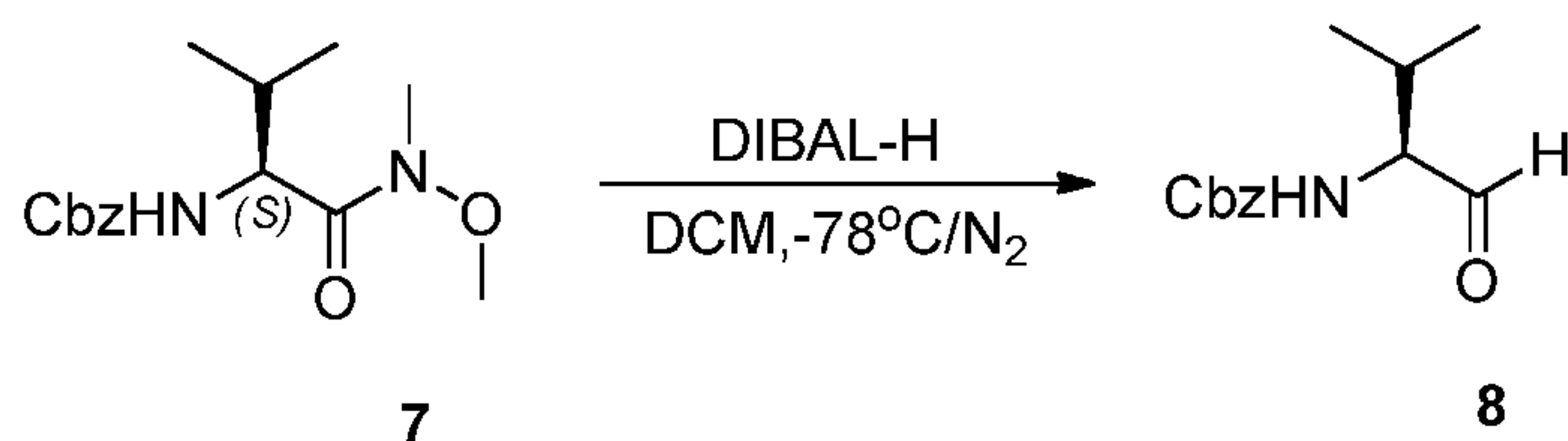
To a solution of compound **3** (18.00 g, 108.36 mmol) in anhydrous THF (300 mL) was added NaH (5.2 g, 130.03 mmol) at 0°C . The mixture was stirred at 0°C for 1 h, then compound **4** (25.64 g, 130.03 mmol) was added slowly into the mixture. The reaction mixture was stirred at 0°C for 0.5 h. The mixture was filtered, concentrated, and purified by column chromatography on silica gel (PE: EtOAc = 1:1) to give the desired product (20 g, 96 %).

$^1\text{H NMR}$ (400 MHz, CDCl_3) δ 3.84 (s, 3H), 3.81 (s, 3H), 2.25 (s, 3H).

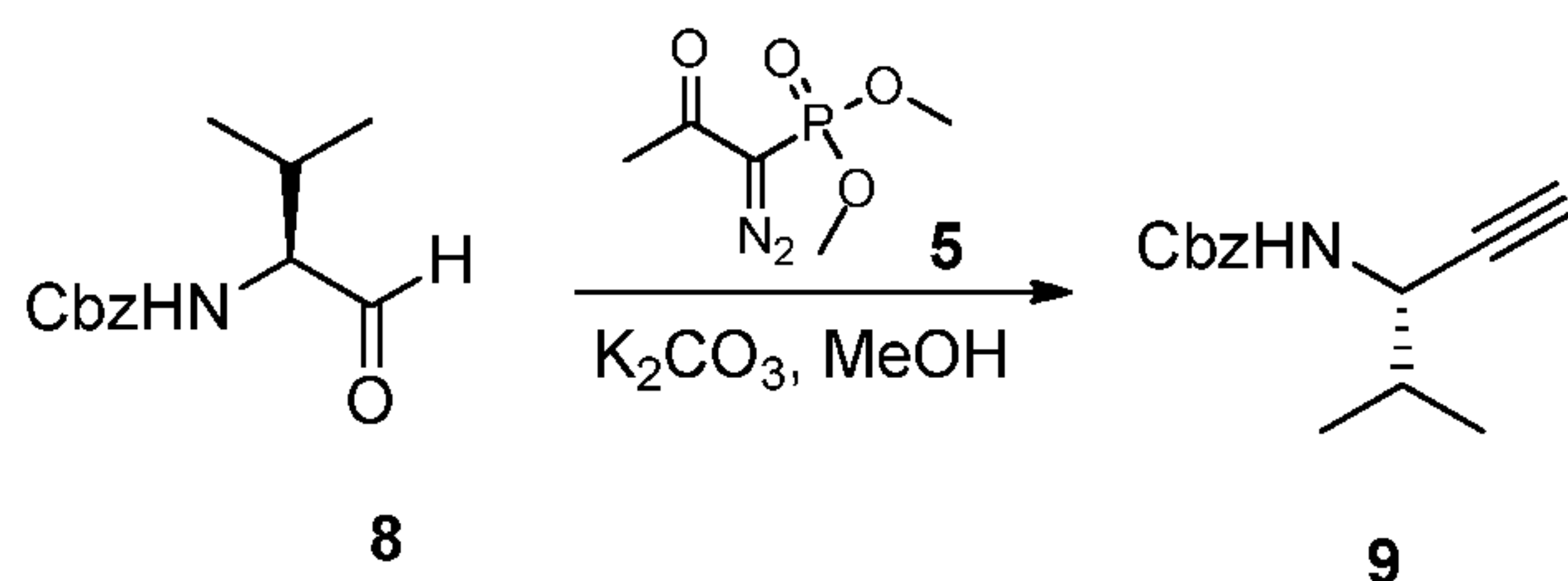


To a mixture of compound **6** (20.0 g, 79.59 mmol) in anhydrous DCM (150 mL) was added Et₃N (24.16 g, 238.77 mmol) and HATU (45.40 g, 119.39 mmol). The mixture was stirred at r.t. for 15 min, then NHMe(OMe) HCl (11.65 g, 119.39 mmol) was added. The reaction mixture was stirred at r.t. overnight. The mixture was diluted with DCM, washed with saturated aq. Na₂CO₃ (100 mL × 3), saturated citric acid (100 mL × 3) and brine (100 mL). The organic layer was dried, concentrated, and purified by column chromatography on silica gel (PE: EtOAc = 10: 1) to give the desired product (20.0 g, 85.4 %).

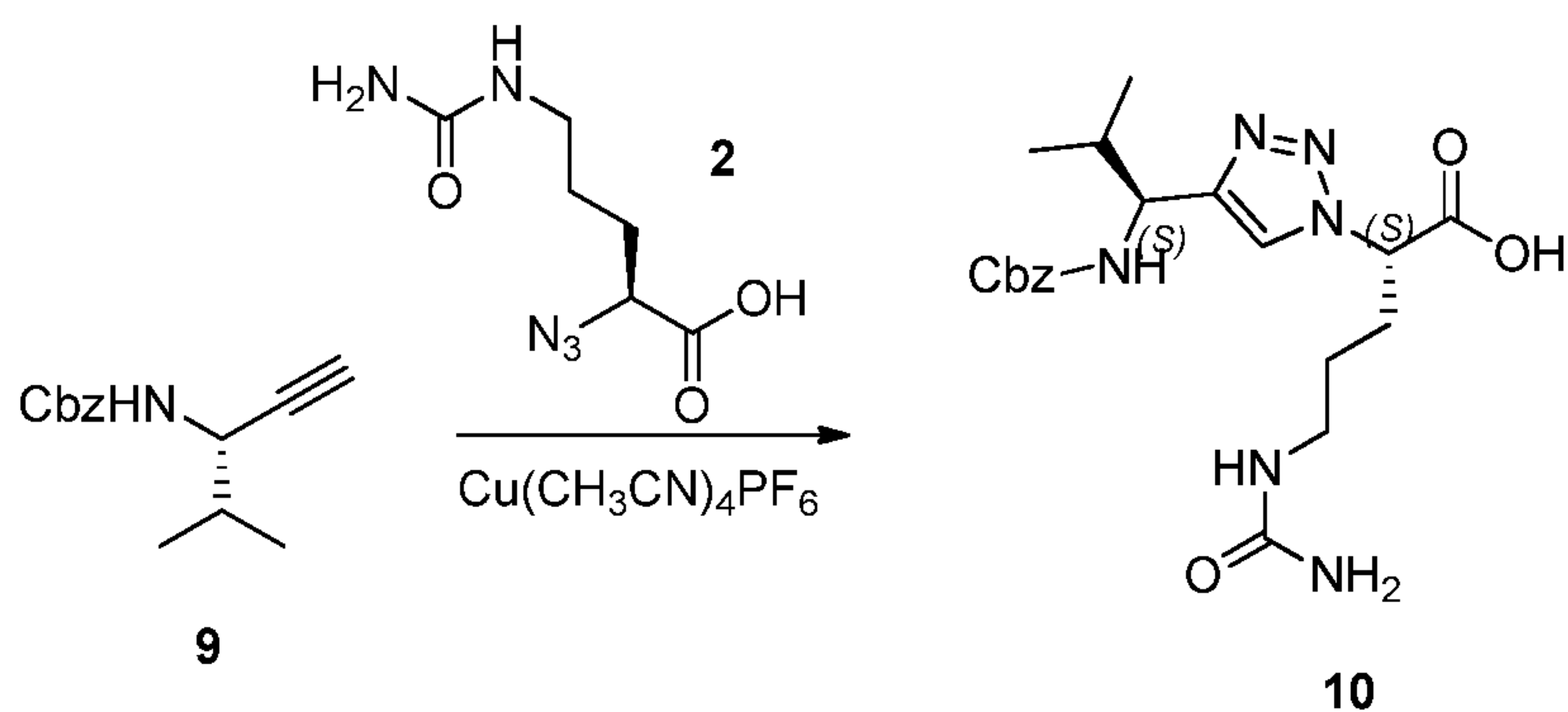
¹H NMR (400 MHz, DMSO-*d*₆) δ 7.97 (s, 1H), 7.73 (d, *J* = 9.2 Hz, 1H), 7.36-7.29 (m, 5H), 6.01 (s, 1H), 5.40 (dd, *J* = 5.2 Hz, 1H), 5.08-4.99 (m, 2H), 4.58 (dd, *J* = 2.8 Hz, 1H), 2.99-2.94 (m, 2H), 2.21-2.02 (m, 4H), 1.02-1.33 (m, 2H), 0.86-0.77 (m, 6H).



Compound **7** (12 g, 40.77 mmol) was dissolved in anhydrous DCM (40 mL) and the resulting solution was cooled to -78°C with a dry ice/acetone bath. DIBAL-H (122.3 mL, 122.3 mmol, 1.0 M in toluene) was added dropwise and the resulting solution was stirred at -78°C for 4 h. Excess hydride was quenched by the addition of MeOH (40 mL) at -78°C and the resulting solution was warmed to r.t. The solution was evaporated to give the compound **8** (~ 9.2 g, 96 %) without further purification.

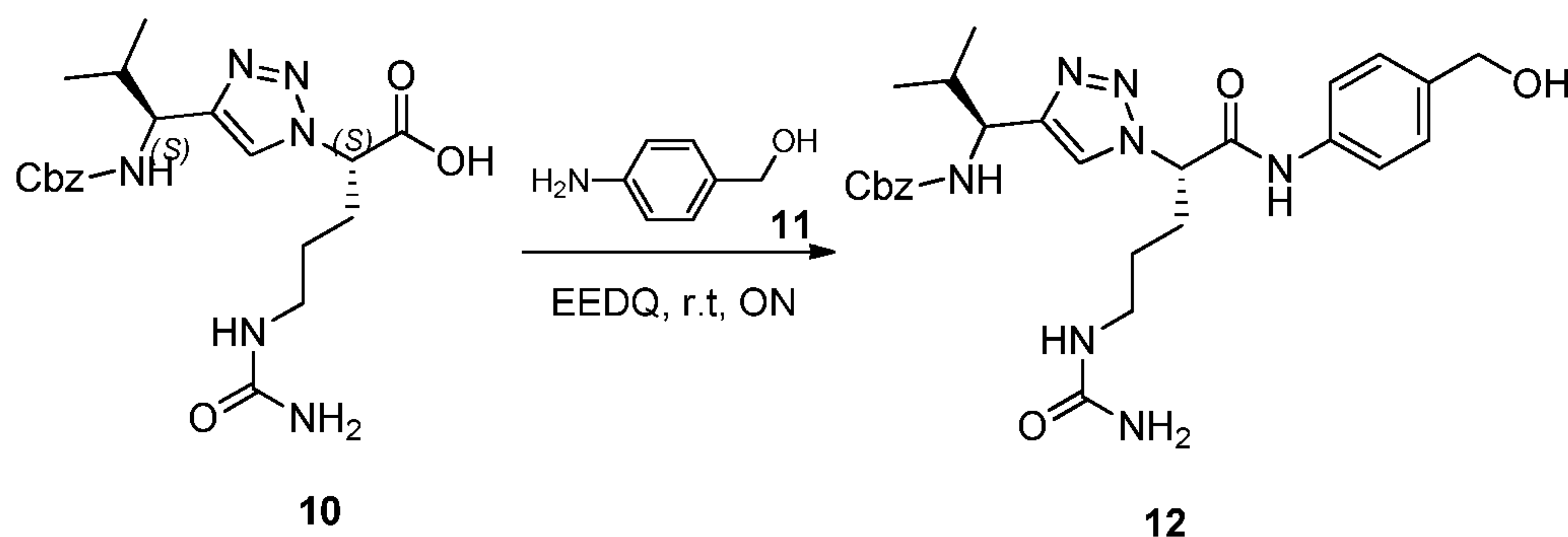


To a solution of compound **8** (crude, ~9.2 g, 39.1 mmol) and compound **5** (11.27 g, 58.65 mmol) in MeOH (150 mL) was added K₂CO₃ (16.2 g, 117.3 mmol). The reaction mixture was stirred at r.t. overnight. The mixture was concentrated in vacuum, and purified by column chromatography on silica gel (PE: EtOAc = 50: 1) to give the desired product (4 g, 44 %).



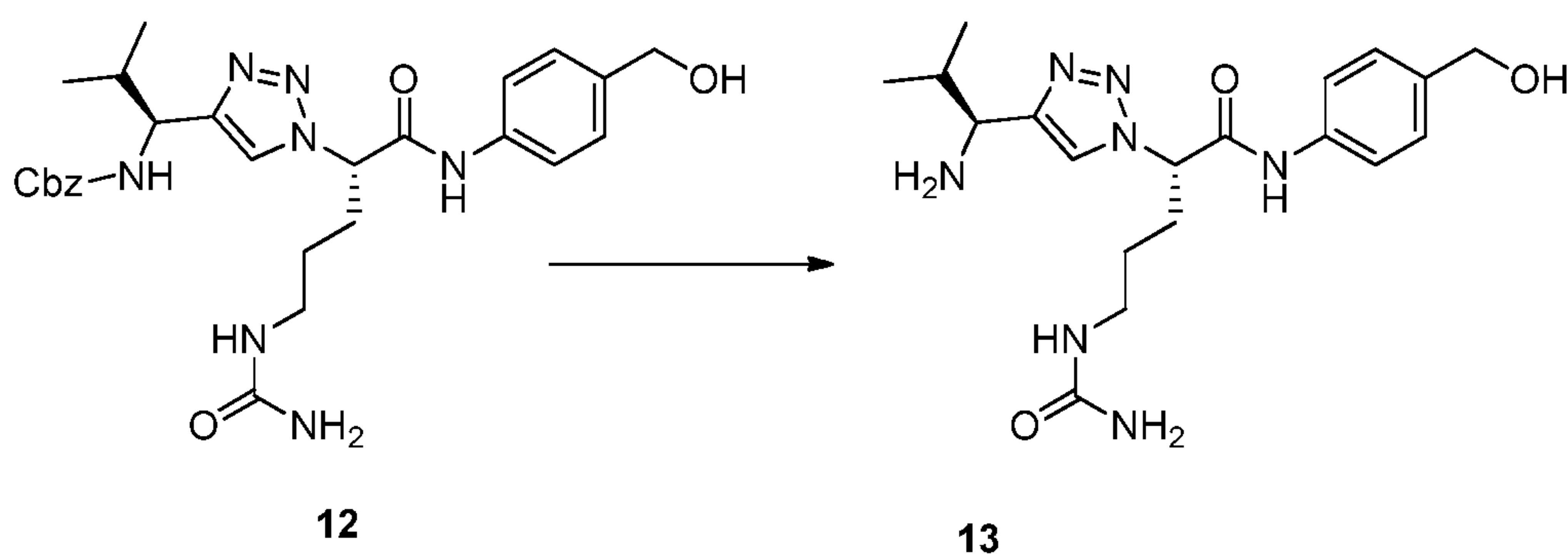
To the solution of compound **9** (4.0 g, 17.29 mmol) and Compound **2** (4.17 g, 20.75 mmol) in DMF (15 mL) was added $\text{Cu}(\text{CH}_3\text{CN})_4\text{PF}_6$ (1.29 g, 3.46 mmol). The reaction mixture was stirred at 60°C for 2 h. The mixture was purified to give compound **10** (5.0 g, 66.8 %).

- 5 ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 7.97 (s, 1H), 7.73 (d, $J = 9.2$ Hz, 1H), 7.36-7.29 (m, 5H), 6.01 (s, 1H), 5.40 (dd, $J = 5.2$ Hz, 1H), 5.08-4.99 (m, 2H), 4.58 (dd, $J = 2.8$ Hz, 1H), 2.99-2.94 (m, 2H), 2.21-2.02 (m, 4H), 1.02-1.33 (m, 2H), 0.86-0.77 (m, 6H).



- 10 To a solution of compound **10** (crude, ~3.8 g, 8.79 mmol) in DMF (15 mL) was added EEDQ (4.34 g, 17.58 mmol) and compound **11** (1.62 g, 13.18 mmol) at 0 °C. The reaction mixture was stirred at r.t. under N_2 overnight. The mixture was purified by prep-HPLC to give compound **12** (650 mg, 13.7 %).

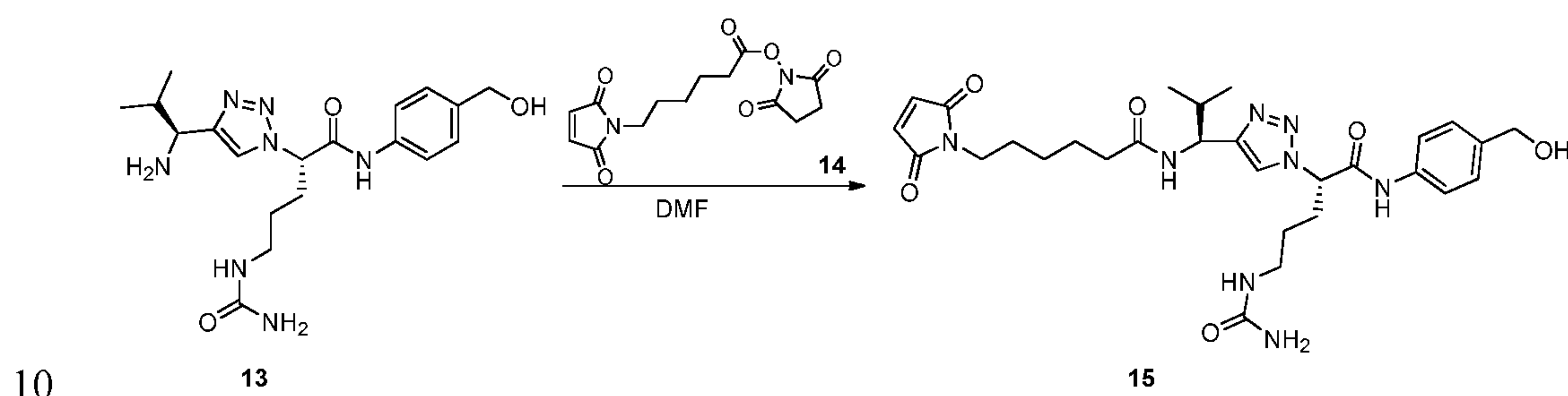
- 15 ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 10.52 (d, $J = 6.8$ Hz, 1H), 8.05 (s, 1H), 7.72 (d, $J = 9.2$ Hz, 2H), 7.33-7.23 (m, 7H), 6.01 (s, 1H), 5.47-5.43 (m, 3H), 5.04-4.96 (m, 2H), 4.59-4.54 (m, 1H), 4.41 (s, 2H), 3.04-2.94 (m, 3H), 2.09-1.97 (m, 4H), 1.24 (t, $J = 6.4$ Hz, 2H), 0.82-0.74 (m, 6H).



To the reaction of compound **12** (650 mg, 1.21 mmol) in MeOH (15 mL) was added Pd/C (300 mg). The reaction mixture was stirred at r.t. under H₂ for 2 h. The reaction mixture was filtered and the filtrate was concentrated to give the **13** (450 mg, 92 %).

5 LCMS (ESI): RT = 0.611 min, M+H⁺ = 404.0. method = 5-95 /1.5 min.

¹H NMR (400 MHz, DMSO-*d*₆) δ 10.55 (s, 1H), 8.03 (d, *J* = 7.6 Hz, 1H), 7.51 (d, *J* = 8.4 Hz, 2H), 7.23 (d, *J* = 8.4, 2H), 6.05 (t, *J* = 5.6 Hz, 1H), 5.46-5.42 (m, 3H), 5.14 (s, 1H), 4.40 (s, 2H), 3.76 (d, *J* = 5.2 Hz, 2H), 3.00-2.93 (m, 3H), 2.09-2.04 (m, 2H), 1.90-1.87 (m, 1H), 1.25-1.21 (m, 2H), 0.82-0.77 (m, 6H).



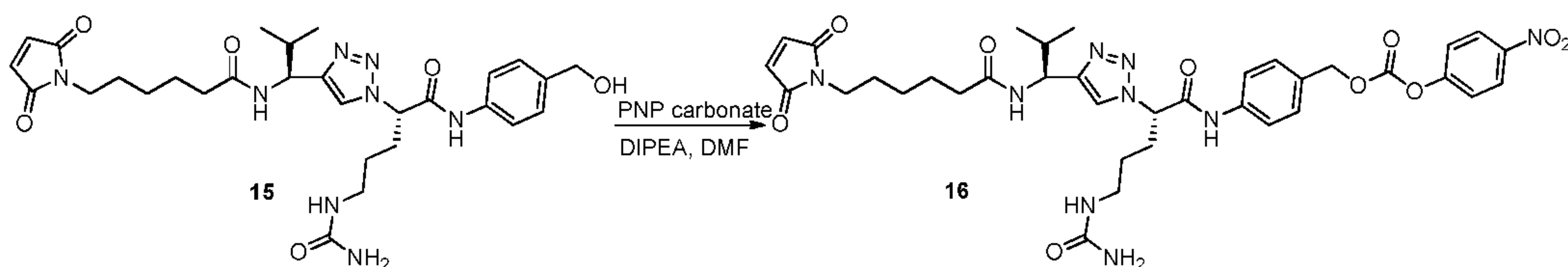
10

Compound **13** (390 mg, 0.965 mmol) and compound **14** (327 mg, 1.06 mmol) were dissolved in DMF (10 mL) at 16°C. The mixture was stirred at r.t. for 2 h. The mixture was concentrated in vacuum and purified by column chromatography (PE/EtOAc=3/1) to give desire product **15** (400 mg, yield: 54%)

15 LCMS: (5-95, AB, 1.5 min), 0.726 min, MS=597.1[M+1];

¹H NMR (400 MHz, DMSO-*d*₆) δ 10.52 (s, 1 H), 8.09 (d, *J* = 9.2 Hz, 1 H), 8.03 (s, 1 H), 7.53 (d, *J* = 8.4 Hz, 2 H), 7.25 (d, *J* = 8.4 Hz, 2 H), 7.00 (s, 2 H), 6.03 - 6.00 (t, *J* = 5.6 Hz, 1 H), 5.45 (s, 1 H), 5.42 (s, 2 H), 5.14 - 5.11 (t, *J* = 5.8 Hz, 1 H), 4.91 - 4.87 (m, 1 H), 4.43 (d, *J* = 5.2 Hz, 2 H), 3.38 (s, 2 H), 3.03 - 2.98 (m, 2 H), 2.14 - 2.05 (m, 4 H), 1.50 - 1.46 (m, 4 H), 1.27 - 1.18 (m, 4 H),

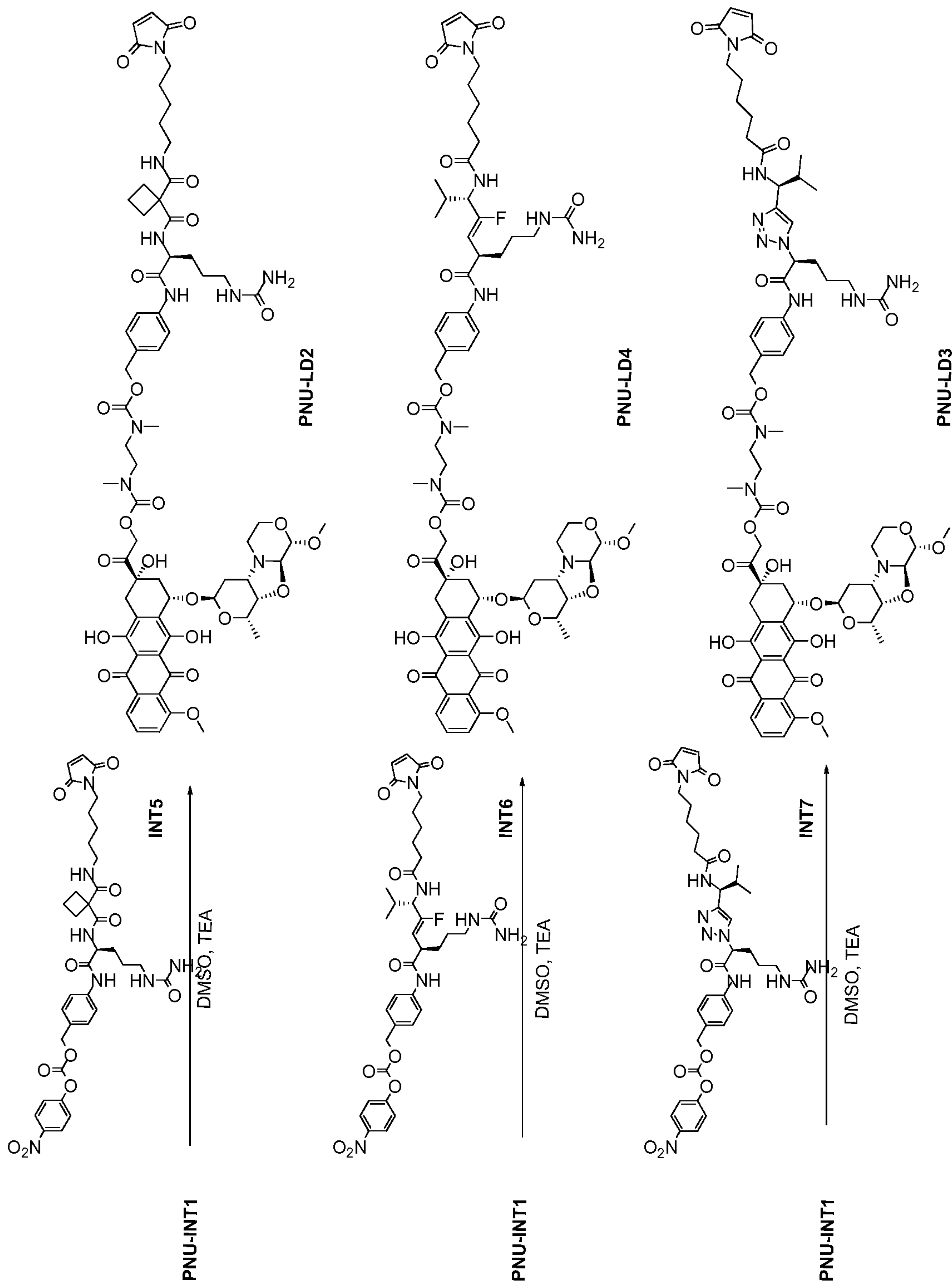
20 0.82 - 0.77 (m, 6 H).



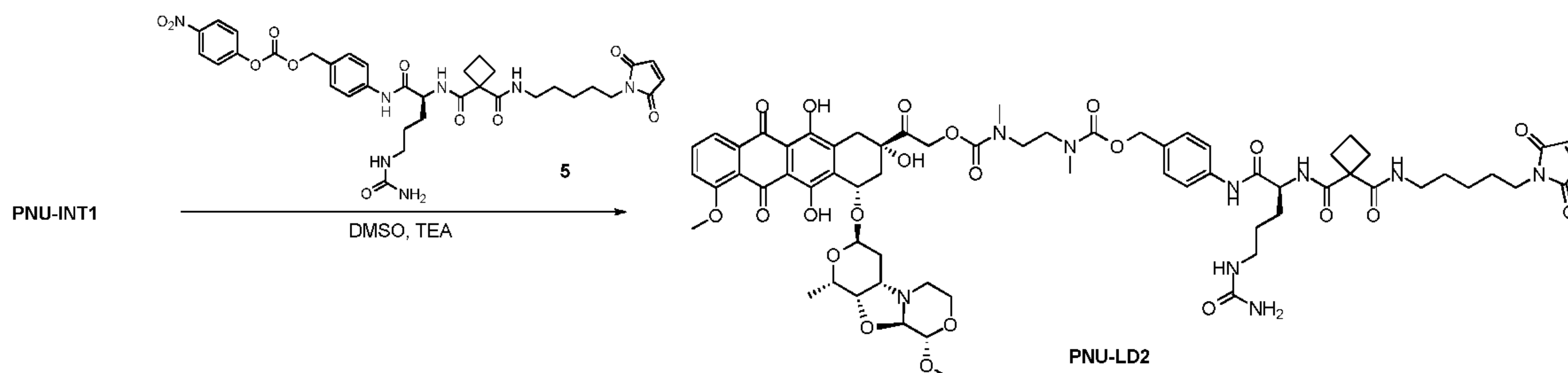
To a solution of compound **15** (30 mg, 0.05 mmol) in dry DMF (2 mL) was added PNP carbonate (46 mg, 0.15 mmol) and DIPEA (13 mg, 0.101 mmol) at 20°C. After the mixture was stirred at 16°C for 16 h under N₂, it was filtered and purified by prep-TLC (DCM/MeOH=10/1) to give **16 (INT7)** (25 mg, yield: 65%).

5

Synthesis of PNU-LD2, PNU-LD3 and PNU-LD4 from the common intermediate.
Scheme 4



Step 1:

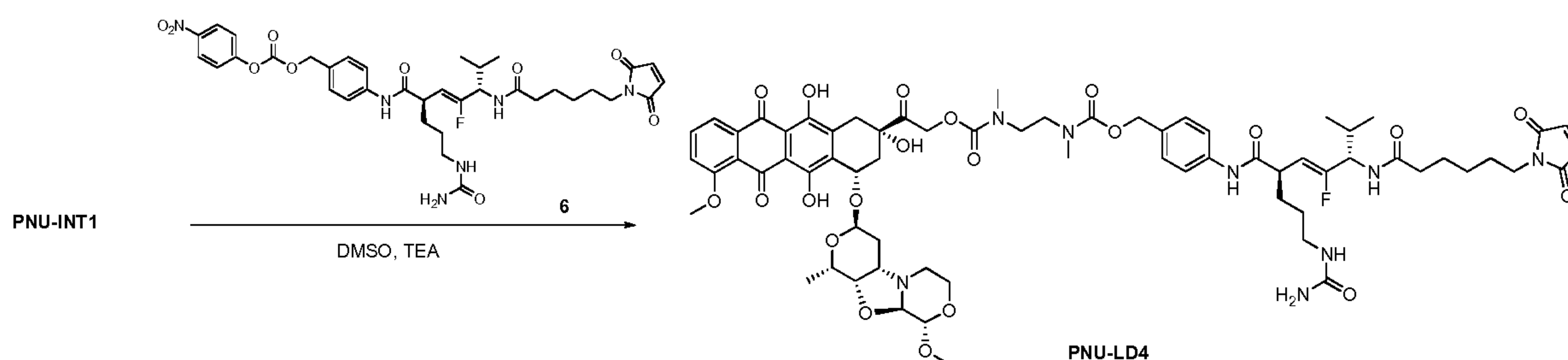


- 5 To a solution of **PNU-INT1** (90.00 mg, 119.08 μmol) and compound **INT5** (131.42 mg, 178.63 μmol) in DMSO (1 mL) was added Et_3N (60.25 mg, 592.42 μmol) at 25°C . After the reaction mixture was stirred at 25°C for 1 h, it was diluted with H_2O (5 mL) and extracted twice with DCM/MeOH (10 mL/1 mL). The organic layer was dried over Na_2SO_4 and concentrated to give the crude product which was purified by prep-TLC (DCM:MeOH = 10:1) to give the desired
- 10 product (50 mg, 31%) [4-[[[(2S)-2-[[1-[5-(2,5-dioxopyrrol-1-yl)pentylcarbamoyl]cyclobutanecarbonyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl N-[2-[[2-[(2S,4S)-4-[[[(1S,3R,4aS,9S,9aR,10aS)-9-methoxy-1-methyl-3,4,4a,6,7,9,9a,10a-octahydro-1H-pyrano[1,2]oxazolo[3,4-b][1,4]oxazin-3-yl]oxy]-2,5,12-trihydroxy-7-methoxy-6,11-dioxo-3,4-dihydro-1H-tetracen-2-yl]-2-oxo-ethoxy]carbonyl-methyl-amino]ethyl]-N-methyl-carbamate
- 15 **PNU-LD2** as a red solid.

LCMS: (10-80, AB, 3.0 min), 1.948 min, MS = 1352.5 $[\text{M}+\text{H}]^+$;

- ^1H NMR (400MHz, CDCl_3) δ 13.82 (s, 1H), 13.20 (s, 1H), 7.96 (d, $J = 7.2$ Hz, 1H), 7.71 (t, $J = 8.0$ Hz, 1H), 7.53 (d, $J = 7.2$ Hz, 1H), 7.31 (d, $J = 8.0$ Hz, 1H), 7.23-7.10 (m, 2H), 6.61 (s, 2H), 5.4 (br, 1H), 5.2-4.4 (m, 10H), 4.01 (s, 6H), 3.53-3.33 (m, 14H), 3.16-2.50 (m, 19H), 1.94-1.18 (m,
- 20 22H).

Step 2:



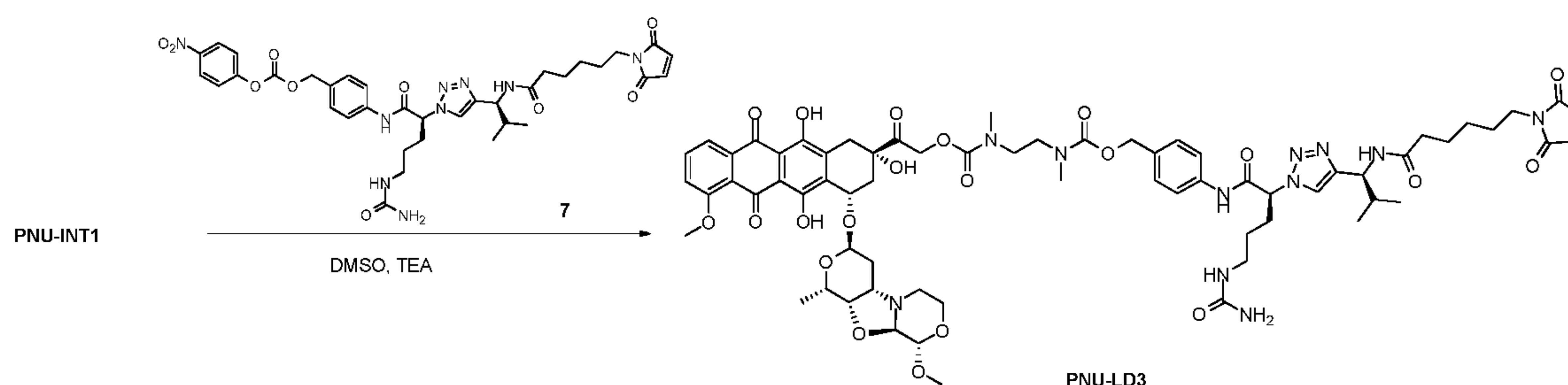
25

To a solution of **PNU-INT1** (11.00 mg, 14.55 μmol) and compound **INT6** (12.00 mg, 16.01 μmol) in DMSO (0.5 mL) was added Et_3N (7.36 mg, 72.75 μmol) at 25°C . The reaction mixture was stirred at 25°C for 1 h. The reaction mixture was diluted with H_2O (3 mL) and extracted twice with

DCM/MeOH (5 mL / 0.5 mL). The organic layer was dried over Na₂SO₄ and concentrated to give the crude product which was purified by prep-TLC (DCM:MeOH=10:1) to give the desired product [4-[[[(Z,2R,5S)-5-[6-(2,5-dioxopyrrol-1-yl)hexanoylamino]-4-fluoro-6-methyl-2-(3-ureidopropyl)hept-3-enoyl]amino]phenyl]methyl N-[2-[[2-[(2S,4S)-4-[[[(1S,3R,4aS,9S,9aR,10aS)-9-methoxy-1-methyl-3,4,4a,6,7,9,9a,10a-octahydro-1H-pyrano[1,2]oxazolo[3,4-b][1,4]oxazin-3-yl]oxy]-2,5,12-trihydroxy-7-methoxy-6,11-dioxo-3,4-dihydro-1H-tetracen-2-yl]-2-oxoethoxy]carbonyl-methyl-amino]ethyl]-N-methyl-carbamate **PNU-LD4** (6 mg, 31.17%) as a red solid.

LCMS: (10-80, AB, 3.0 min), 1.894 min, MS = 1366.5 [M+H]⁺;

10 Step 3:



To a solution of **PNU-INT1** (22.00 mg, 29.11 μmol) and compound **INT7** (23.49 mg, 32.02 μmol) in DMSO (0.5 mL) was added Et₃N (14.73 mg, 145.55 μmol) at 25°C. The reaction mixture was stirred at 25°C for 1 h. The reaction mixture was diluted with H₂O (3 mL) and extracted twice with DCM/MeOH (5 mL/0.5 mL). The organic layer was dried over Na₂SO₄ and concentrated to give the crude product which was purified by prep-TLC (DCM:MeOH=10:1) to give the desired product [4-[[[(2S)-2-[4-[(1S)-1-[6-(2,5-dioxopyrrol-1-yl)hexanoylamino]-2-methyl-propyl]triazol-1-yl]-5-ureido-pentanoyl]amino]phenyl]methyl N-[2-[[2-[(2S,4S)-4-[[[(1S,3R,4aS,9S,9aR,10aS)-9-methoxy-1-methyl-3,4,4a,6,7,9,9a,10a-octahydro-1H-pyrano[1,2]oxazolo[3,4-b][1,4]oxazin-3-yl]oxy]-2,5,12-trihydroxy-7-methoxy-6,11-dioxo-3,4-dihydro-1H-tetracen-2-yl]-2-oxoethoxy]carbonyl-methyl-amino]ethyl]-N-methyl-carbamate **PNU-LD3** (14 mg, 34.89%) as a red solid.

LCMS: (10-80, AB, 3.0 min), 1.938 min, MS = 1378.5 [M+H]⁺;

25

Method of Preparing ADCs

Preparation of cysteine engineered antibodies for conjugation by reduction and reoxidation

Under certain conditions, the cysteine engineered antibodies may be made reactive for conjugation with linker-drug intermediates of the invention, by treatment with a reducing agent such as DTT (Cleland's reagent, dithiothreitol) or TCEP (tris(2-carboxyethyl)phosphine hydrochloride; Getz et al (1999) Anal. Biochem. Vol 273:73-80; Soltec Ventures, Beverly, MA). Full length, cysteine

engineered monoclonal antibodies (ThioMabs) expressed in CHO cells (Gomez et al (2010) Biotechnology and Bioeng. 105(4):748-760; Gomez et al (2010) Biotechnol. Prog. 26:1438-1445) were reduced, for example with about a 50 fold excess of DTT overnight at room temperature to reduce disulfide bonds which may form between the newly introduced cysteine residues and the cysteine present in the culture media.

Light chain amino acids are numbered according to Kabat (Kabat et al., *Sequences of proteins of immunological interest*, (1991) 5th Ed., US Dept of Health and Human Service, National Institutes of Health, Bethesda, MD). Heavy chain amino acids are numbered according to the EU numbering system (Edelman et al (1969) Proc. Natl. Acad. of Sci. 63(1):78-85), except where noted as the Kabat system. Single letter amino acid abbreviations are used.

Full length, cysteine engineered monoclonal antibodies (ThioMabs) expressed in CHO cells bear cysteine adducts (cystines) or glutathionylated on the engineered cysteines due to cell culture conditions. To liberate the reactive thiol groups of the engineered cysteines, the ThioMabs are dissolved in 500 mM sodium borate and 500 mM sodium chloride at about pH 8.0 and reduced with about a 50-100 fold excess of 1 mM TCEP (tris(2-carboxyethyl)phosphine hydrochloride (Getz et al (1999) Anal. Biochem. Vol 273:73-80; Soltec Ventures, Beverly, MA) for about 1-2 hrs at 37 °C. Alternatively, DTT can be used as reducing agent. The formation of inter-chain disulfide bonds was monitored either by non-reducing SDS-PAGE or by denaturing reverse phase HPLC PLRP column chromatography. The reduced ThioMab is diluted and loaded onto a HiTrap SP FF column in 10 mM sodium acetate, pH 5, and eluted with PBS containing 0.3M sodium chloride, or 50 mM Tris-Cl, pH 7.5 containing 150 mM sodium chloride.

Disulfide bonds were reestablished between cysteine residues present in the parent Mab by carrying out reoxidation. The eluted reduced ThioMab is treated with 15X or 2 mM dehydroascorbic acid (dhAA) at pH 7 for 3 hours or for 3 hrs in 50 mM Tris-Cl, pH 7.5, or with 2 mM aqueous copper sulfate (CuSO₄) at room temperature overnight. Other oxidants, i.e. oxidizing agents, and oxidizing conditions, which are known in the art may be used. Ambient air oxidation may also be effective. This mild, partial reoxidation step forms intrachain disulfides efficiently with high fidelity. The buffer is exchanged by elution over Sephadex G25 resin and eluted with PBS with 1mM DTPA. The thiol/Ab value is checked by determining the reduced antibody concentration from the absorbance at 280 nm of the solution and the thiol concentration by reaction with DTNB (Aldrich, Milwaukee, WI) and determination of the absorbance at 412 nm.

Liquid chromatography/Mass Spectrometric Analysis was performed on a TSQ Quantum Triple quadrupole™ mass spectrometer with extended mass range (Thermo Electron, San Jose California). Samples were chromatographed on a PRLP-S®, 1000 A, microbore column (50mm ×

2.1mm, Polymer Laboratories, Shropshire, UK) heated to 75 °C. A linear gradient from 30-40% B (solvent A: 0.05% TFA in water, solvent B: 0.04% TFA in acetonitrile) was used and the eluent was directly ionized using the electrospray source. Data were collected by the Xcalibur® data system and deconvolution was performed using ProMass® (Novatia, LLC, New Jersey). Prior to
5 LC/MS analysis, antibodies or drug conjugates (50 micrograms) were treated with PNGase F (2 units/ml; PROzyme, San Leandro, CA) for 2 hours at 37 °C to remove N-linked carbohydrates.

Hydrophobic Interaction Chromatography (HIC) samples were injected onto a Butyl HIC NPR column (2.5 micron particle size, 4.6 mm × 3.5 cm) (Tosoh Bioscience) and eluted with a linear gradient from 0 to 70% B at 0.8 ml/min (A: 1.5 M ammonium sulfate in 50 mM potassium
10 phosphate, pH 7, B: 50 mM potassium phosphate pH 7, 20% isopropanol). An Agilent 1100 series HPLC system equipped with a multi wavelength detector and Chemstation software was used to resolve and quantitate antibody species with different ratios of drugs per antibody. Cysteine engineered antibodies of the present invention can be prepared according the general method described above.

15 Conjugation of linker-drug intermediates to antibodies (procedure 1)

Engineered antibody cysteines were blocked as mixed disulfides with glutathione and/or cysteine as expressed in CHO cells. These cysteines had to be "deblocked" prior to conjugation.

Deblocked antibody (5-12 mg/mL) in 20 mM succinate, 150 mM NaCl, 2 mM EDTA was brought to 75-100 mM Tris, pH 7.5-8 (using 1M Tris). Co-solvent (DMSO, DMF, or DMA) was added to
20 the antibody solution, followed by linker-drug (in DMSO or DMF) to give a final %-organic solvent of 10-13% and final concentration of linker-drug 2.5-10X relative to antibody concentration. Reactions were allowed to proceed at room temperature for 1-12 hours (until maximum conjugation was achieved). Conjugation reactions were purified via cation exchange chromatography and/or gel filtration using disposable columns (S maxi or Zeba, respectively).
25 Additional purification by preparative gel filtration (S200 columns) was performed if the crude conjugate was significantly aggregated according to analytical SEC (e.g., >10%). Conjugates were subsequently exchanged into formulation buffer (20 mM His-acetate, pH 5.5, 240 mM sucrose) using either gel filtration or dialysis. Tween-20 was subsequently added to the purified conjugate to reach a final concentration of 0.02%. Final conjugate concentrations ranged from 2.4 to 7.5
30 mg/mL (%Yield: 34-81% from deblocked antibody). Conjugates were analyzed by LCMS to obtain a measurement of the drug-antibody ratio (DAR), which ranged from 1.3 to 2.1 (average: 1.8). Conjugates were also analyzed for presence of high-molecular weight aggregates using analytical SEC (Zenix or Shodex columns); final, purified conjugates displayed aggregation ranging from 0-10%. Conjugates were also assessed for endotoxin contamination, which, in all

cases, did not exceed 1.3 EU/mg. Free, unconjugated drug did not exceed 1% of the final conjugate.

Conjugation of linker-drug intermediates to antibodies (procedure 2, alternative procedure)

After the reduction and reoxidation procedures of the above example, the antibody is dissolved in
5 PBS (phosphate buffered saline) buffer and chilled on ice. An excess, from about 1.5 molar to 20
equivalents of a linker-drug intermediate with a thiol-reactive functional group such as maleimido
or bromo-acetamide, is dissolved in DMSO, diluted in acetonitrile and water, and added to the
chilled reduced, reoxidized antibody in PBS. After about one hour, an excess of maleimide is
added to quench the reaction and cap any unreacted antibody thiol groups. The conjugation
10 mixture may be loaded and eluted through a HiTrap SP FF column to remove excess drug-linker
intermediate and other impurities. The reaction mixture is concentrated by centrifugal
ultrafiltration and the cysteine engineered antibody drug conjugate is purified and desalted by
elution through G25 resin in PBS, filtered through 0.2 μ m filters under sterile conditions, and
frozen for storage.

15 The ADCs of the present invention can be prepared according to the procedure described in the
above section.

ASSAYS

Select linkers were then tested and found active in *in vitro* and *in vivo* assays. The cleavage data is
shown in the table below

20

Cathepsin B cleavage Assay

Like peptide linkers, non-peptide linkers for ADC is expect to be cleavable in lysosome in order
for proper drug release. As a digestive organelle of the cell, lysosome is enriched with some
proteases which show optimal hydrolytic activity at an acidic pH. Cathepsin B is a representative
25 lysosomal protease and has been shown to contribute to the activation of ADC peptide linkers
(ref). As an initial screen, an assay was developed using purified cathepsin B to identify cleavable
linker-drug constructs that are suitable for conjugation with antibody. Norfloxacin was used to
represent the drug component of the linker-drug. The percentage of cleavage relative to the
control peptides (such as Val-Cit) was measured at a given time point as well as the kinetic
30 parameters of the cleavage reaction (K_m and V_{max}). Detailed description of the assay is shown
below. From this assay, a variety of proteolytically active and structurally diverse linkers were
identified and later used in making ADCs.

Cathepsin B cleavage activity using experimental linker-drugs as substrate was measured by monitoring the release of Norfloxacin using LC/MS. Varying concentrations of linker-drug (3-fold serial dilutions) were incubated in 20 uL reactions containing 20 nM Cathepsin B (EMD Millipore cat. #219364, human liver), 10 mM MES pH 6.0, 1 mM DTT, 0.03% CHAPS, and 25 nM Norfloxacin-d5 internal standard (Santa Cruz Biotechnology, cat. #sc-301482). Reactions were incubated for 1 hour at 37°C, followed by addition of 60 uL of 2% formic acid to quench the reactions. Samples were analyzed by injecting 2 uL of stopped reactions on a Waters Acquity UPLC BEH Phenyl column (2.1 mm x 50 mm, Waters cat. #186002884). Samples were purified using a linear 2 minute gradient (0% to 80%) of acetonitrile, 0.1% formic acid on a Water Acquity UPLC. Norfloxacin and Norfloxacin-d5 internal standard were detected using an AB Sciex QTrap 5500 triple quadrupole mass spectrometer operating in positive MRM mode (Norfloxacin 320→233 m/z, Norfloxacin-d5 325 →233 m/z). The quantified norfloxacin (normalized with internal standard) was plotted against linker-drug concentration, and the resulting plot was curve fitted with a Michaelis-Menten fit using GraphPad Prism software for the kinetic constants K_m and V_{max} .

In vitro cell proliferation assay

Efficacy of ADC was measured by a cell proliferation assay employing the following protocol (CELLTITER GLO™ Luminescent Cell Viability Assay, Promega Corp. Technical Bulletin TB288; Mendoza et al (2002) Cancer Res. 62:5485-5488):

1. An aliquot of 100 µl of cell culture containing about 10^4 cells (SKBR-3, BT474, MCF7 or MDA-MB-468) in medium was deposited in each well of a 96-well, opaque-walled plate.
2. Control wells were prepared containing medium and without cells.
3. ADC was added to the experimental wells and incubated for 3-5 days.
4. The plates were equilibrated to room temperature for approximately 30 minutes.
5. A volume of CELLTITER GLO™ Reagent equal to the volume of cell culture medium present in each well was added.
6. The contents were mixed for 2 minutes on an orbital shaker to induce cell lysis.
7. The plate was incubated at room temperature for 10 minutes to stabilize the luminescence signal.
8. Luminescence was recorded and reported in graphs as RLU = relative luminescence units.

Data are plotted as the mean of luminescence for each set of replicates, with standard deviation error bars. The protocol is a modification of the CELLTITER GLO™ Luminescent Cell

Media: SK-BR-3 grow in 50/50/10%FBS/glutamine/250 µg/mL G-418 OVCAR-3 grow in RPMI/20%FBS/glutamine

5 *In vivo* assay

1. The efficacy of the anti-CD33 antibody-drug conjugates (ADCs) was investigated in a mouse xenograft model of HL-60 or EOL-1 (human acute myeloid leukemia). The HL-60 cell line was obtained from ATCC (American Type Culture Collection; Manassas, VA) and EOL-1 cell line was originated from DSMZ (German Collection of Microorganisms and Cell Cultures;
10 Braunschweig, Germany).

Female C.B-17 SCID mice (Charles River Laboratories; Hollister, CA) were each inoculated subcutaneously in the flank area with five million cells of HL-60 or EOL-1. When the xenograft tumors reached an average tumor volume of 100-300 mm³ (referred to as Day 0), animals were
15 randomized into groups of 7-10 mice each and received a single intravenous injection of the ADCs. Approximately 4 hours prior to administration of ADCs, animals were dosed intraperitoneally with excess amount (30mg/kg) of anti-gD control antibody to block possible nonspecific antibody binding sites on the tumor cells. Tumors and body weights of mice were measured 1-2 times a week throughout the study. Mice were promptly euthanized when body
20 weight loss was >20% of their starting weight. All animals were euthanized before tumors reached 3000 mm³ or showed signs of impending ulceration.

25 2. The efficacy of the anti-Napi2B antibody-drug conjugates (ADCs) was investigated in a mouse xenograft model of OVCAR3-X2.1 (human ovarian cancer). The OVCAR3 cell line was obtained from ATCC (American Type Culture Collection; Manassas, VA) and a sub-line OVCAR3-X2.1 was generated at Genentech for optimal growth in mice.

30 Female C.B-17 SCID-beige mice (Charles River Laboratories; San Diego, CA) were each inoculated in the thoracic mammary fat pad area with ten million OVCAR3-X2.1 cells. When the xenograft tumors reached an average tumor volume of 100-300 mm³ (referred to as Day 0), animals were randomized into groups of 7-10 mice each and received a single intravenous injection of the ADCs. Tumors and body weights of mice were measured 1-2 times a week
35 throughout the study. Mice were promptly euthanized when body weight loss was >20% of their

starting weight. All animals were euthanized before tumors reached 3000 mm³ or showed signs of impending ulceration.

3. The efficacy of the anti-CD22 antibody-drug conjugates (ADCs) is investigated in a mouse xenograft model of BJAB-luc (human Burkitt's lymphoma) or WSU-DLCL2 (human diffuse large B-cell lymphoma). The BJAB cell line is obtained from DSMZ (German Collection of Microorganisms and Cell Cultures; Braunschweig, Germany), and a sub-line BJAB-luc is generated at Genentech to stably express the luciferase gene. The WSU-DLCL2 cell line is also originated from DSMZ.

10

Female C.B-17 SCID mice (Charles River Laboratories; Hollister, CA) are each inoculated subcutaneously in the flank area with 20 million cells of BJAB-luc or WSU-DLCL2. When the xenograft tumors reached an average tumor volume of 100-300 mm³ (referred to as Day 0), animals are randomized into groups of 7-10 mice each and received a single intravenous injection of the ADCs. Tumors and body weights of mice are measured 1-2 times a week throughout the study. Mice are promptly euthanized when body weight loss is >20% of their starting weight. All animals are euthanized before tumors reached 3000 mm³ or showed signs of impending ulceration.

20

4. The efficacy of the anti-Her2 antibody-drug conjugates (ADCs) is investigated in a mouse allograft model of MMTV-HER2 Founder #5 (murine mammary tumor). The MMTV-HER2 Founder #5 (Fo5) model (developed at Genentech) is a transgenic mouse model in which the human HER2 gene, under transcriptional regulation of the murine mammary tumor virus promoter (MMTV-HER2), is overexpressed in mammary epithelium. The overexpression causes spontaneous development of mammary tumors that overexpress the human HER2 receptor. The mammary tumor from one of the founder animals (founder #5, Fo5) has been propagated in FVB mice (Charles River Laboratories) by serial transplantation of tumor fragments.

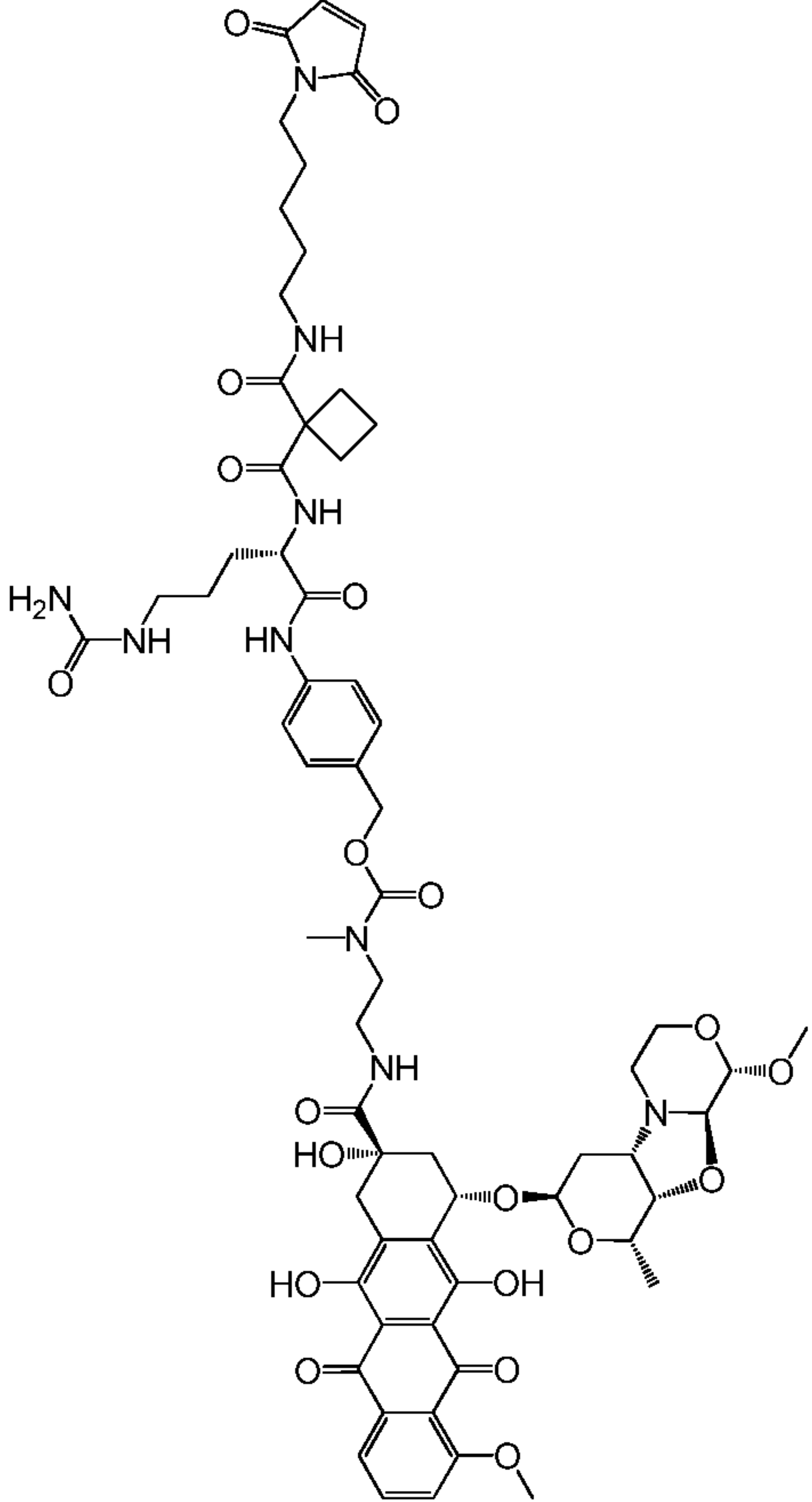
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For efficacy studies, the Fo5 transgenic mammary tumor is surgically transplanted into the thoracic mammary fat pad of female nu/nu mice (Charles River Laboratories; Hollister, CA) as tumor fragments of approximately 2mm x 2mm in size. When the allograft tumors reached an average tumor volume of 100-300 mm³ (referred to as Day 0), animals are randomized into groups of 7-10 mice each and received a single intravenous injection of the ADCs. Tumors and body weights of mice are measured 1-2 times a week throughout the study. Mice are promptly euthanized when body weight loss is >20% of their starting weight. All animals are euthanized before tumors reached 3000 mm³ or showed signs of impending ulceration.

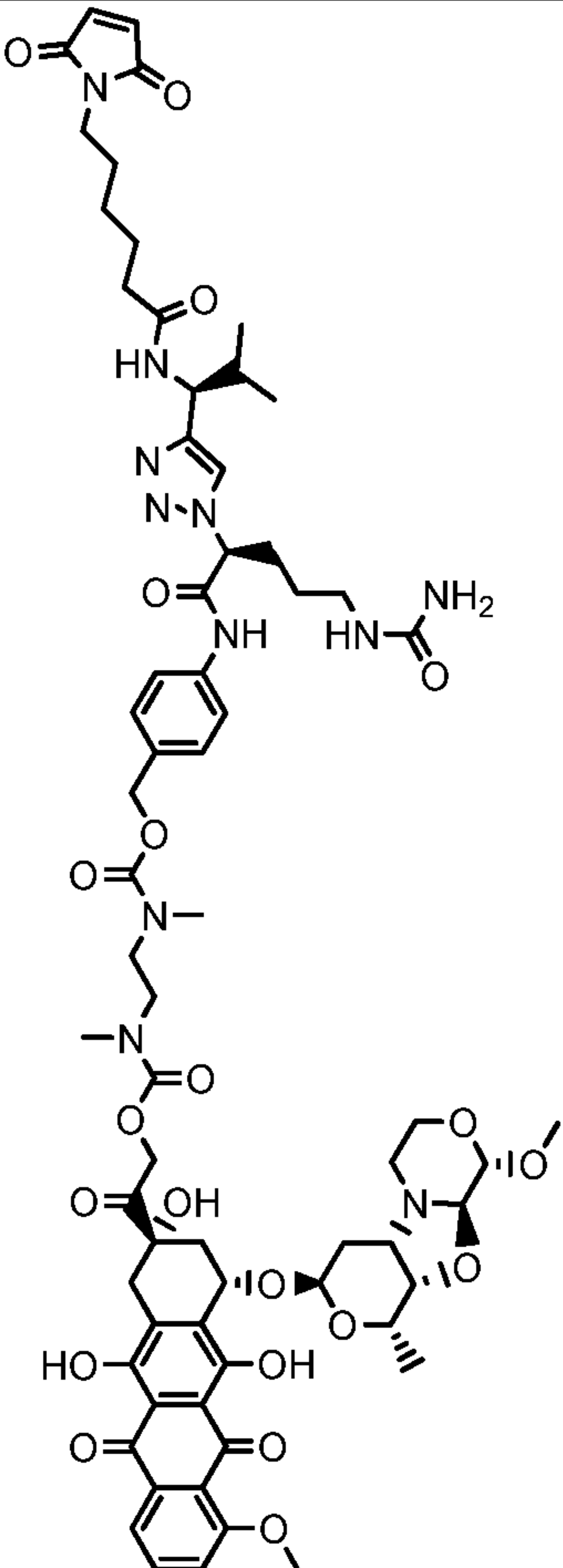
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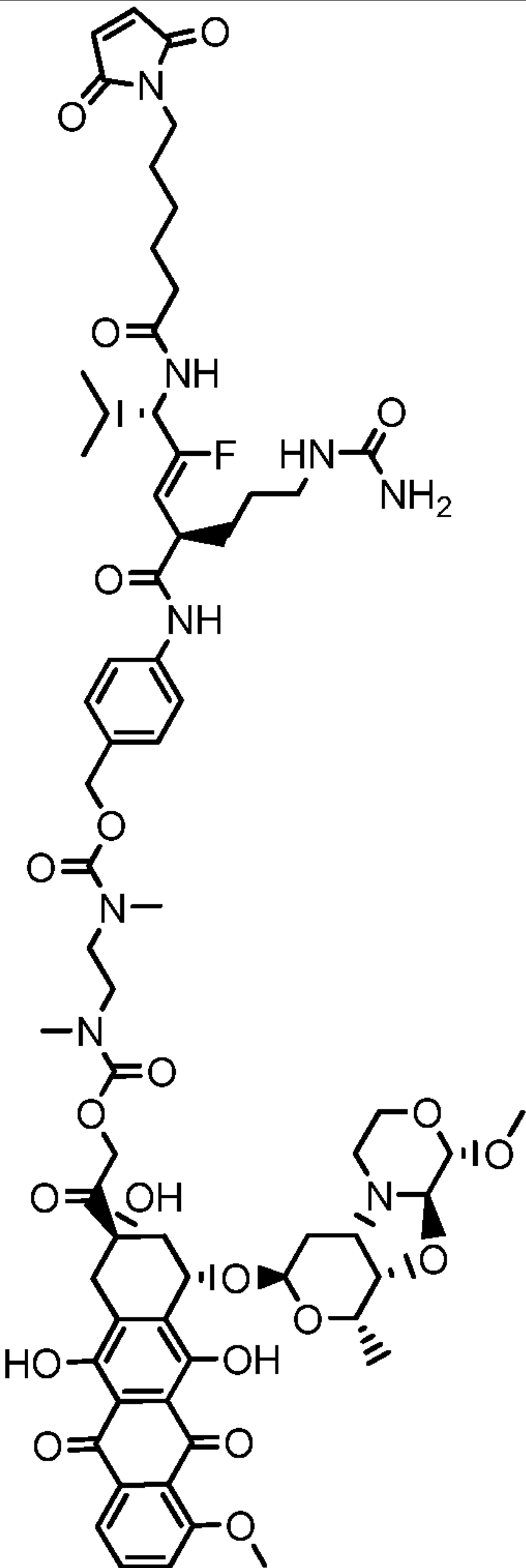
BIOLOGICAL DATA

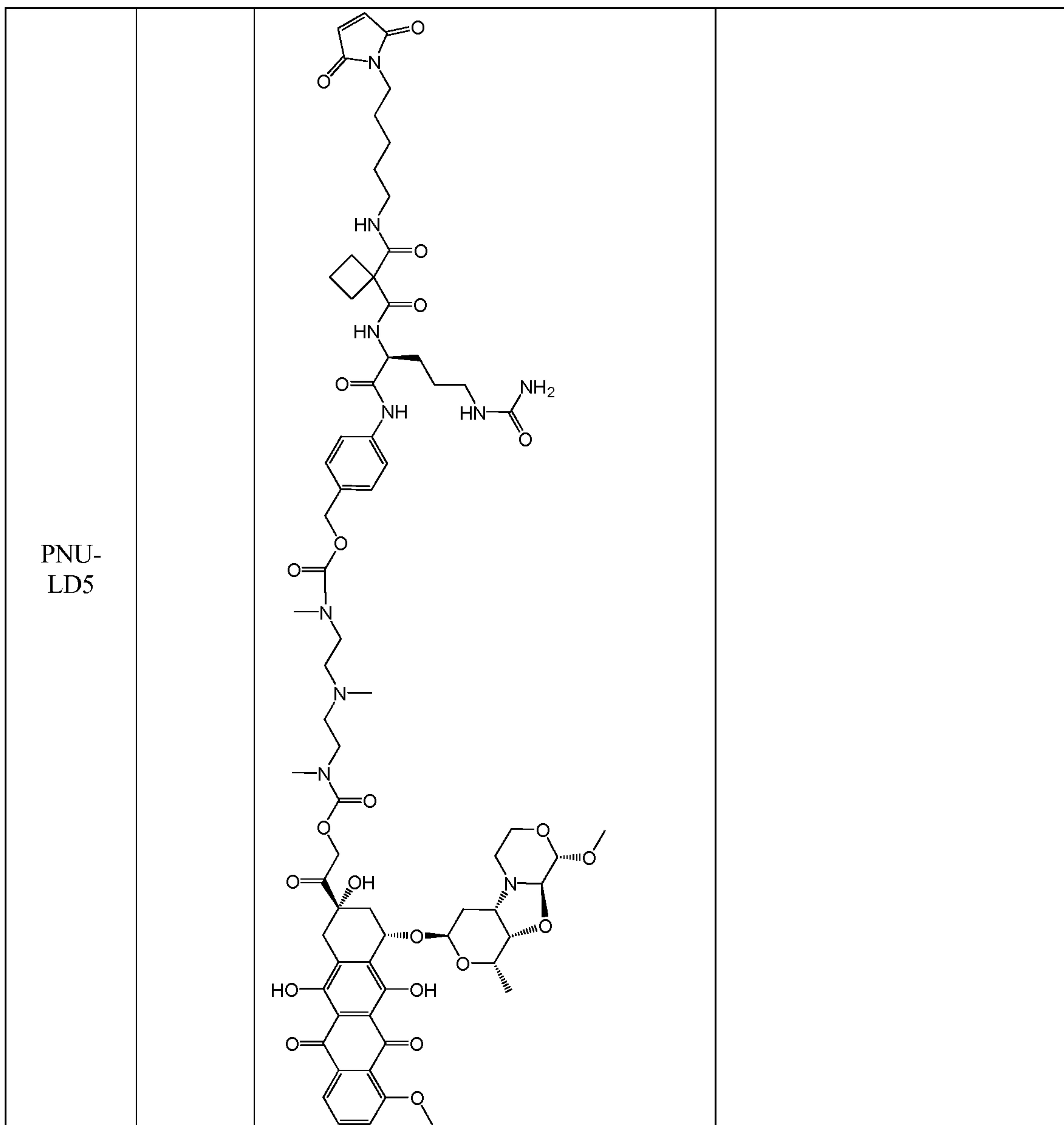
ADC Linker-Drug structures made according to the general procedure described herein

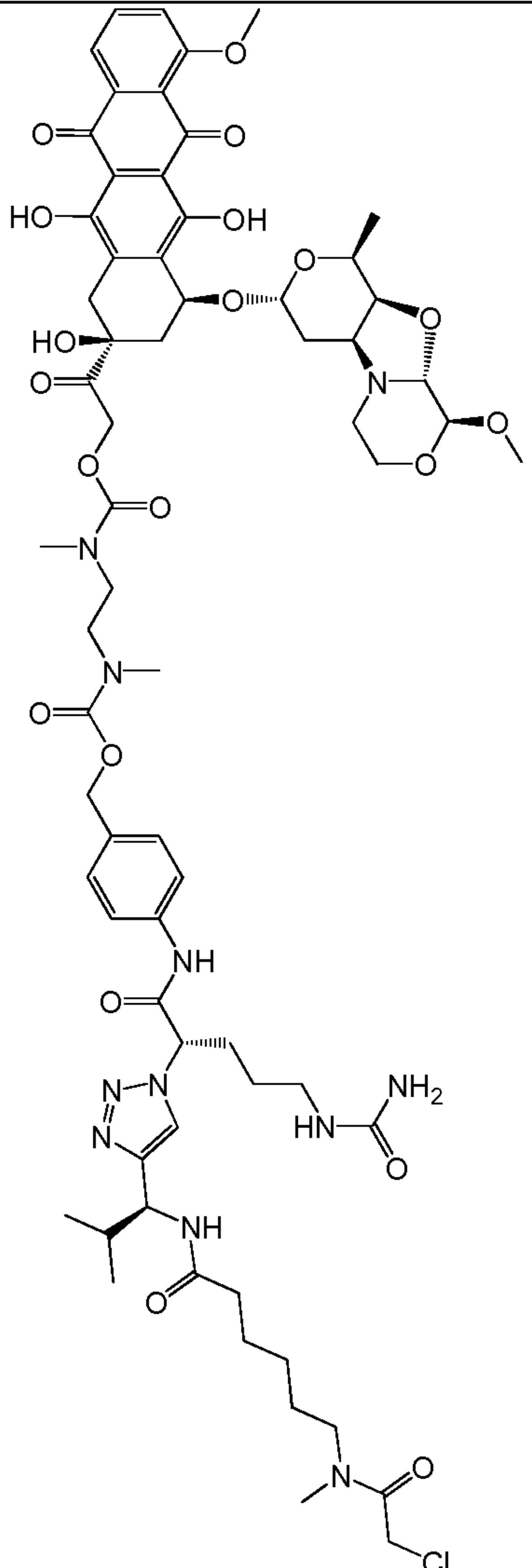
Example	Corresponding ADC	Structure	Name
PNU-LD1			<p>[4-[[[(2S)-2-[[1-[5-(2,5-dioxopyrrol-1-yl)pentylcarbamoyl]cyclobutane carbonyl]amino]-5-ureidopentanoyl]amino]phenyl]methyl N-[2-[[[(2S,4S)-4-[[[(1S,3R,4aS,9S,9aR,10aS)-9-methoxy-1-methyl-3,4,4a,6,7,9,9a,10a-octahydro-1H-pyrano[1,2]oxazolo[3,4-b][1,4]oxazin-3-yl]oxy]-2,5,12-trihydroxy-7-methoxy-6,11-dioxo-3,4-dihydro-1H-tetracene-2-carbonyl]amino]ethyl]-N-methyl-carbamate</p>

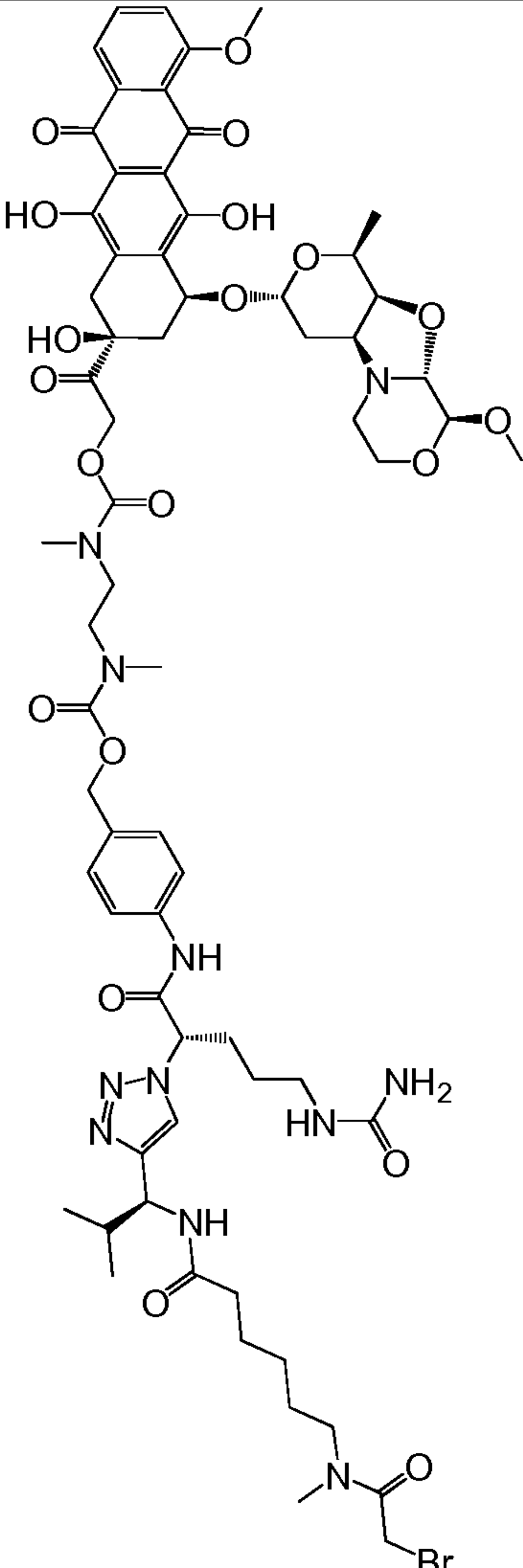
<p>PNU-LD2</p>	<p>CD33 PNU ADC2-2 and MUC16 PNU ADC2-4</p>		<p>[4-[[[(2S)-2-[[1-[5-(2,5-dioxopyrrol-1-yl)pentylcarbamoyl]cyclobutane carbonyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl N-[2-[[2-[(2S,4S)-4-[[[(1S,3R,4aS,9S,9aR,10aS)-9-methoxy-1-methyl-3,4,4a,6,7,9,9a,10a-octahydro-1H-pyrano[1,2]oxazolo[3,4-b][1,4]oxazin-3-yl]oxy]-2,5,12-trihydroxy-7-methoxy-6,11-dioxo-3,4-dihydro-1H-tetracen-2-yl]-2-oxoethoxy]carbonyl-methyl-amino]ethyl]-N-methyl-carbamate</p>
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<p>PNU-LD3</p>	<p>NaPi2b PNU ADC3-1 and CD33 PNU ADC3-2</p>		<p>[4-[[[(2S)-2-[4-[(1S)-1-[6-(2,5-dioxopyrrol-1-yl)hexanoylamino]-2-methylpropyl]triazol-1-yl]-5-ureidopentanoyl]amino]phenyl]methyl N-[2-[[2-[(2S,4S)-4-[[[(1S,3R,4aS,9S,9aR,10aS)-9-methoxy-1-methyl-3,4,4a,6,7,9,9a,10a-octahydro-1H-pyrano[1,2]oxazolo[3,4-b][1,4]oxazin-3-yl]oxy]-2,5,12-trihydroxy-7-methoxy-6,11-dioxo-3,4-dihydro-1H-tetracen-2-yl]-2-oxoethoxy]carbonyl-methylamino]ethyl]-N-methylcarbamate</p>
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<p>PNU-LD4</p>	<p>NaPi2b PNU ADC4-1, and CD33 PNU ADC4-2</p>		<p>[4-[[[(Z,2R,5S)-5-[6-(2,5-dioxopyrrol-1-yl)hexanoylamino]-4-fluoro-6-methyl-2-(3-ureidopropyl)hept-3-enoyl]amino]phenyl]methyl N-[2-[[2-[(2S,4S)-4-[[[(1S,3R,4aS,9S,9aR,10aS)-9-methoxy-1-methyl-3,4,4a,6,7,9,9a,10a-octahydro-1H-pyrano[1,2]oxazolo[3,4-b][1,4]oxazin-3-yl]oxy]-2,5,12-trihydroxy-7-methoxy-6,11-dioxo-3,4-dihydro-1H-tetracen-2-yl]-2-oxoethoxy]carbonyl-methyl-amino]ethyl]-N-methyl-carbamate</p>
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PNU-LD6			<p>[4-[[[(2S)-2-[4-[(1S)-1-[6-[(2-chloroacetyl)-methyl-amino]hexanoylamino]-2-methyl-propyl]triazol-1-yl]-5-ureido-pentanoyl]amino]phenyl]methyl N-[2-[[2-[(2S,4S)-4-[[[(1S,3R,4aS,9S,9aR,10aS)-9-methoxy-1-methyl-3,4,4a,6,7,9,9a,10a-octahydro-1H-pyrano[1,2]oxazolo[3,4-b][1,4]oxazin-3-yl]oxy]-2,5,12-trihydroxy-7-methoxy-6,11-dioxo-3,4-dihydro-1H-tetracen-2-yl]-2-oxo-ethoxy]carbonyl-methyl-amino]ethyl]-N-methyl-carbamate</p>
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PNU-LD7			<p>[4-[[[(2S)-2-[4-[(1S)-1-[6-[(2-bromoacetyl)-methyl-amino]hexanoylamino]-2-methyl-propyl]triazol-1-yl]-5-ureido-pentanoyl]amino]phenyl]methyl N-[2-[[2-[(2S,4S)-4-[[[(1S,3R,4aS,9S,9aR,10aS)-9-methoxy-1-methyl-3,4,4a,6,7,9,9a,10a-octahydro-1H-pyrano[1,2]oxazolo[3,4-b][1,4]oxazin-3-yl]oxy]-2,5,12-trihydroxy-7-methoxy-6,11-dioxo-3,4-dihydro-1H-tetracen-2-yl]-2-oxo-ethoxy]carbonyl-methyl-amino]ethyl]-N-methyl-carbamate</p>
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SEQUENCES

NaPi2b humanized antibody:

5

In one embodiment, the NaPi2b antibody of ADCs of the present invention comprises three light chain hypervariable regions and three heavy chain hypervariable regions (SEQ ID NO:1-6), the sequences of which are shown below.

10 In one embodiment, the NaPi2b antibody of ADCs of the present invention comprises the variable light chain sequence of SEQ ID NO: 7 and the variable heavy chain sequence of SEQ ID NO: 8

In one embodiment, the NaPi2b antibody of ADCs of the present invention comprises the light chain sequence of SEQ ID NO: 9 and the heavy chain sequence of SEQ ID NO: 10

10H1.11.4B HVR-L1	RSSETLVHSSGNTYLE	Seq ID No: 1
10H1.11.4B HVR-L2	RVSNRFS	Seq ID No: 2
10H1.11.4B HVR-L3	FQGSFNPLT	Seq ID No: 3
10H1.11.4B HVR-H1	GFSFSDFAMS	Seq ID No: 4
10H1.11.4B HVR-H2	ATIGRVAFHTYYPDSMKG	Seq ID No: 5
10H1.11.4B HVR-H3	ARHRGFDVGHFDF	Seq ID No: 6
10H1.11.4B V _L	DIQMTQSPSSLSASVGDRVTITCRSSETLVHSSGNTYLEWYQQK PGKAPKLLIYRVSNRFSGVPSRFSGSGSGTDFTLTISLQPEDFAT YYCFQGSFNPLTFGQGTKVEIKR	SEQ ID NO: 7
10H1.11.4B V _H	EVQLVESGGGLVQPGGSLRLSCAASGFSFSDFAMSWVRQAPGK GLEWVATIGRVAFHTYYPDSMKGRTISRDNSKNTLYLQMNSL RAEDTAVYYCARHRGFDVGHFDFWVGQGLVTVSS	SEQ ID NO: 8
10H1.11.4B Light Chain	DIQMTQSPSSLSASVGDRVTITCRSSETLVHSSGNTYLEWYQQK PGKAPKLLIYRVSNRFSGVPSRFSGSGSGTDFTLTISLQPEDFAT YYCFQGSFNPLTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTA SIVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDEST YSLSSITLTLTKADYEEKHKVYACEVTHQGLSSPVTKSFNRGEC	SEQ ID NO: 9
10H1.11.4B Heavy Chain	EVQLVESGGGLVQPGGSLRLSCAASGFSFSDFAMSWVRQAPGK GLEWVATIGRVAFHTYYPDSMKGRTISRDNSKNTLYLQMNSL RAEDTAVYYCARHRGFDVGHFDFWVGQGLVTVSSCSTKGPSVF PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKK VEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSIVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR EPQVYITLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCSVMHEA LHNHYTQKSLSLSPGK	SEQ ID NO: 10

Anti-CD33 humanized antibody:

In one embodiment, the anti-CD33 antibody of ADCs of the present invention comprises three light chain hypervariable regions and three heavy chain hypervariable regions, the sequences (SEQ ID NO:11-16) of which are shown below

5

In one embodiment, the anti-CD33 antibody of ADCs of the present invention comprises the variable light chain sequence of SEQ ID NO: 17 and the variable heavy chain sequence of SEQ ID NO: 18

15G15.33-HVR L1	RSSQSLLSHNGYNYLD	SEQ ID NO:11
15G15.33-HVR L2	LGVNSVS	SEQ ID NO:12
15G15.33-HVR L3	MQALQTPWT	SEQ ID NO:13
15G15.33-HVR H1	NHAIS	SEQ ID NO:14
15G15.33-HVR H2	GIPIFGTANYAQKFQG	SEQ ID NO:15
15G15.33-HVR H3	EWADVFDI	SEQ ID NO:16
15G15.33 V _L	EIVLTQSPLSLPVTGPGEPAISCRSSQSLLSHNGYNYLDWYLQK PGQSPQLLIYLGVNSVSGVPDRFSGSGSGTDFTLKISRVEADV GVYYCMQALQTPWTFGQGTKVEIK	SEQ ID NO:17
15G15.33 V _H	QVQLVQSGAEVKKPGSSVKVSCKASGGIFSNHAISWVRQAPG QGLEWMGGIPIFGTANYAQKFQGRVTITADESTSTAFMELSS LRSEDTAVYYCAREWADVFDIWGQGMVTVSS	SEQ ID NO:18

10

In one embodiment, the anti-CD33 antibody of ADCs of the present invention comprises the light chain sequence of SEQ ID NO: 19 and the heavy chain sequence of SEQ ID NO: 20

15 In one embodiment, the anti-CD33 antibody of ADCs of the present invention comprises three light chain hypervariable regions and three heavy chain hypervariable regions, the sequences (Seq ID NO: 19-24) of which are shown below.

20 In one embodiment, the anti-CD33 antibody of ADCs of the present invention comprises the variable light chain sequence of SEQ ID NO: 25 and the variable heavy chain sequence of SEQ ID NO: 26

In one embodiment, the anti-CD33 antibody of ADCs of the present invention comprises the variable light chain sequence of SEQ ID NO: 27 and the variable heavy chain sequence of SEQ ID NO: 28

In one embodiment, the anti-CD33 antibody of ADCs of the present invention comprises the variable light chain sequence of SEQ ID NO: 29 and the variable heavy chain sequence of SEQ ID NO: 30

In one embodiment, the anti-CD33 antibody of ADCs of the present invention comprises the variable light chain sequence of SEQ ID NO: 31 and the variable heavy chain sequence of SEQ ID NO: 32

9C3-HVR L1	RASQGIRNDLG	Seq ID NO:19
9C3-HVR L2	AASSLQS	Seq ID NO:20
9C3-HVR L3	LQHNSYPWT	Seq ID NO:21
9C3-HVR H1	GNYSMS	Seq ID NO:22
9C3-HVR H2	LIYSGDSTYYADSVKG	Seq ID NO:23
9C3-HVR H3	DGYYVSDMVV	Seq ID NO:24
9C3 V _L	DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQK PGKAPKRLIYAASSLQSGVPSRFSGSGSGTEFTLTISSLQP EDFATYYCLQHNSYPWTFGQGTKLEIK	Seq ID NO:25
9C3 V _H	EVQLVESGGALIQPGGSLRLSCVASGFTISGNYMSWVRQ APGKGLEWVSLIYSGDSTYYADSVKGRFNISRDISKNTVY LQMNSLRVEDTAVYYCVRDGYVSDMVVWGKGTTVT VSS	Seq ID NO:26
9C3.2 V _L	DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQK PGKAPKRLIYAASSLQSGVPSRFSGSGSGTEFTLTISSLQP EDFATYYCLQHNSYPWTFGQGTKLEIK	Seq ID NO:27
9C3.2 V _H	EVQLVESGGALIQPGGSLRLSCVASGFTISGNYMSWVRQ APGKGLEWVSLIYSGDSTYYADSVKGRFTISRDISKNTVY LQMNSLRVEDTAVYYCVRDGYVSDMVVWGKGTTVT VSS	Seq ID NO:28
9C3.3 V _L	DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQK PGKAPKRLIYAASSLQSGVPSRFSGSGSGTEFTLTISSLQP EDFATYYCLQHNSYPWTFGQGTKLEIK	Seq ID NO:29
9C3.3 V _H	EVQLVESGGALIQPGGSLRLSCVASGFTISGNYMSWVRQ APGKGLEWVSLIYSGDSTYYADSVKGRFSISRDISKNTVY LQMNSLRVEDTAVYYCVRDGYVSDMVVWGKGTTVT VSS	Seq ID NO:30
9C3.4 V _L	DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQK PGKAPKRLIYAASSLQSGVPSRFSGSGSGTEFTLTISSLQP EDFATYYCLQHNSYPWTFGQGTKLEIK	Seq ID NO:31
9C3.4 V _H	EVQLVESGGALIQPGGSLRLSCVASGFTISGNYMSWVRQ APGKGLEWVSLIYSGDSTYYADSVKGRFAISRDISKNTVY LQMNSLRVEDTAVYYCVRDGYVSDMVVWGKGTTVT VSS	Seq ID NO:32

Anti-CD22 humanized antibody:

In one embodiment, the anti-CD22 antibody of ADCs of the present invention comprises three light chain hypervariable regions and three heavy chain hypervariable regions (SEQ ID NO: 41-546), the sequences of which are shown below.

In one embodiment, the anti-CD22 antibody of ADCs of the present invention comprises the variable light chain sequence of SEQ ID NO: 47 and the variable heavy chain sequence of SEQ ID NO: 48

10

In one embodiment, the anti-CD22 antibody of ADCs of the present invention comprises the light chain sequence of SEQ ID NO: 49 and the heavy chain sequence of SEQ ID NO: 50

h10F4.V3.K149C HVR-L1	RSSQSIVHSVGNTFLE	Seq ID No: 41
h10F4.V3.K149C HVR-L2	KVSNRFS	Seq ID No: 42
h10F4.V3.K149C HVR-L3	FQGSQFPYT	Seq ID No: 43
h10F4.V3.K149C HVR-H1	GYEFSRSWMN	Seq ID No: 44
h10F4.V3.K149C HVR-H2	RIYPGDGD TNYS GKFKG	Seq ID No: 45
h10F4.V3.K149C HVR-H3	DGSSWDWYFDV	Seq ID No: 46
h10F4.V3.K149C V _L	DIQMTQSPSSLSASVGDRVTITCRSSQSIVHSVGNTFLEWYQQKPGKAPKLLIYKVS NRFS GVP SRFS GSGS GTDFTLTIS SLQPEDFATY YCFQGSQFPYTFGQGTKVEIKR	SEQ ID NO: 47
h10F4.V3.K149C	EVQLVESGGGLVQP GGS LRLS CAASGYEFSRSWMNWVRQAPG KGLEWVGR IY PGD GD TNYS GKFKGRFTISADTSKNTAYLQMNS	SEQ ID NO: 48

V _H	LRAEDTAVYYCARDGSSWDWYFDVWGQGTLVTVSS	
h10F4.V3.K149C Light Chain	DIQMTQSPSSLSASVGDRVTITCRSSQSIVHSVGNTFLEWYQQKP GKAPKLLIYKVSNRFSGVPSRFSGSGSGTDFTLTISSLQPEDFATY YCFQGSQFPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQWCVDNALQSGNSQESVTEQDSKDSTY SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	SEQ ID NO: 49
h10F4.V3.K149C Heavy Chain	EVQLVESGGGLVQPGGSLRLSCAASGYEFSRSWMNWVRQAPG KGLEWVGRIPGDGDTNYSKGKFKGRFTISADTSKNTAYLQMNS LRAEDTAVYYCARDGSSWDWYFDVWGQGTLVTVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVD KKVEPKSCDKTHTCPPCPAPELGGPSVFLFPPKPKDTLMISRTP EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGK	SEQ ID NO: 50

ADC in vitro Data

The following ADCs were tested in *in vitro* assays described above and were found to be active. The activities of said ADCs are illustrated in the table below.

5

Code	Antibody ID	EOL-1 IC ₅₀ (ng/mL)
CD33 PNU ADC2-2	15G15.33	6.9
MUC16 PNU ADC2-4		337
NaPi2b PNU ADC3-1	10H1.11.4B	24.2
CD33 PNU ADC3-2	15G15.33	2.0
NaPi2b PNU ADC4-1	10H1.11.4B	218
CD33 PNU ADC4-2	15G15.33	0.6

ADC in vivo data

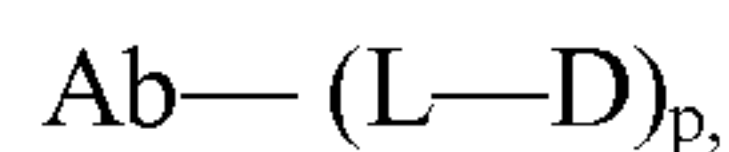
The following ADCs were tested in *in vivo* assays described above and were found to be active. The activities of said ADCs are illustrated in Figures 1-2 and the description below.

5 Figure 1 shows efficacy comparison of CD33 ADCs in SCID mice with HL-60 human acute myeloid leukemia tumors. CD33 PNU ADC3-2 showed dose-dependent inhibition of tumor growth compared with vehicle group. 5 ug/m² drug dose of ADC3-2 resulted in similar tumor growth delay as ADC2-2 at 15 ug/m² drug dose. Tumor remission was achieved when CD33 PNU ADC3-2 was given at 15 ug/m² drug dose. The non-targeting control NaPi2b PNU ADC3-1 had
10 minimal effect on the tumor growth.

Figure 2 shows efficacy comparison of CD33 ADCs in SCID mice with HL-60 human acute myeloid leukemia tumors. CD33 PNU ADC4-2 showed dose-dependent inhibition of tumor growth compared with vehicle group. The anti-tumor activity of CD33 PNU ADC4-2 was
15 comparable with CD33 PNU ADC2-2, resulting in tumor growth delay at drug dose of 10ug/m² (= 0.4 mg/kg of antibody dose). Tumor regression was achieved when CD33 PNU ADC4-2 was given at drug dose of 20ug/m². The non-targeting control NaPi2b PNU ADC4-1 had no effect on tumor growth.

Claims

1. An antibody-drug conjugate represented by Formula (I)



Ab is an antibody;

L is a peptidomimetic linker represented by the following formula

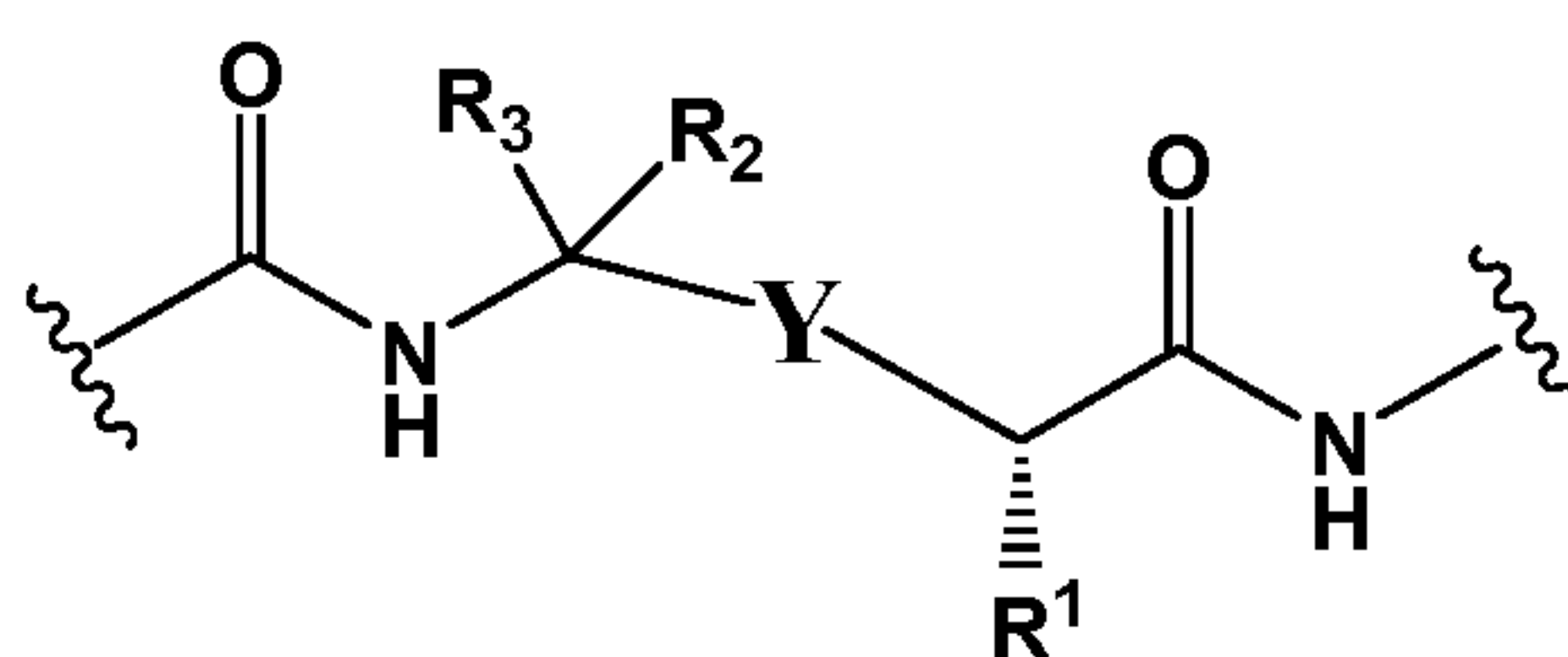
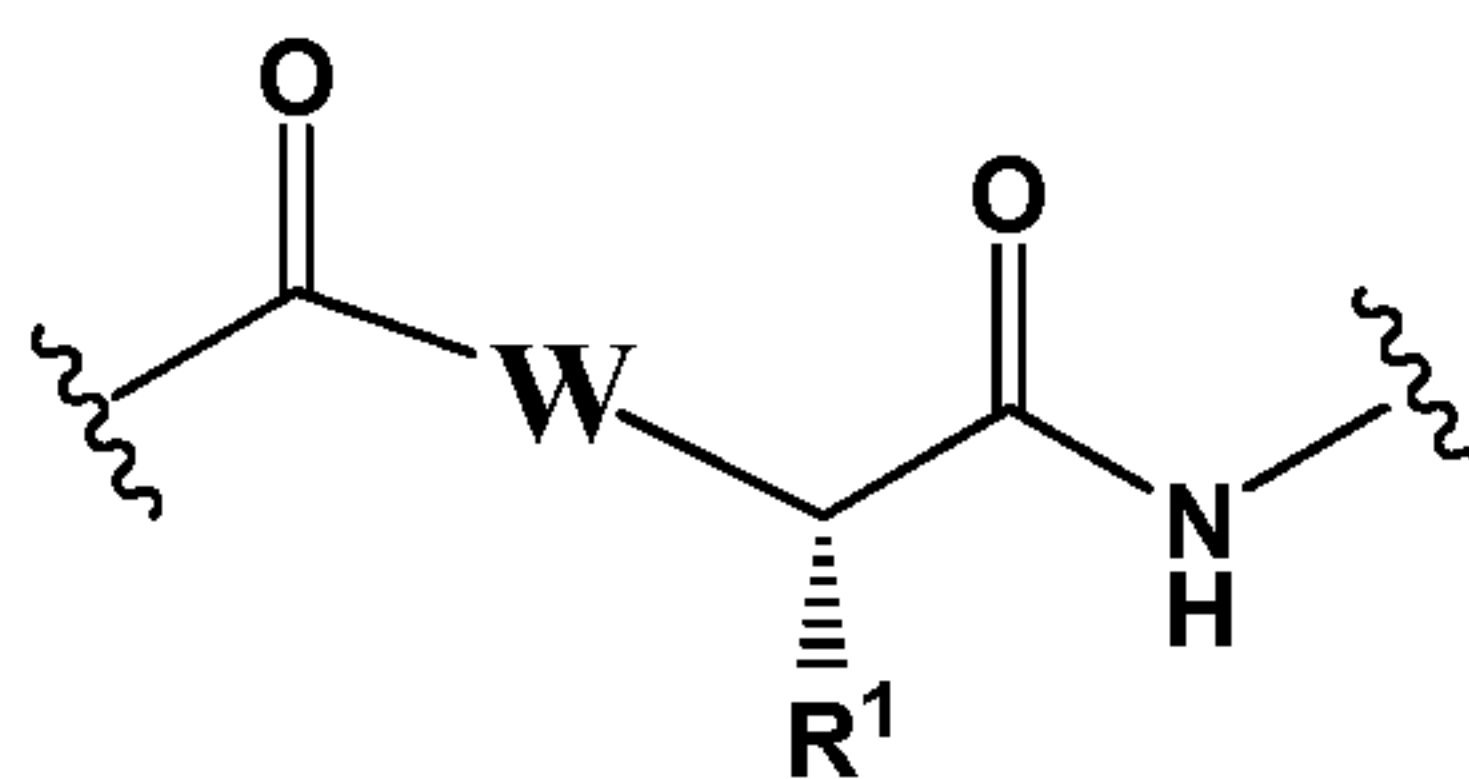


wherein

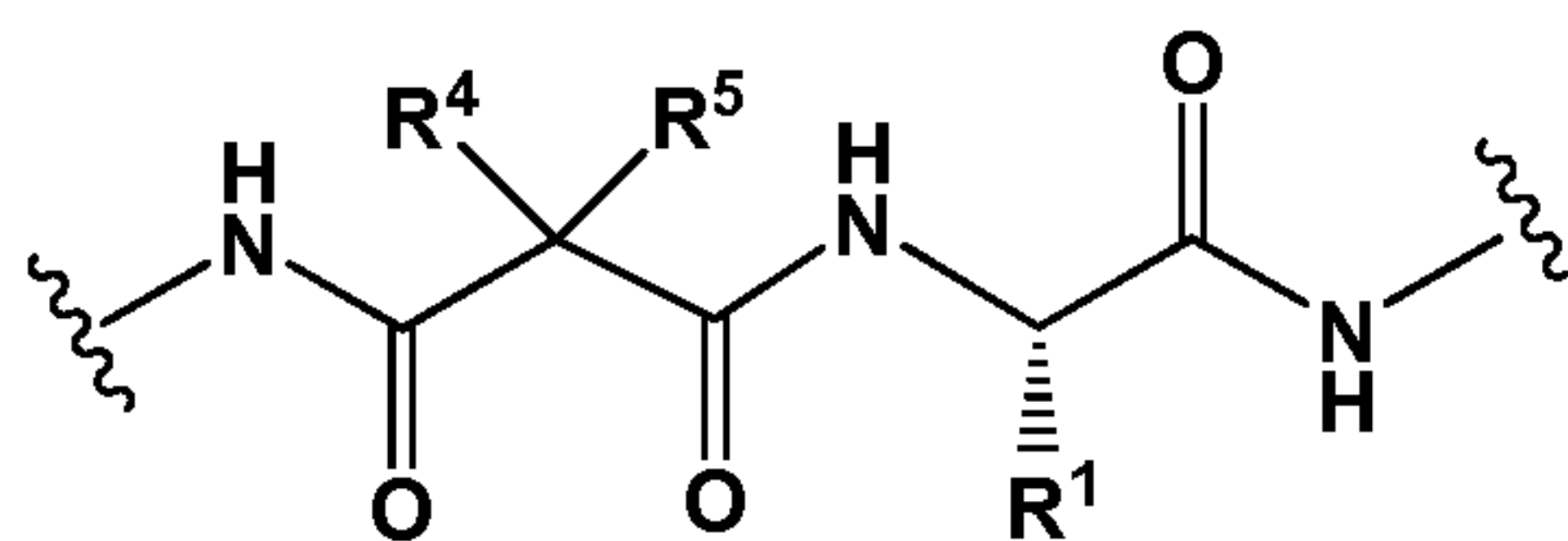
Str is a stretcher unit covalently attached to Ab;

Sp is a bond or spacer unit covalently attached to a drug moiety;

PM is a non-peptide chemical moiety selected from the group consisting of:



and



W is -NH-heterocycloalkyl- or heterocycloalkyl;

Y is heteroaryl, aryl, -C(O)C₁-C₆alkylene, C₁-C₆alkenyl, C₁-C₆alkylene or -C₁-C₆alkylene-NH-;

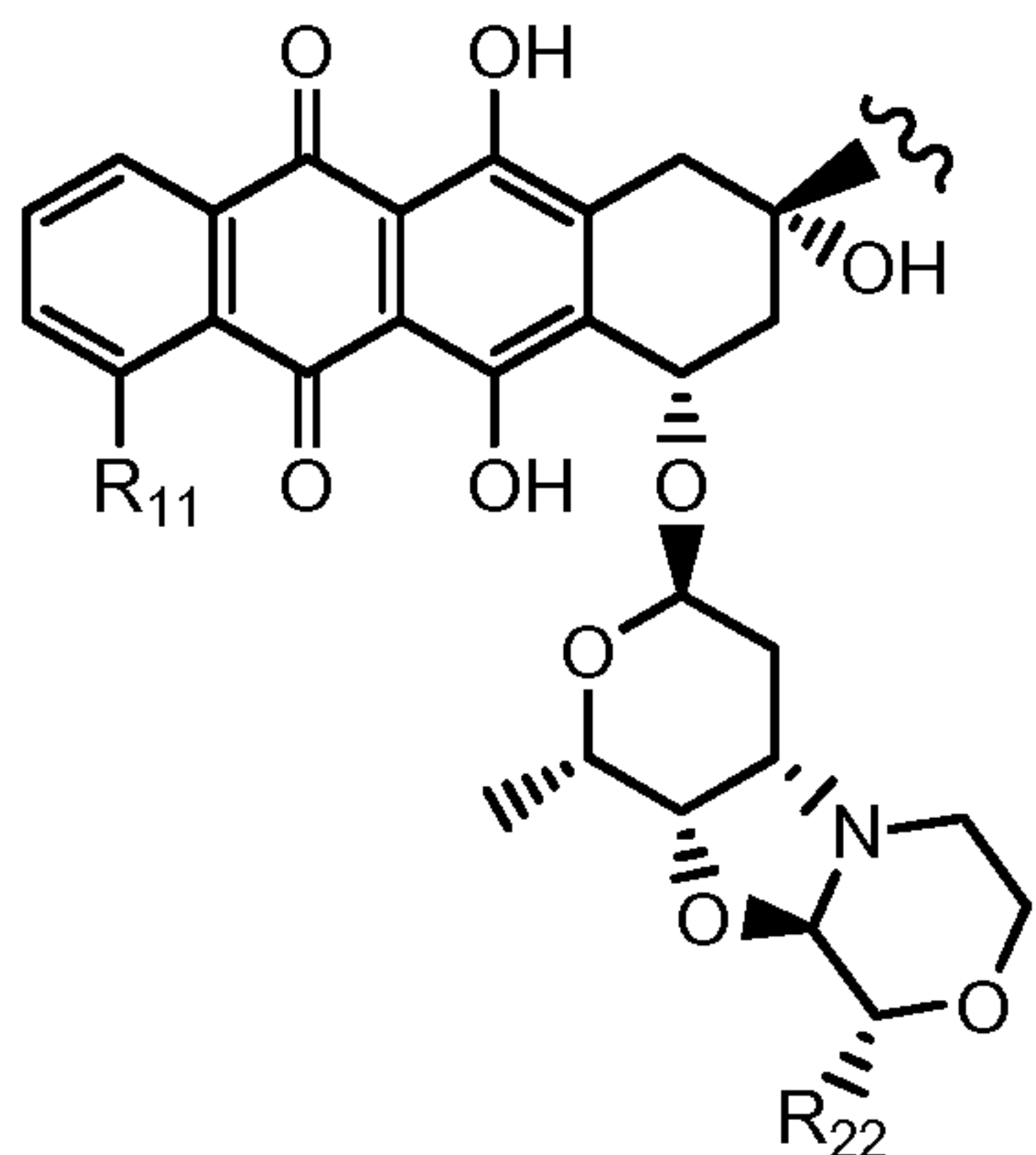
each R¹ is independently C₁-C₁₀alkyl, C₁-C₁₀alkenyl, (C₁-C₁₀alkyl)NHC(NH)NH₂ or (C₁-C₁₀alkyl)NHC(O)NH₂;

R³ and R² are each independently H, C₁-C₁₀alkyl, C₁-C₁₀alkenyl, arylalkyl or heteroarylalkyl, or R³ and R² together may form a C₃-C₇cycloalkyl;

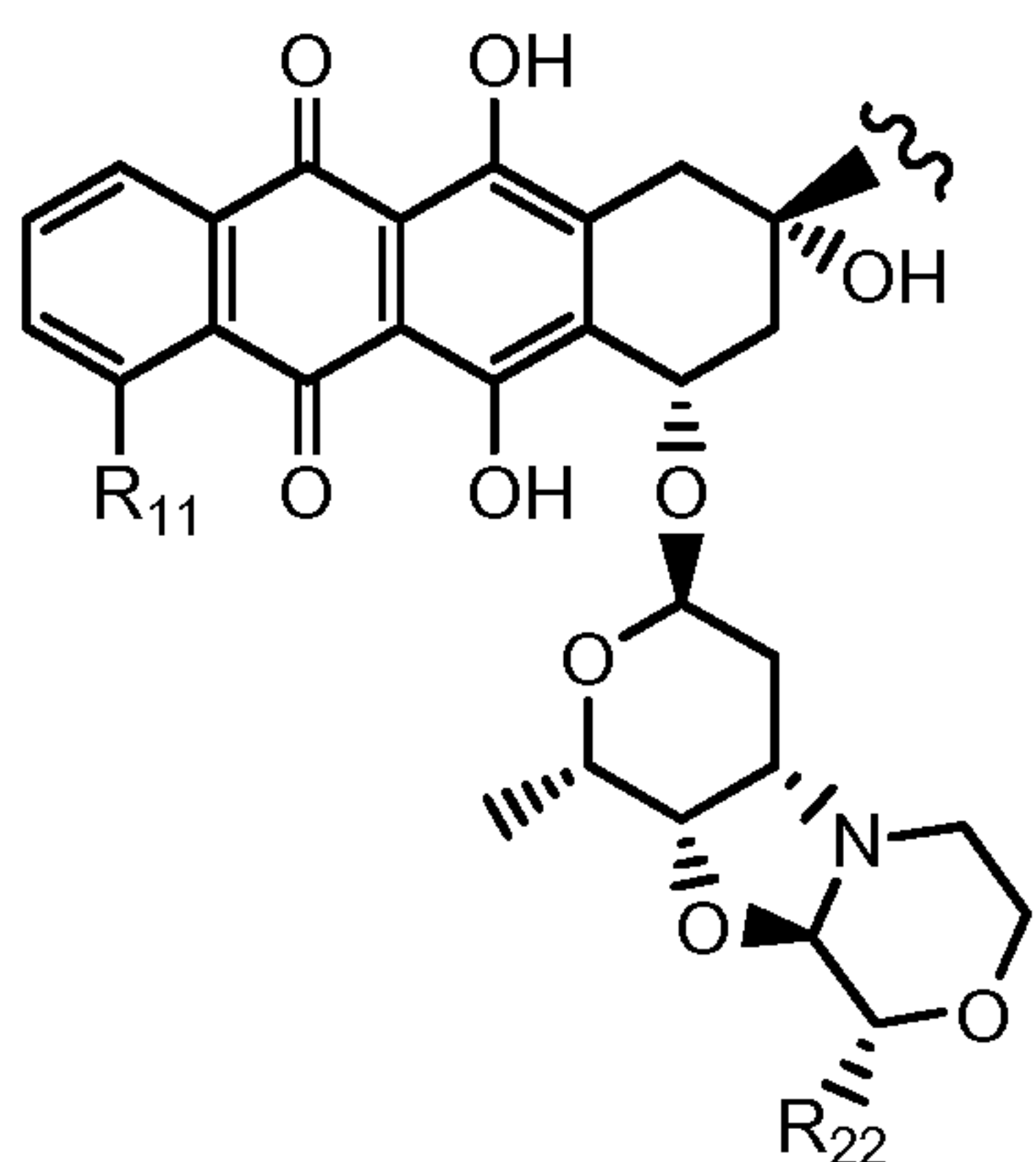
R⁴ and R⁵ are each independently C₁-C₁₀alkyl, C₁-C₁₀alkenyl, arylalkyl, heteroarylalkyl, (C₁-C₁₀alkyl)OCH₂-, or R⁴ and R⁵ together may form a C₃-C₇cycloalkyl ring;

p is an integer from 1 to 8;

D is a drug moiety of Formula (Ia) or (Ib) represented by the following structures:



(Ia)

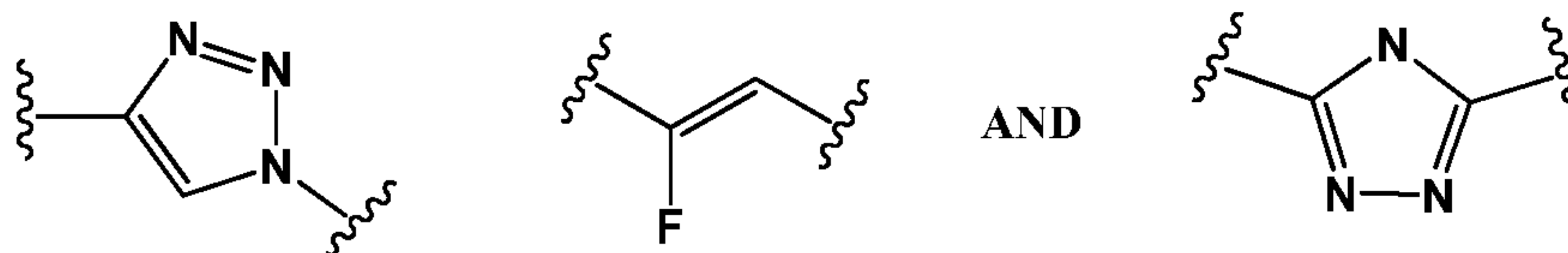


(Ib)

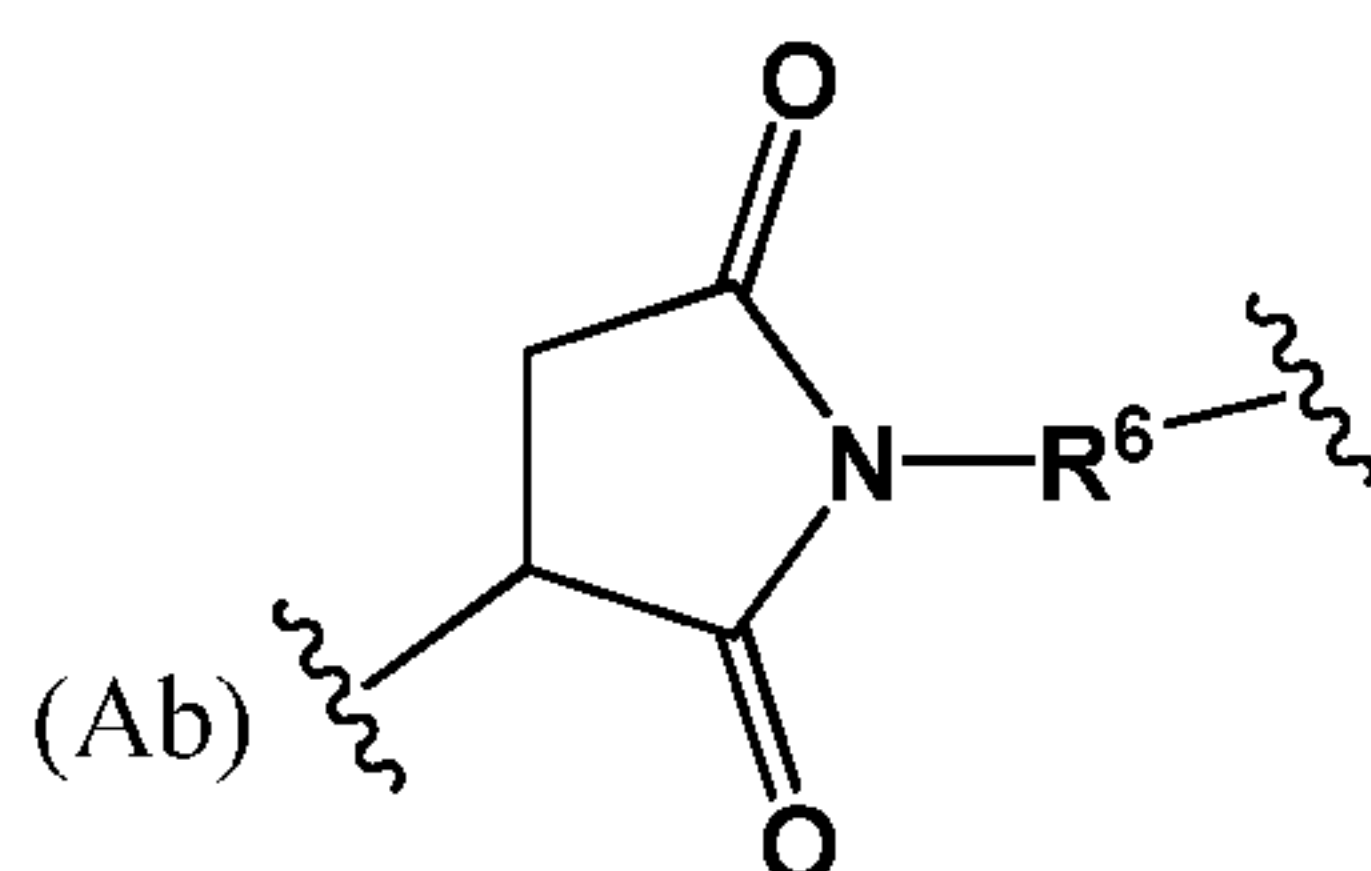
wherein R^{11} is hydrogen atom, hydroxy or methoxy group and R^{22} is a C_1 - C_5 alkoxy group, or a pharmaceutically acceptable salt thereof.

2. The antibody-drug conjugate of claim 1 wherein Y is heteroaryl; R^4 and R^5 together form a cyclobutyl ring.

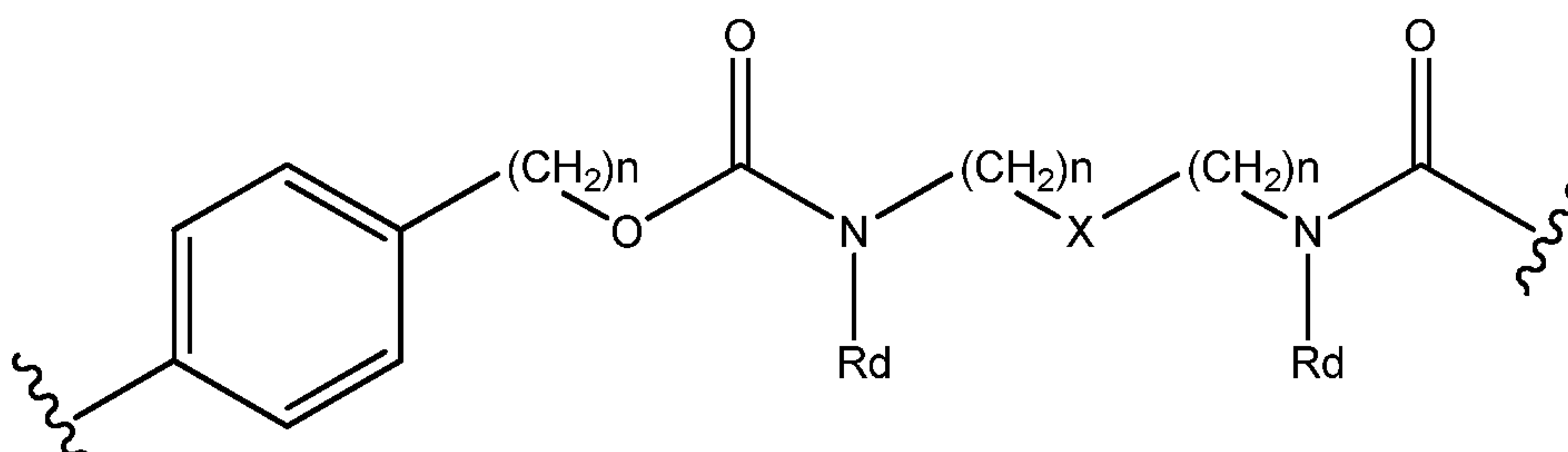
3. The antibody-drug conjugate of claim 1, wherein Y is a moiety selected from the group consisting of



4. An antibody-drug conjugate of claim 1, wherein Str is a chemical moiety represented by the following formula:

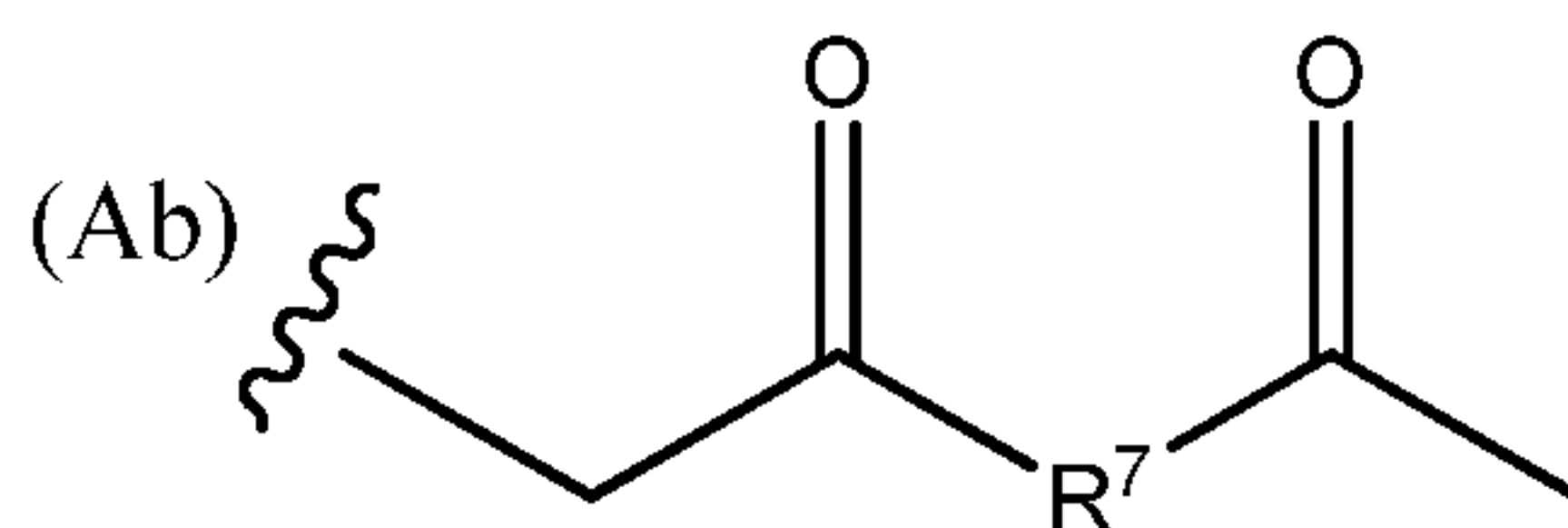


wherein R^6 is selected from the group consisting of C_1 - C_{10} alkylene, C_1 - C_{10} alkenyl, C_3 - C_8 cycloalkyl, $(C_1$ - C_8 alkylene)O-, and C_1 - C_{10} alkylene- $C(O)N(R^a)$ - C_2 - C_6 alkylene, where each alkylene may be substituted by one to five substituents selected from the group consisting of halo, trifluoromethyl, difluoromethyl, amino, alkylamino, cyano, sulfonyl, sulfonamide, sulfoxide, hydroxy, alkoxy, ester, carboxylic acid, alkylthio, aryl, arylalkyl, C_3 - C_8 cycloalkyl, C_4 - C_7 heterocycloalkyl, heteroarylalkyl and heteroaryl each R^a is independently H or C_1 - C_6 alkyl; Sp is $—Ar—R^b—$, wherein Ar is aryl or heteroaryl, R^b is $(C_1$ - C_{10} alkylene)O- or Sp is the following formula

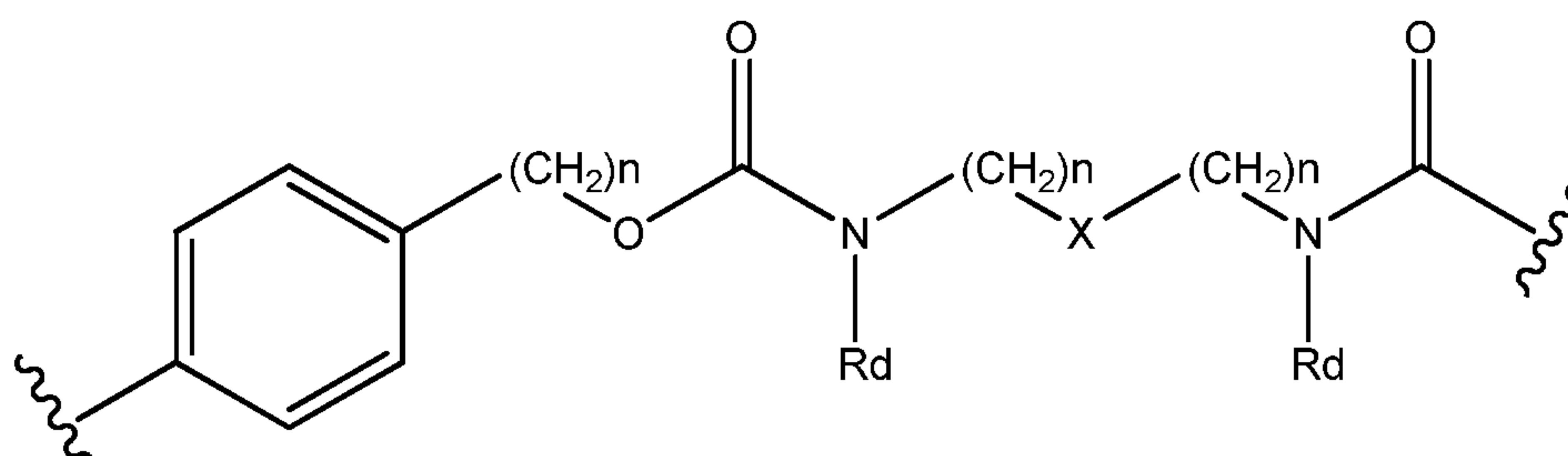


wherein
each n is independently 1-6;
X is N, CH_2 or a bond; and
each R^d is independently H or C_1 - C_3 alkyl.

5. The antibody-drug conjugate compound of claim 1, wherein Str has the formula:



wherein R^7 is selected from C_1 - C_{10} alkylene, C_1 - C_{10} alkenyl, $(C_1$ - C_{10} alkylene)O-, $N(R^c)$ - $(C_2$ - C_6 alkylene)- $N(R^c)$ and $N(R^c)$ - $(C_2$ - C_6 alkylene); where each R^c is independently H or C_1 - C_6 alkyl; Sp is $—Ar—R^b—$, wherein Ar is aryl or heteroaryl, R^b is $(C_1$ - C_{10} alkylene)O- or Sp is the following formula



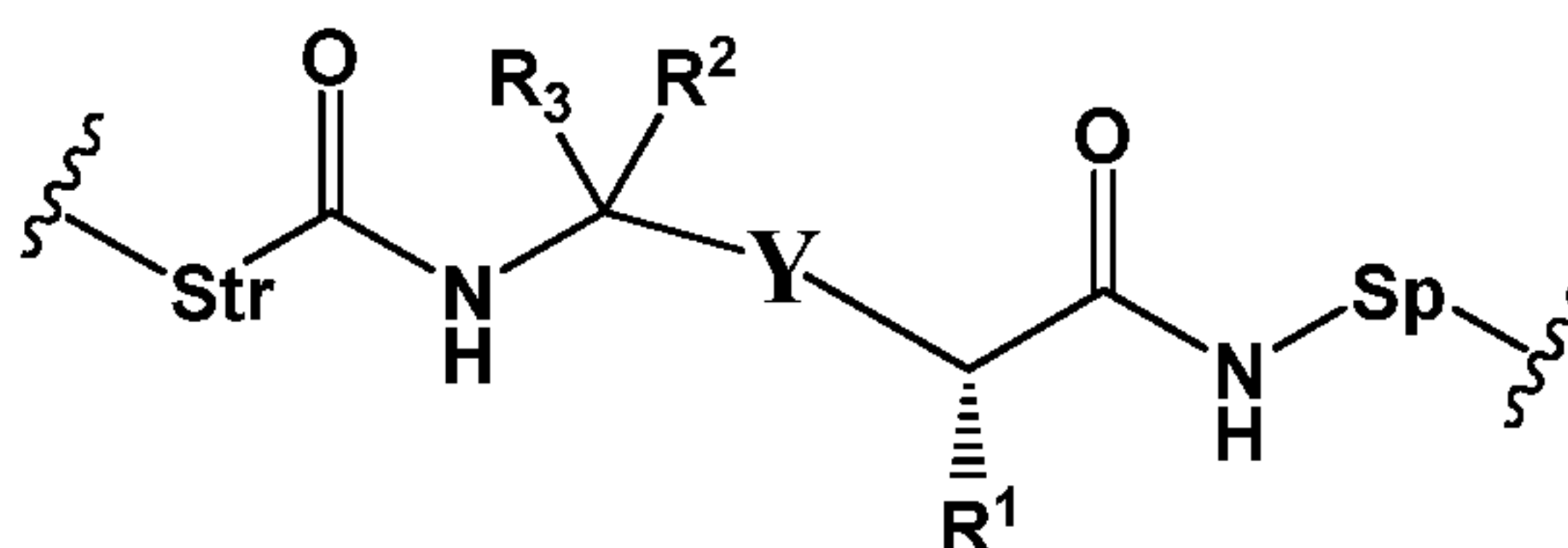
wherein

each n is independently 1-6;

X is N, CH_2 or a bond; and

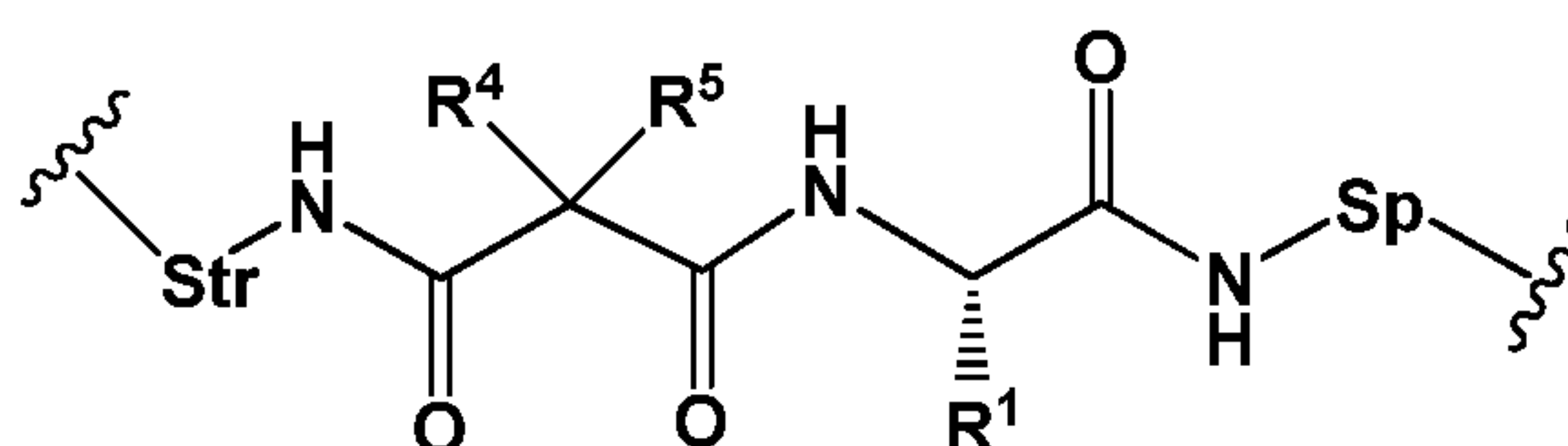
each R^d is independently H or C_1 - C_3 alkyl.

6. The antibody-drug conjugate compound of claim 4, wherein L is non-peptide chemical moiety represented by the following formula



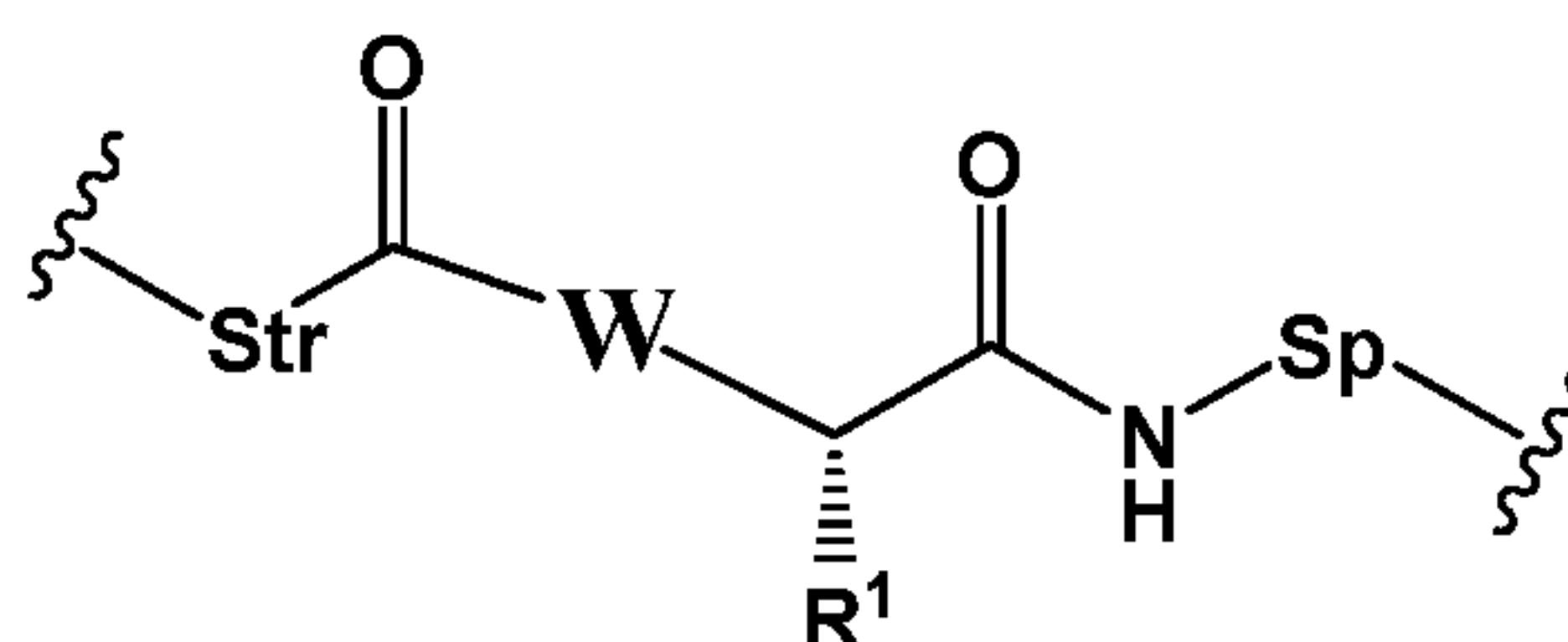
R^1 is C_1 - C_6 alkyl, C_1 - C_6 alkenyl, $(\text{C}_1$ - C_6 alkyl) $\text{NHC}(\text{NH})\text{NH}_2$ or $(\text{C}_1$ - C_6 alkyl) $\text{NHC}(\text{O})\text{NH}_2$;
 R^3 and R^2 are each independently H, C_1 - C_{10} alkyl.

7. The antibody-drug conjugate compound of claim 4, wherein L is non-peptide chemical moiety represented by the following formula



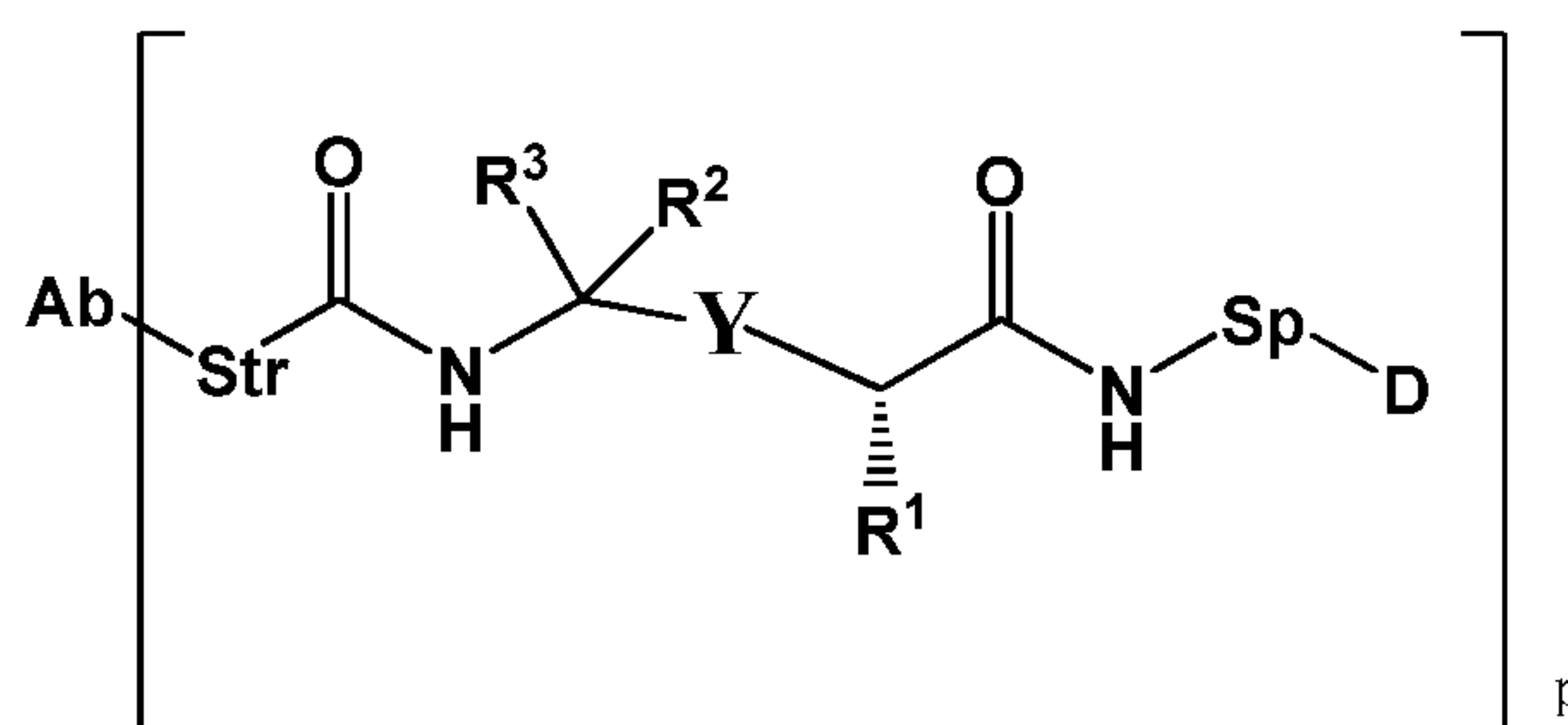
R^1 is C_1 - C_6 alkyl, $(\text{C}_1$ - C_6 alkyl) $\text{NHC}(\text{NH})\text{NH}_2$ or $(\text{C}_1$ - C_6 alkyl) $\text{NHC}(\text{O})\text{NH}_2$;
 R^4 and R^5 together form a C_3 - C_7 cycloalkyl ring.

8. The antibody-drug conjugate compound of claim 4, wherein L is non-peptide chemical moiety represented by the following formula



R^1 is C_1 - C_6 alkyl, $(C_1$ - C_6 alkyl) $NHC(NH)NH_2$ or $(C_1$ - C_6 alkyl) $NHC(O)NH_2$.

9. The antibody-drug conjugate compound of claim 4 represented by the following formula:



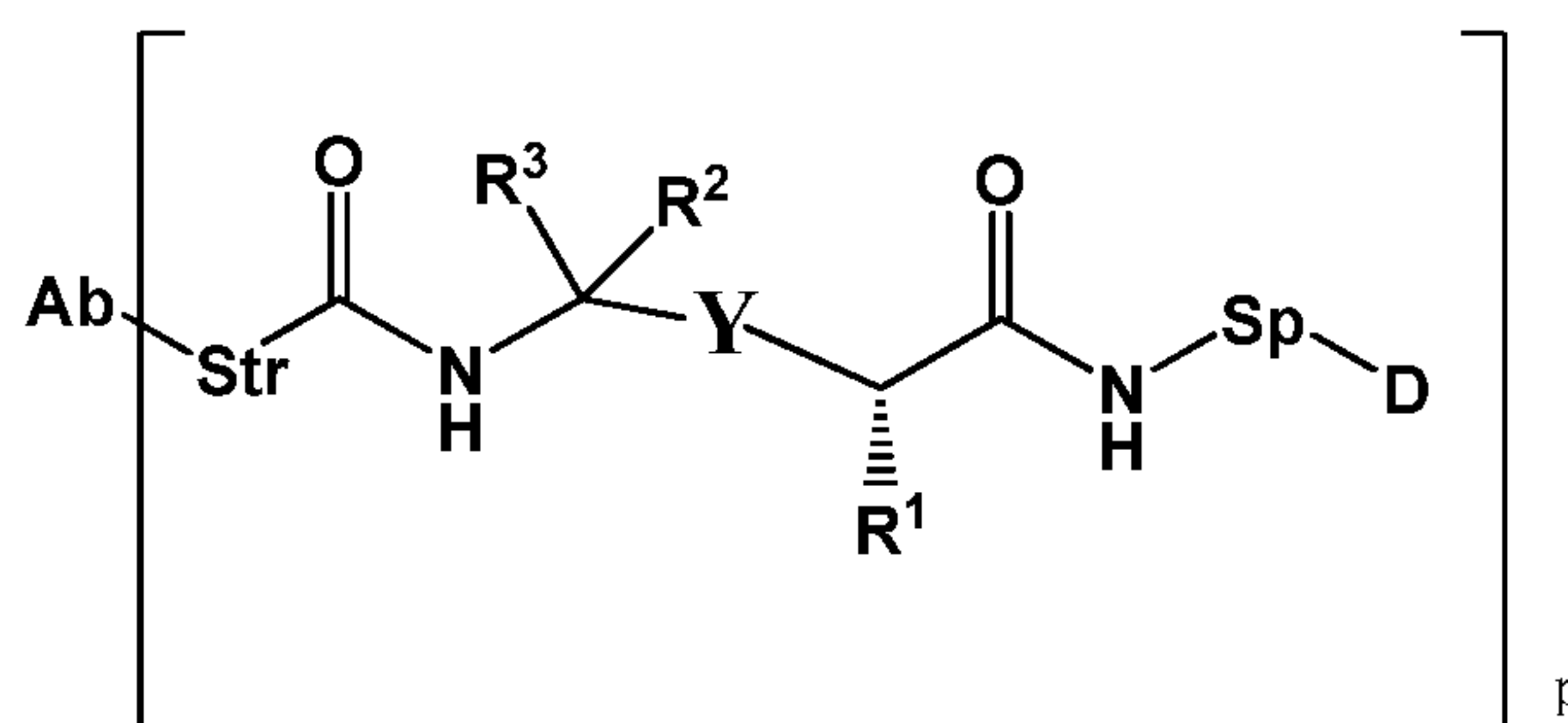
(I)(A1)

wherein

R^6 is selected from the group consisting of C_1 - C_{10} alkylene, and C_1 - C_{10} alkylene- $C(O)N(R^a)$ - C_2 - C_6 alkylene, where each alkylene may be substituted by one to five substituents selected from the group consisting of halo, trifluoromethyl, difluoromethyl, amino, alkylamino, cyano, sulfonyl, sulfonamide, sulfoxide, hydroxy, alkoxy, ester, carboxylic acid, alkylthio, aryl, arylalkyl, C_3 - C_8 cycloalkyl, C_4 - C_7 heterocycloalkyl, heteroarylalkyl and heteroaryl each R^a is independently H or C_1 - C_6 alkyl;

p is 1, 2, 3 or 4.

10. The antibody-drug conjugate compound of claim 7 represented by the following formula:



(I)(A1)

wherein

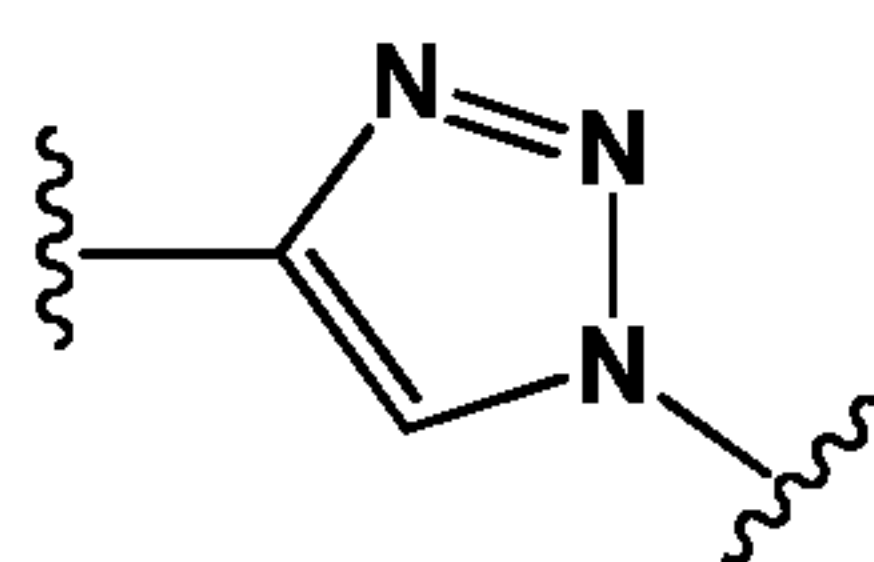
R^6 is selected from the group consisting of C_1 - C_{10} alkylene, and C_1 - C_{10} alkylene- $C(O)N(R^a)$ - C_2 - C_6 alkylene, where each alkylene may be substituted by one to five substituents selected from the

group consisting of halo, trifluoromethyl, difluoromethyl, amino, alkylamino, cyano, sulfonyl, sulfonamide, sulfoxide, hydroxy, alkoxy, ester, carboxylic acid, alkylthio, aryl, arylalkyl, C₃-C₈cycloalkyl, C₄-C₇heterocycloalkyl, heteroarylalkyl and heteroaryl each R^a is independently H or C₁-C₆alkyl;

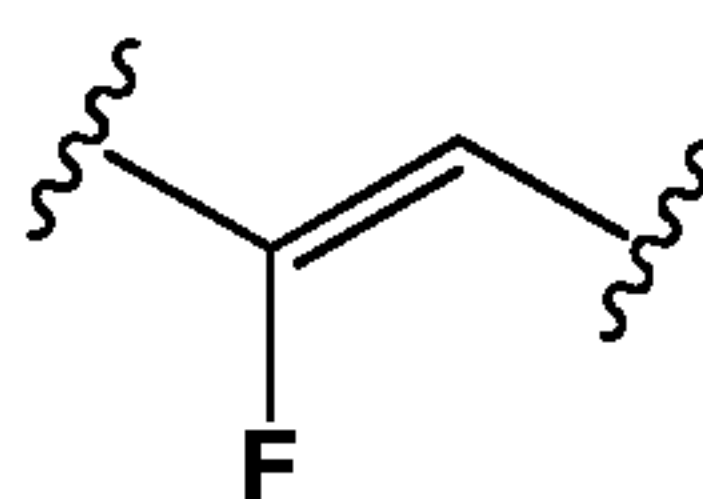
p is 1, 2, 3 or 4.

11. The antibody-drug conjugate compound of any one of claims 4, wherein Y is heteroaryl, aryl or alkenyl; R⁶ is C₁-C₁₀alkylene.

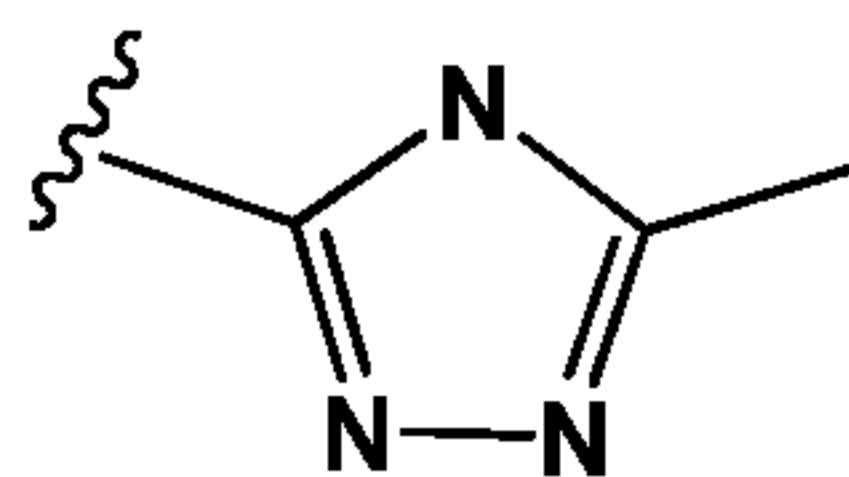
12. The antibody-drug conjugate compound of claim 11, wherein Y is



13. The antibody-drug conjugate compound of claim 11, wherein Y is

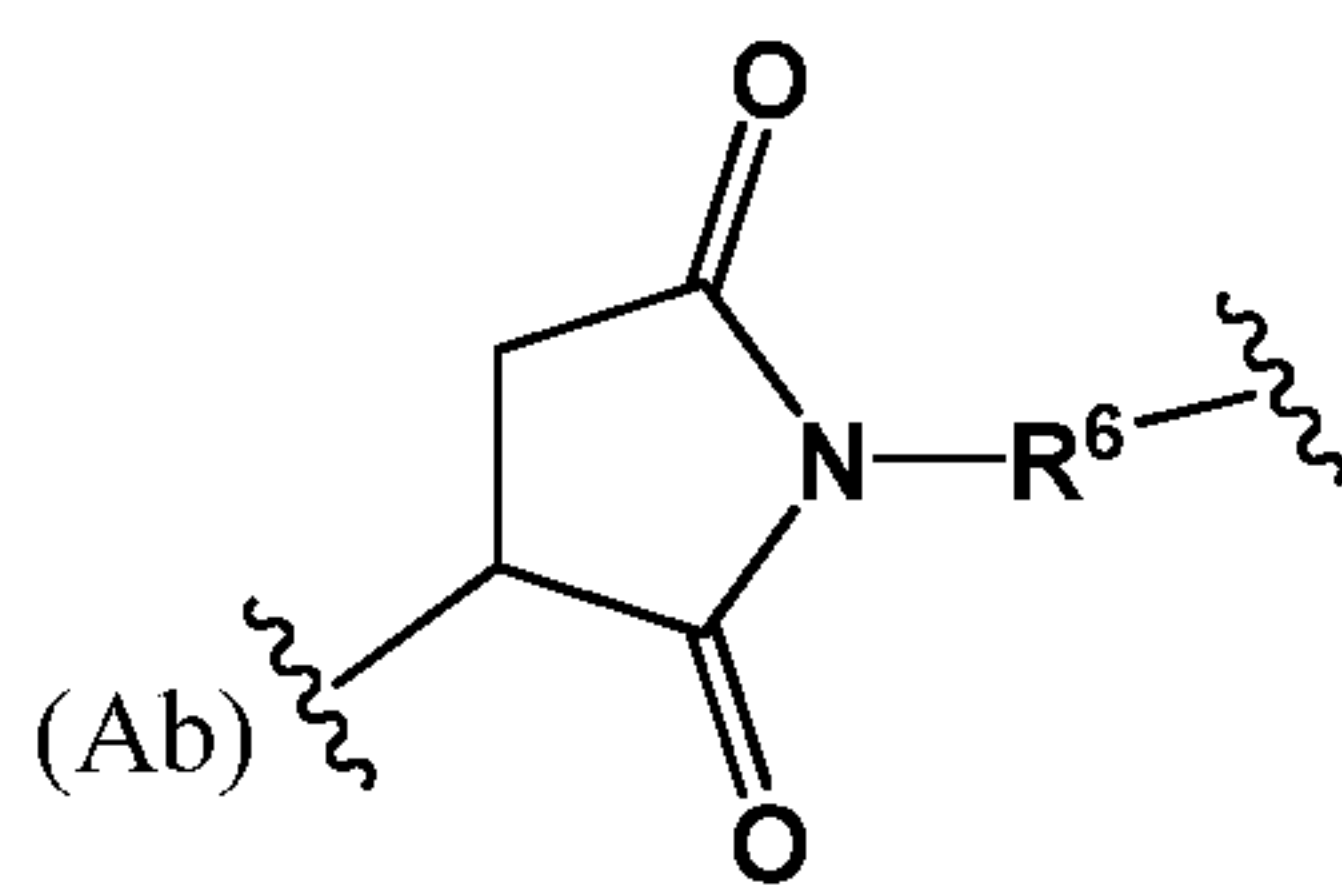


14. The antibody-drug conjugate compound of claim 11, wherein Y is



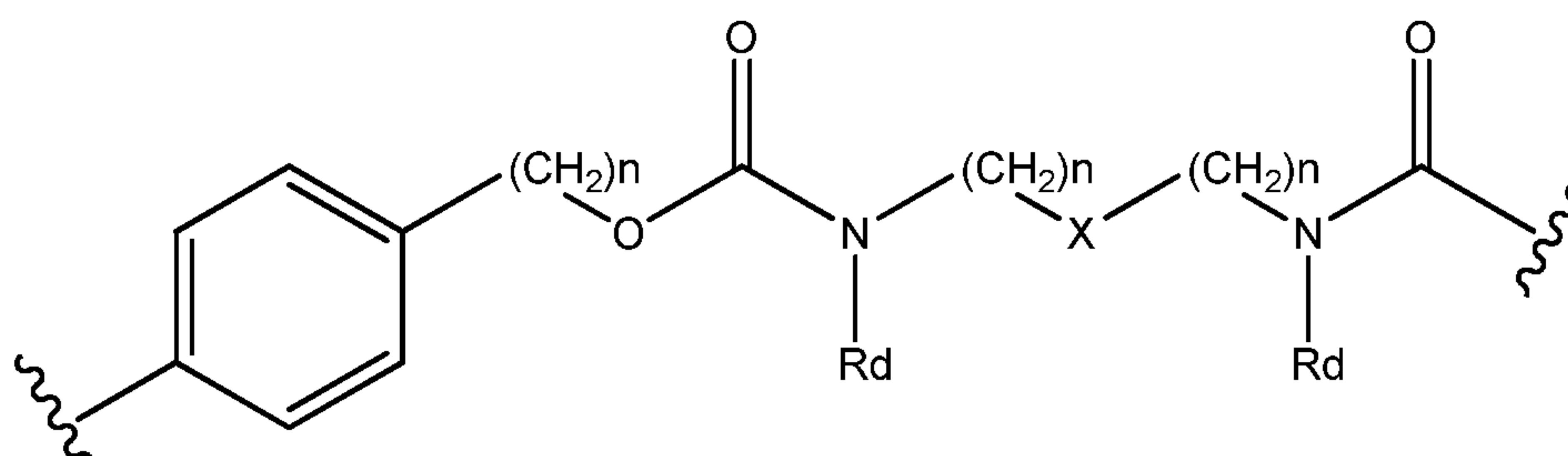
15. A compound of any one of claims 9, wherein

Str is a chemical moiety represented by the following formula:



R⁶ is C₁-C₆alkylene;

Sp is the following formula



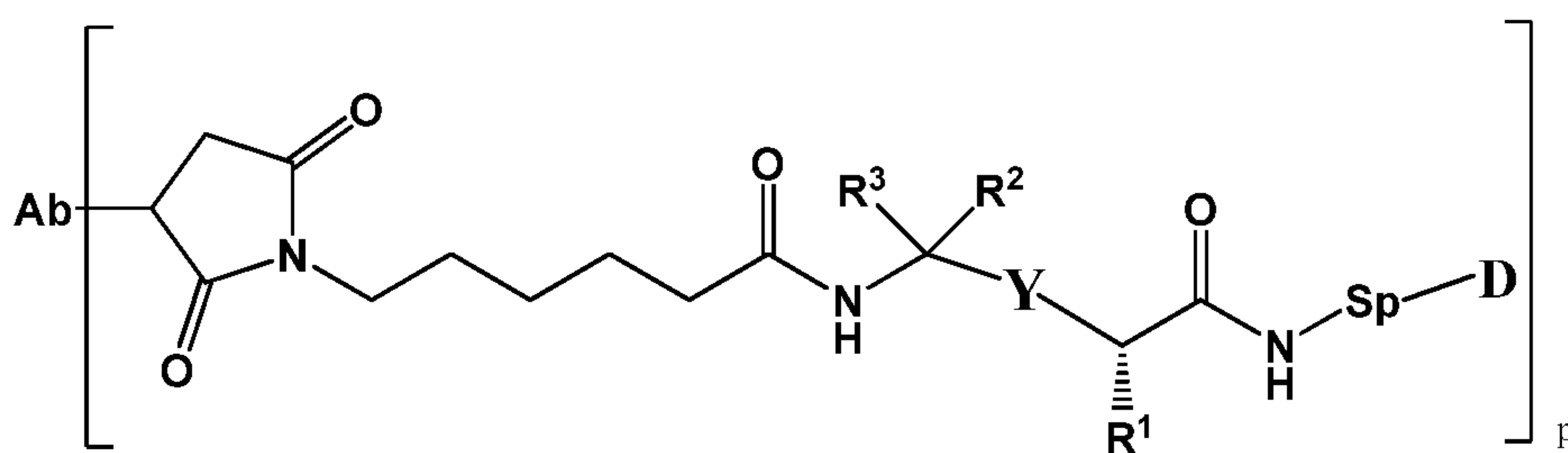
wherein

each n is independently 1-6;

X is N, CH_2 or a bond; and

each R^d is independently H or C_1 - C_3 alkyl.

16. The antibody-drug conjugate compound of claim 9, which is represented by the following formula:



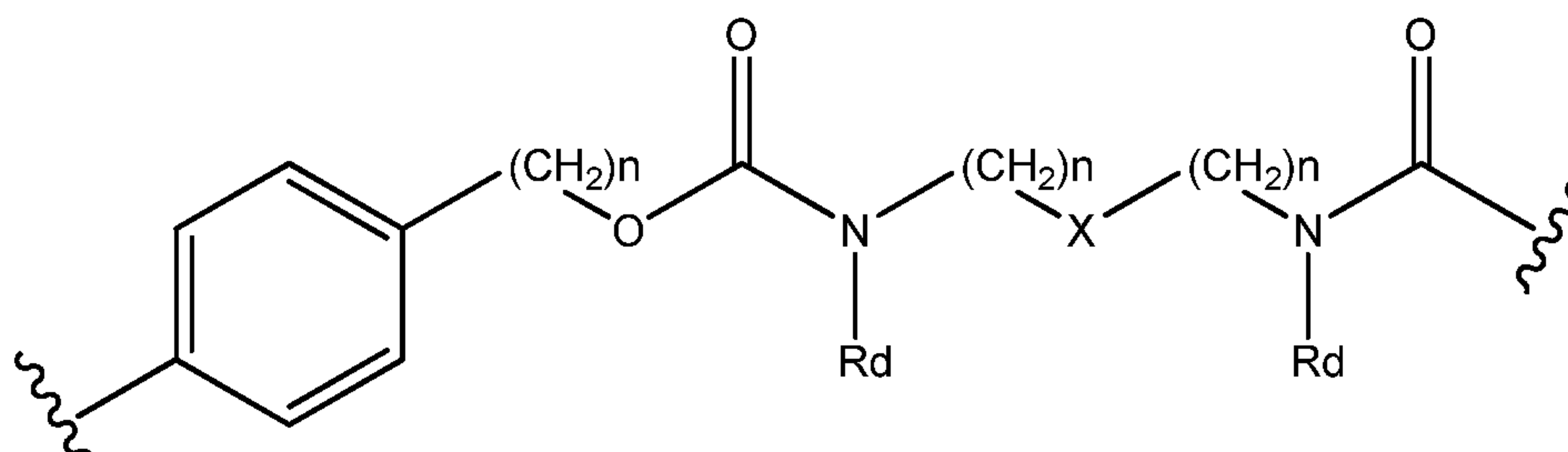
(I)(A2)

wherein

R^1 is C_1 - C_6 alkyl- NH_2 , $(\text{C}_1$ - C_6 alkyl) $\text{NHC}(\text{NH})\text{NH}_2$ or $(\text{C}_1$ - C_6 alkyl) $\text{NHC}(\text{O})\text{NH}_2$;

p is 1, 2, 3 or 4;

Sp is the following formula



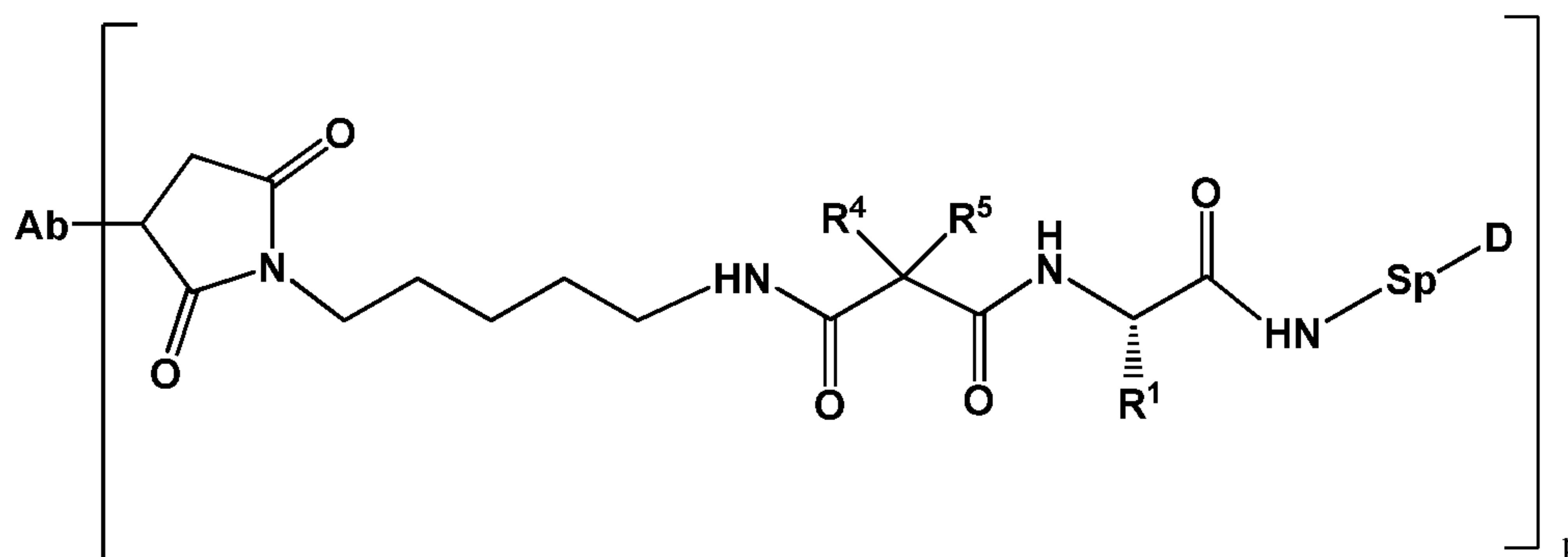
wherein

each n is independently 1-6;

X is N, CH_2 or a bond; and

each R^d is independently H or C_1 - C_3 alkyl.

17. The antibody-drug conjugate compound of claim 7, which is represented by the following formula:



(I)(B2)

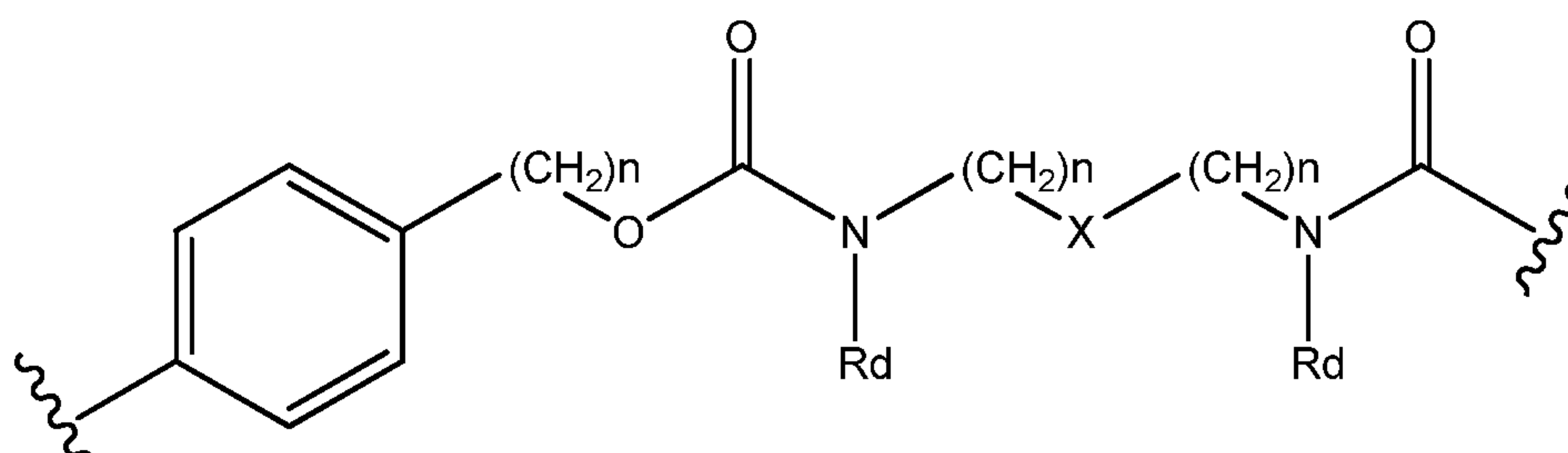
wherein

p is 1, 2, 3 or 4;

R^1 is C_1 - C_6 alkyl- NH_2 , $(C_1$ - C_6 alkyl) $NHC(NH)NH_2$ or $(C_1$ - C_6 alkyl) $NHC(O)NH_2$;

R^4 and R^5 are each independently C_1 - C_6 alkyl, wherein said alkyl are unsubstituted, or R^4 and R^5 may form a C_3 - C_7 cycloalkyl ring; and

Sp is the following formula



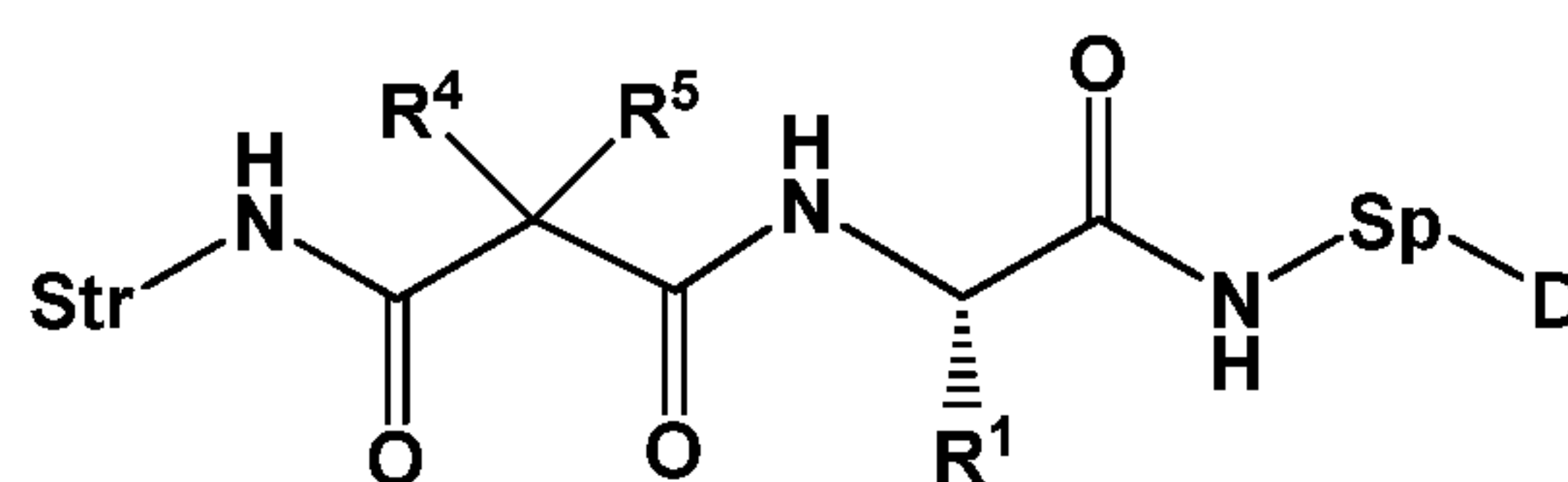
wherein

each n is independently 1-6;

X is N, CH_2 or a bond; and

each R^d is independently H or C_1 - C_3 alkyl.

18. A non-peptide compound of Formula (I)(B)(LD1):



(I)(D)(LD1)

wherein

Str is a stretcher unit which can be covalently attached to an antibody;

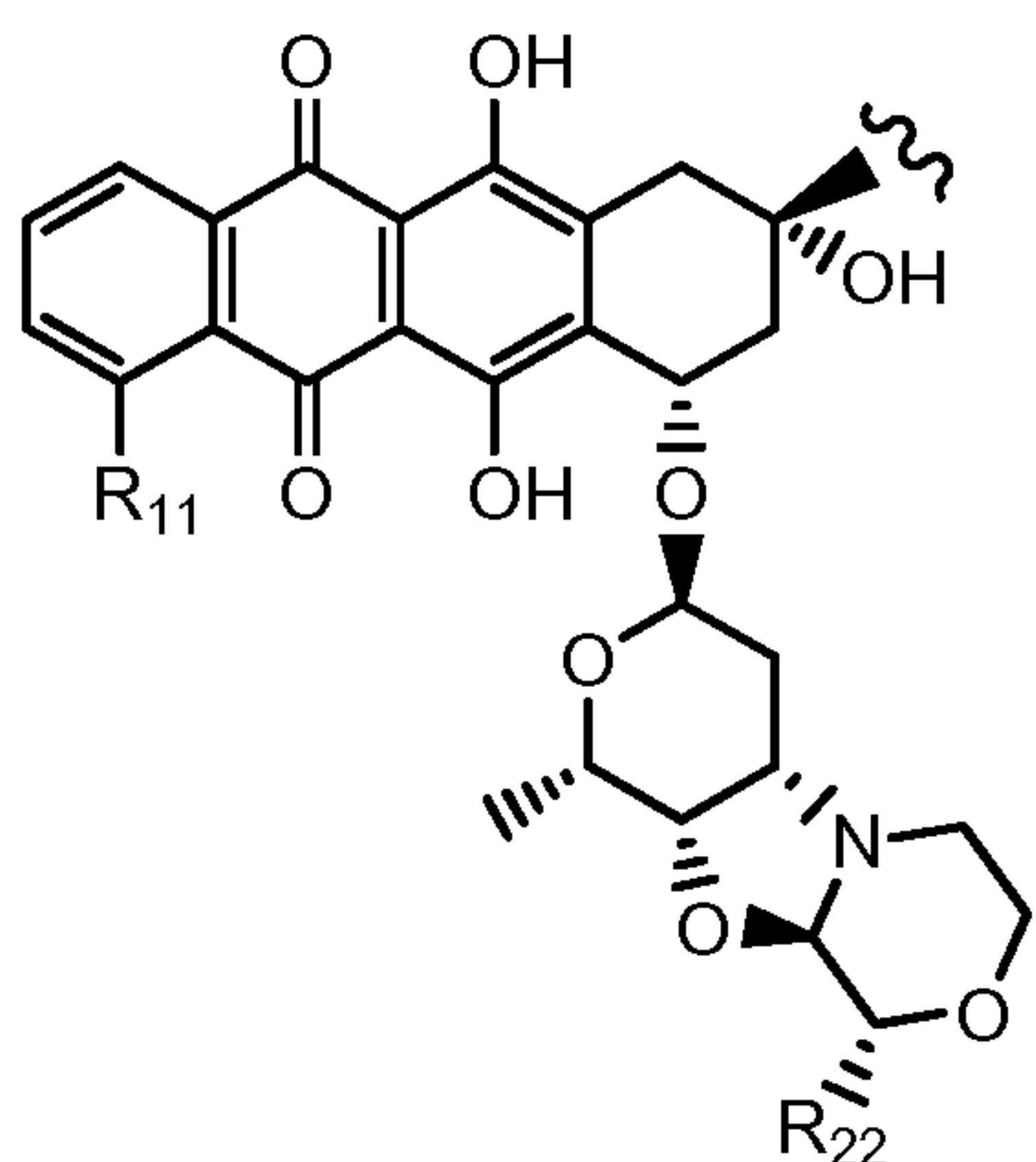
Sp is a bond or a spacer unit covalently attached to a drug moiety;

R¹ is C₁-C₁₀alkyl, (C₁-C₁₀alkyl)NHC(NH)NH₂ or (C₁-C₁₀alkyl)NHC(O)NH₂;

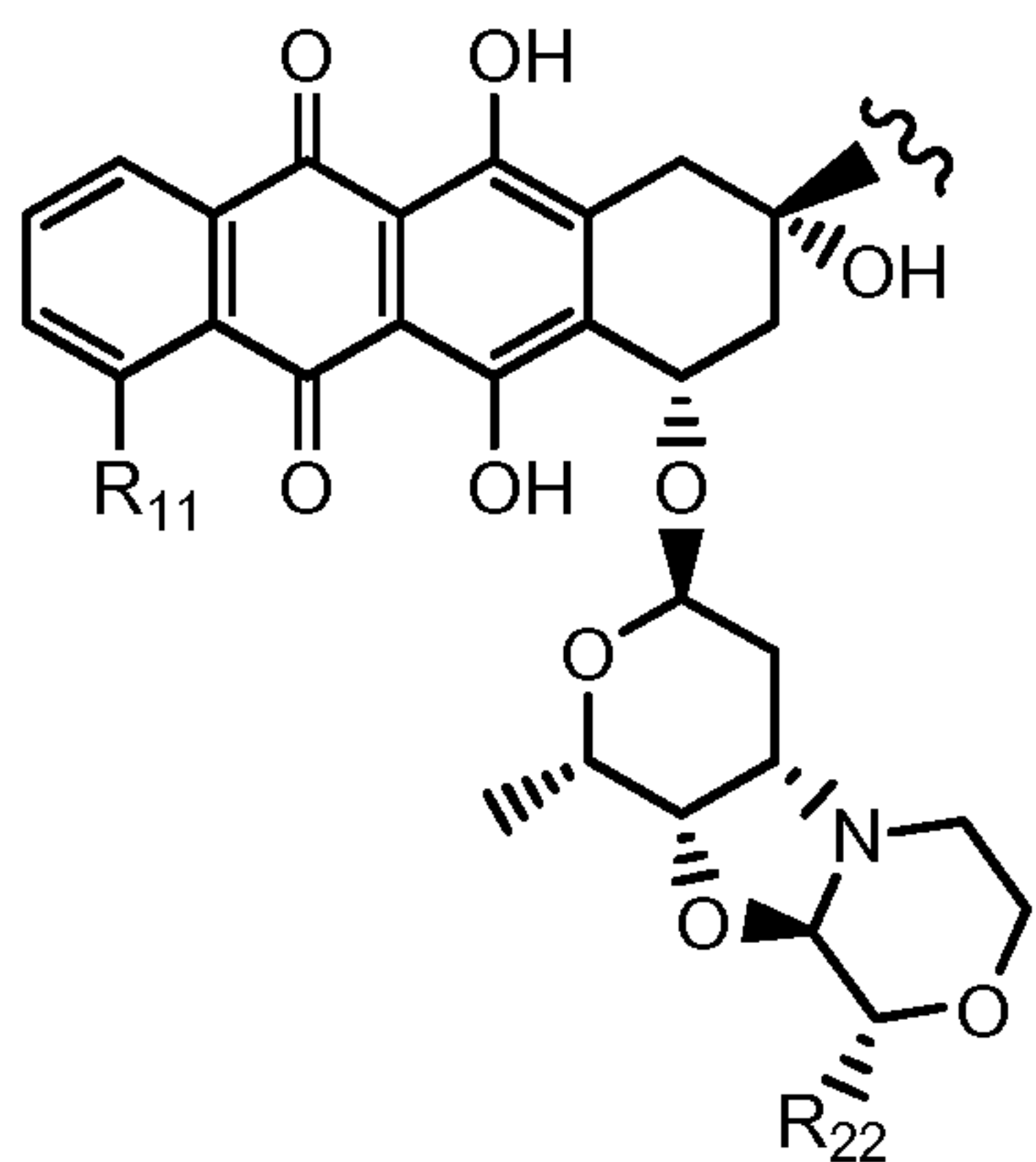
R⁴ and R⁵ are each independently C₁-C₁₀alkyl, arylalkyl, heteroarylalkyl, (C₁-C₁₀alkyl)OCH₂-, or

R⁴ and R⁵ may form a C₃-C₇cycloalkyl ring;

D is a drug moiety of Formula (Ia) or (Ib) represented by the following structures:



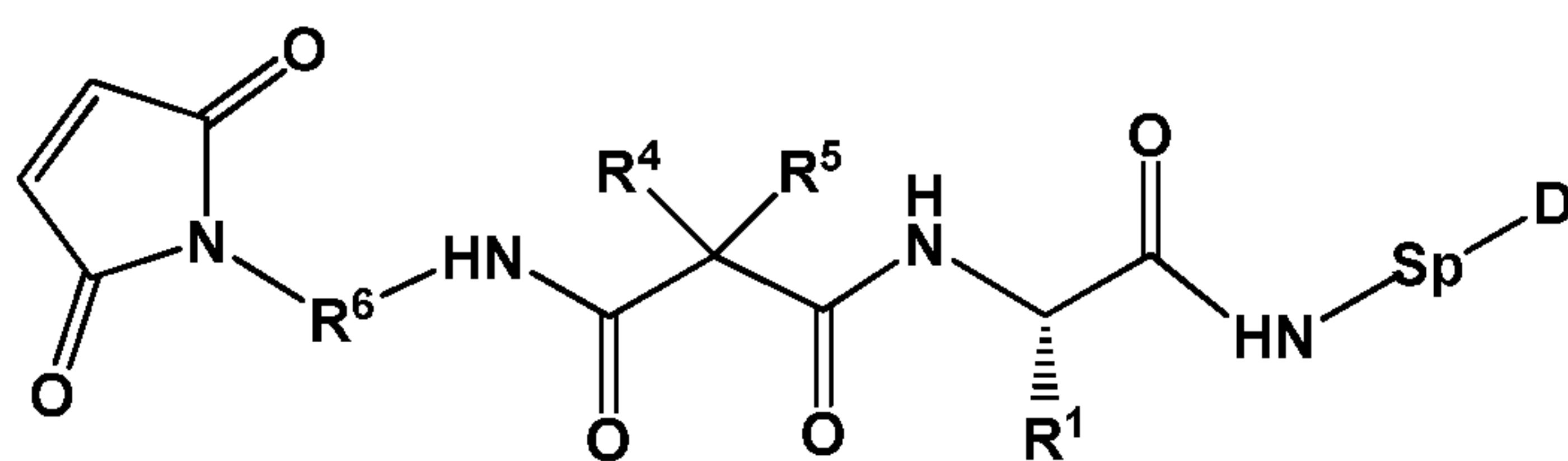
(Ia)



(Ib)

wherein R¹¹ is hydrogen atom, hydroxy or methoxy group and R²² is a C₁-C₅ alkoxy group, or a pharmaceutically acceptable salt thereof.

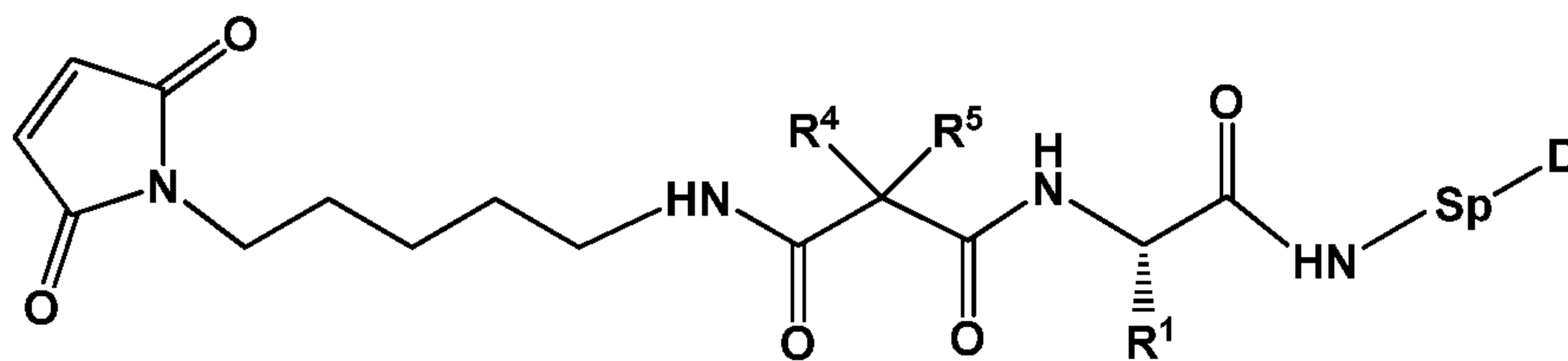
19. The compound of claim 18 represented by the following formula



(I)(B)(LD2)

wherein R_6 is C_1 - C_{10} alkylene; R^4 and R^5 together form a C_3 - C_7 cycloalkyl ring.

20. The compound of claim 18 represented by the following formula



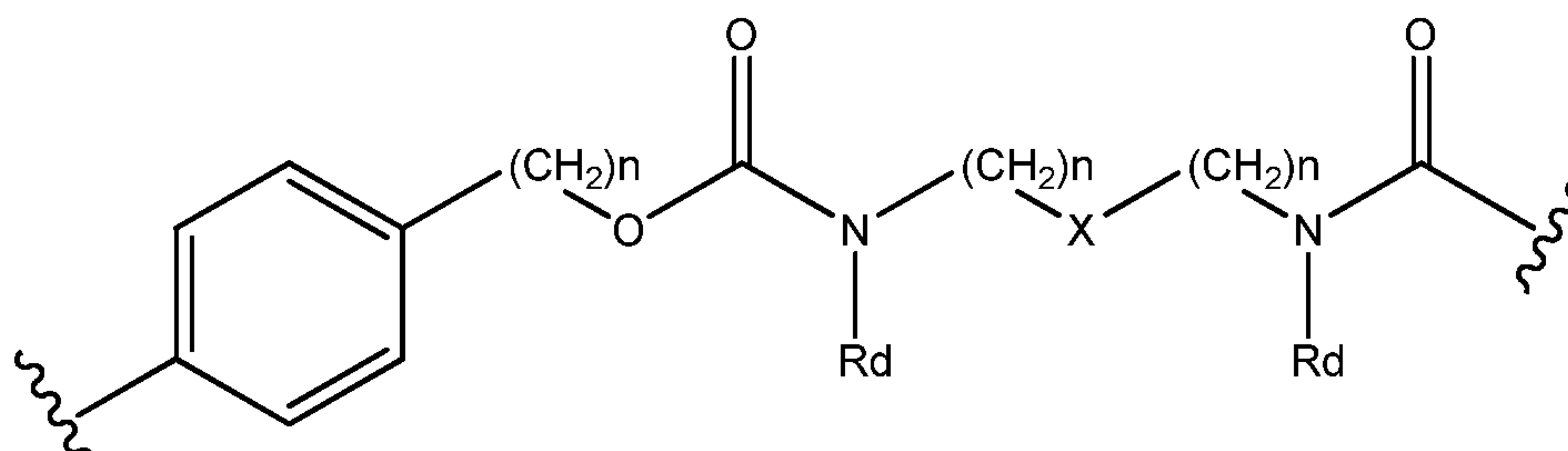
(I)(B)(LD3)

wherein

R^1 is C_1 - C_6 alkyl- NH_2 , $(C_1$ - C_6 alkyl) $NHC(NH)NH_2$ or $(C_1$ - C_6 alkyl) $NHC(O)NH_2$;

R^4 and R^5 are each independently C_1 - C_6 alkyl, wherein said alkyl are unsubstituted, or R^4 and R^5 may form a C_3 - C_7 cycloalkyl ring; and

Sp is the following formula



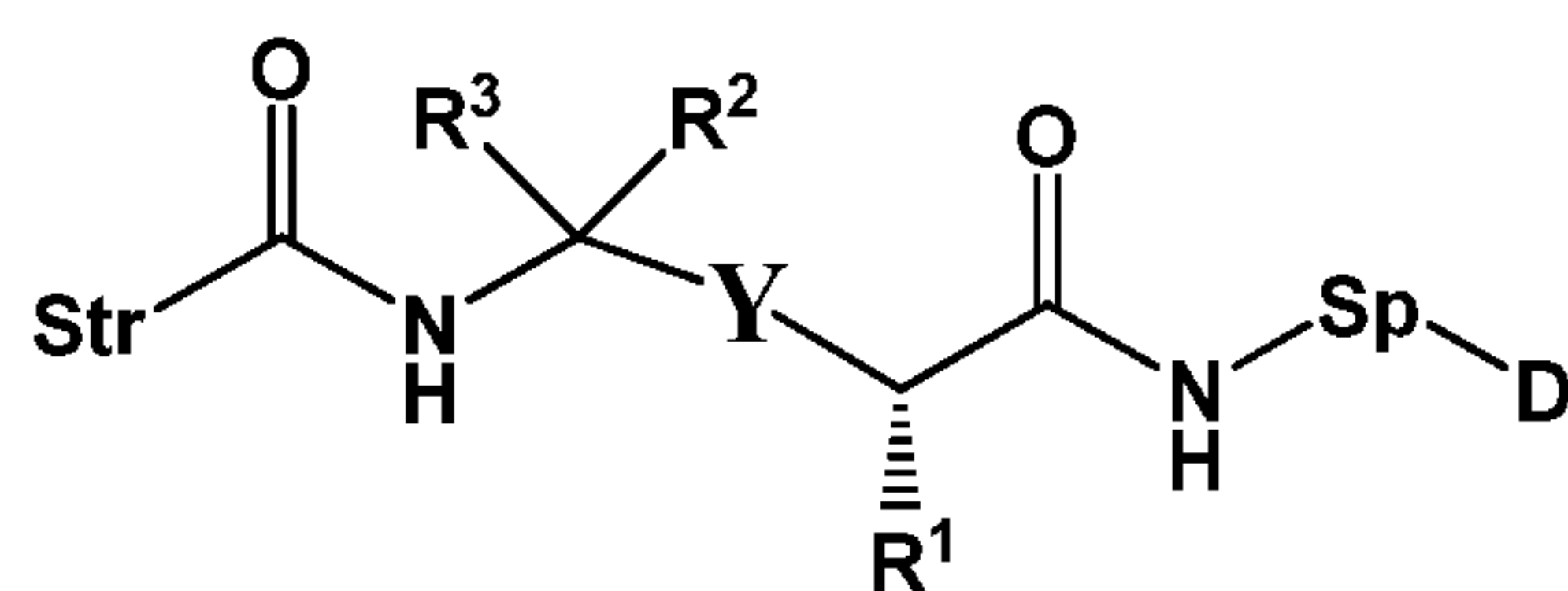
wherein

each n is independently 1-6;

X is N , CH_2 or a bond; and

each R^d is independently H or C_1 - C_3 alkyl.

21. A compound of Formula:



(I)(A)(LD1)

wherein

Str is a stretcher unit which can be covalently attached to an antibody;

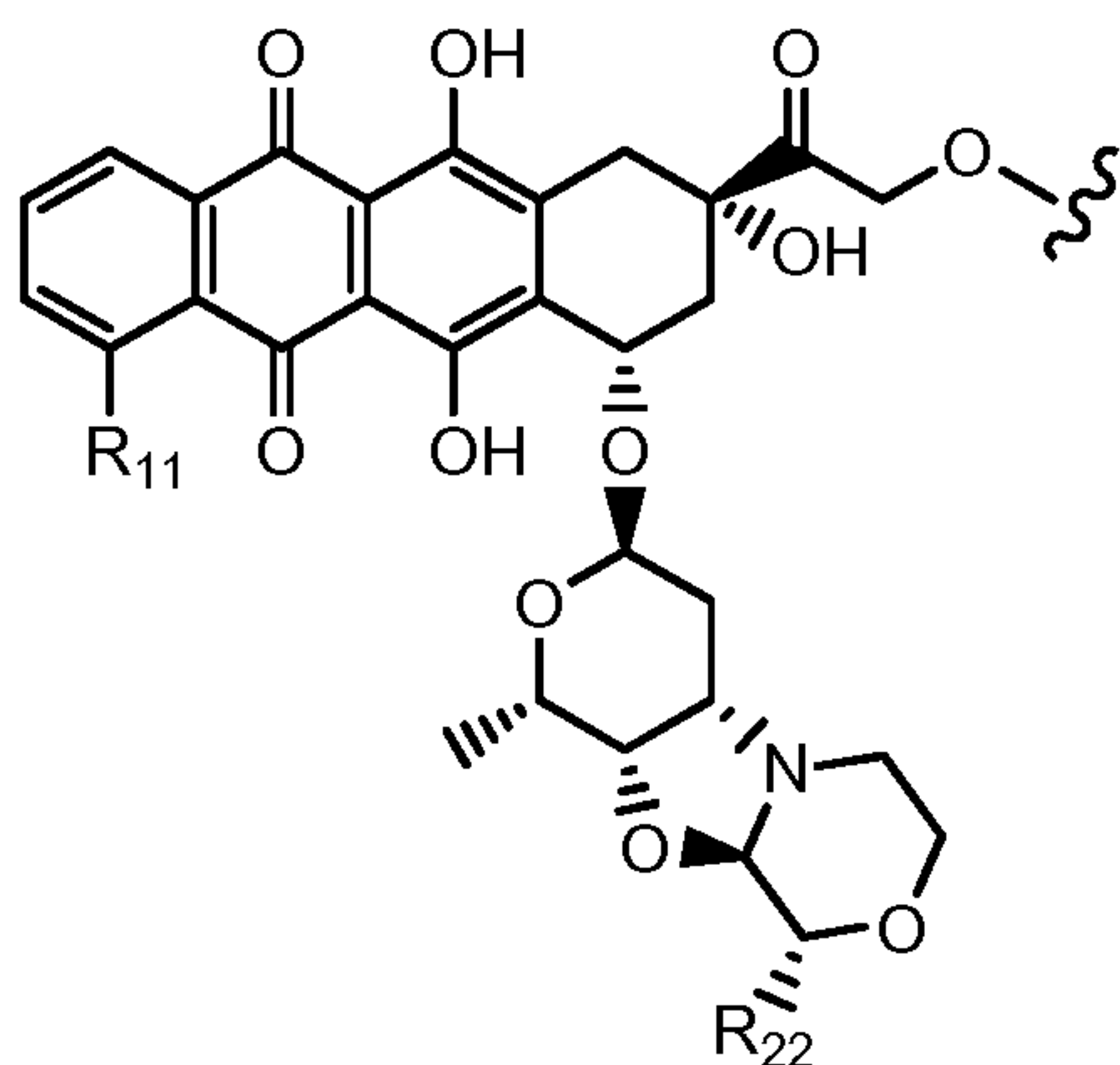
Sp is an optional spacer unit covalently attached to a drug moiety;

Y is heteroaryl, aryl, $-C(O)C_1-C_6$ alkenyl, C_1-C_6 alkenyl or $-C_1-C_6$ alkenyl-NH-;

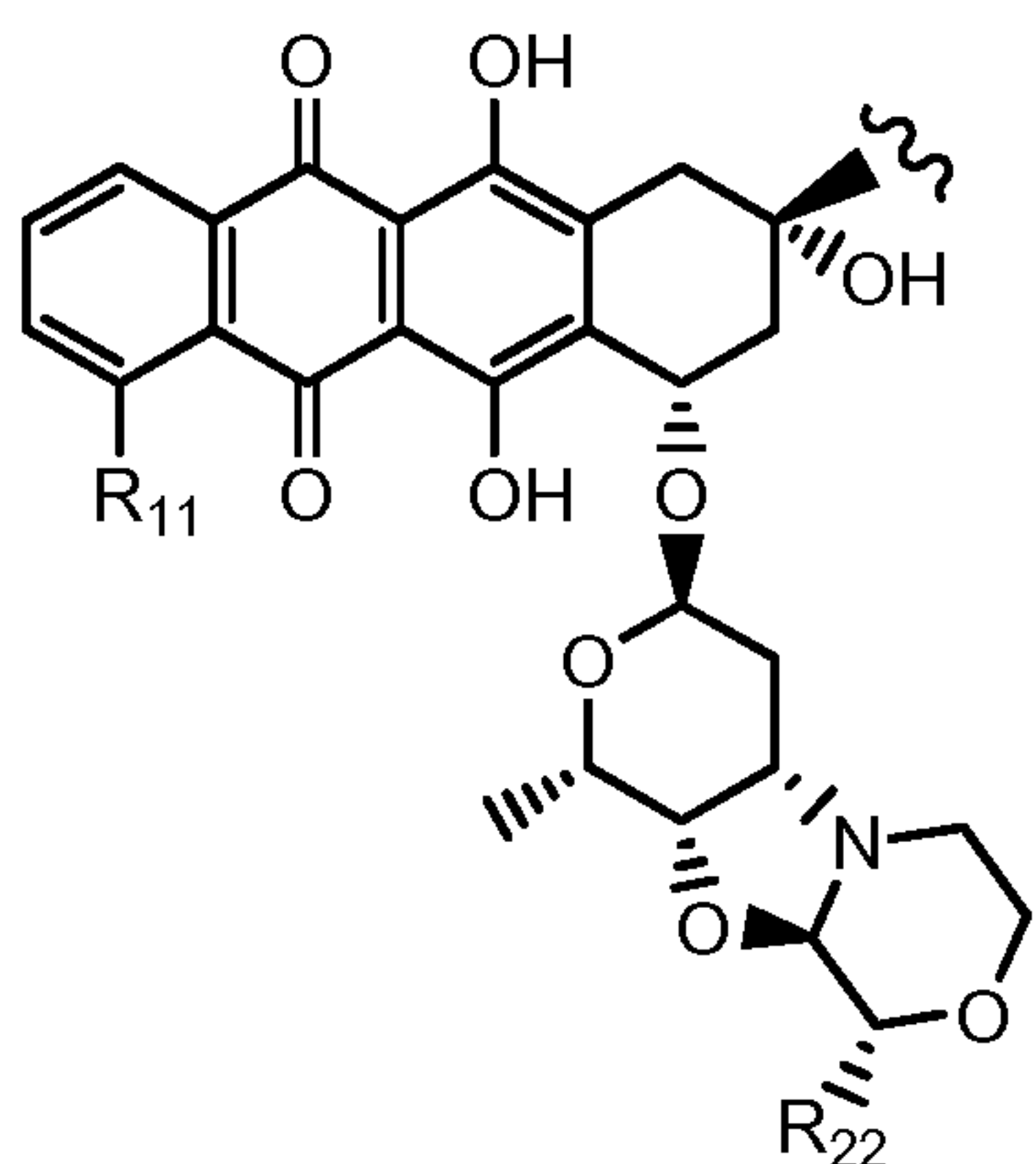
R^1 is C_1-C_{10} alkyl, $(C_1-C_{10}alkyl)NHC(NH)NH_2$ or $(C_1-C_{10}alkyl)NHC(O)NH_2$;

R^3 and R^2 are each independently H, C_1-C_{10} alkyl, arylalkyl or heteroarylalkyl, or R^3 and R^2 together may form a C_3-C_7 cycloalkyl;

D is a drug moiety of Formula (Ia) or (Ib) represented by the following structures



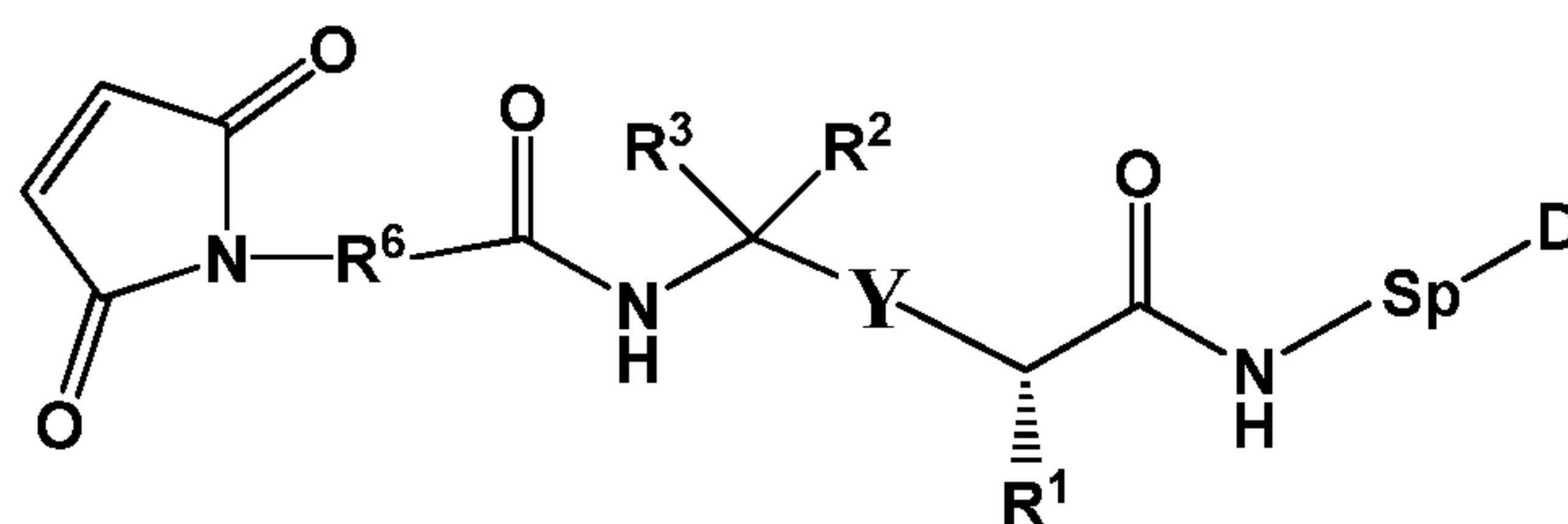
(Ia) and



(Ib)

wherein R^{11} is hydrogen atom, hydroxy or methoxy group and R^{22} is a C_1-C_5 alkoxy group, or a pharmaceutically acceptable salt thereof.

22. The compound of claim 21 represented by the following formula:



(I)(A)(LD2)

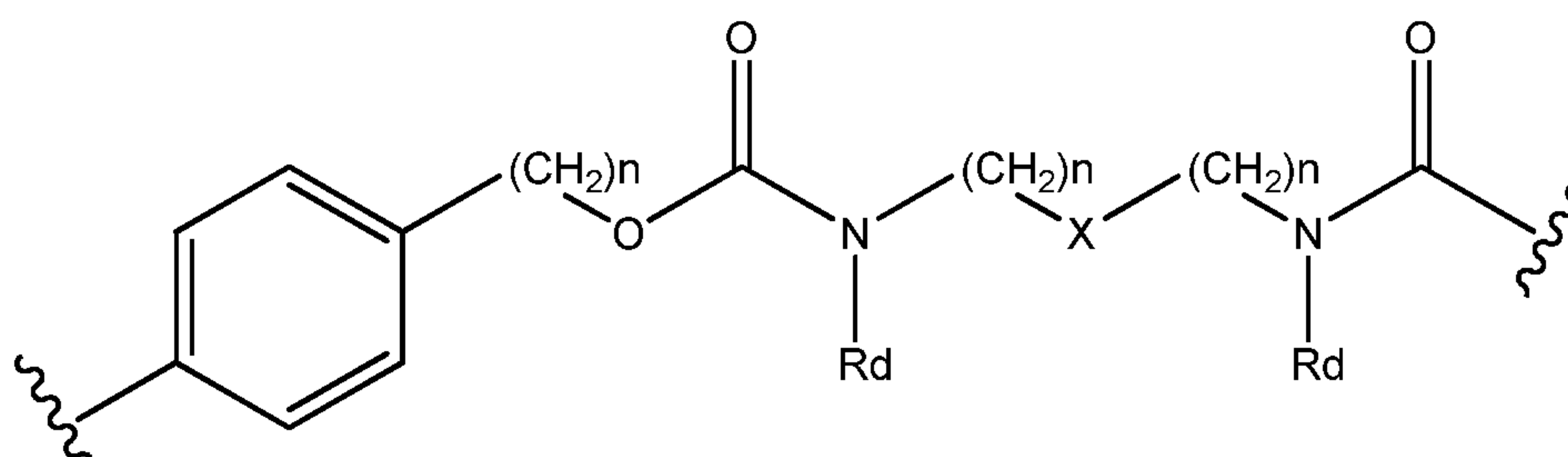
wherein

R^1 is C_1 - C_{10} alkyl, $(C_1$ - C_{10} alkyl) $NHC(NH)NH_2$ or $(C_1$ - C_{10} alkyl) $NHC(O)NH_2$;

R^3 and R^2 are each independently H, C_1 - C_{10} alkyl, arylalkyl or heteroarylalkyl, or R^3 and R^2 together may form a C_3 - C_7 cycloalkyl;

R_6 is C_1 - C_{10} alkylene; and

Sp is the following formula



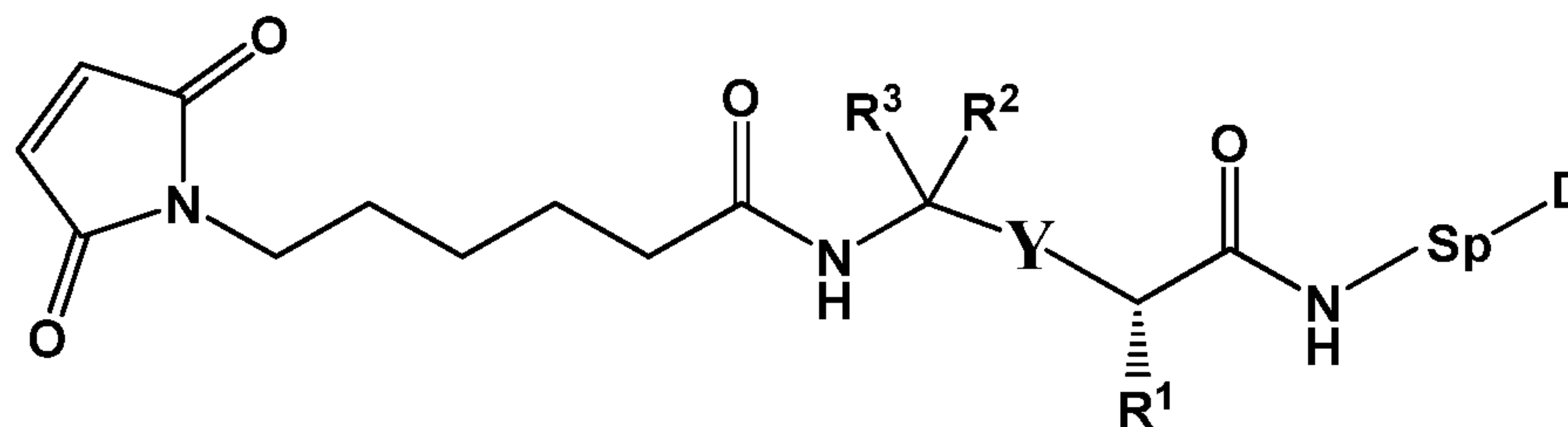
wherein

each n is independently 1-6;

X is N, CH_2 or a bond; and

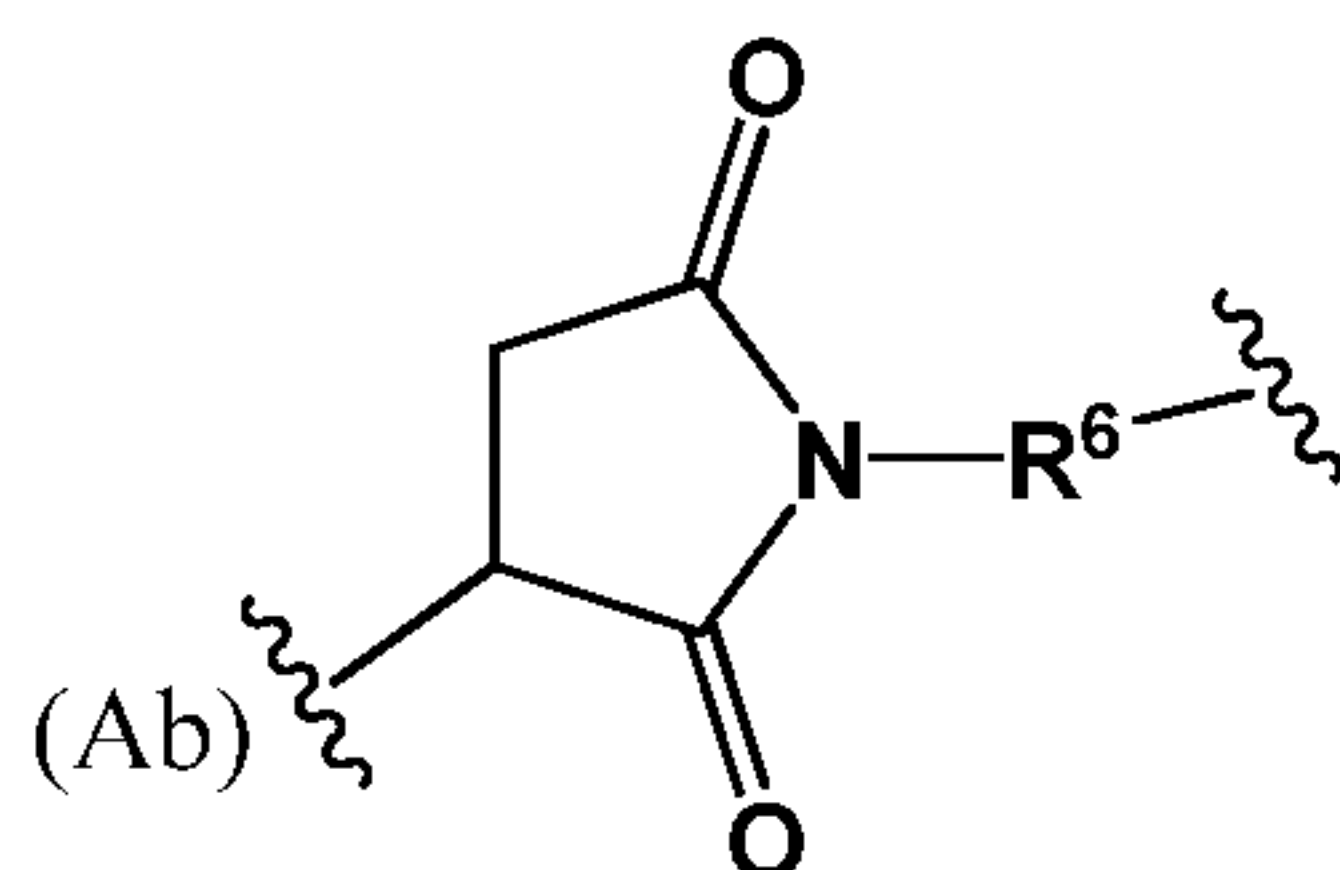
each R^d is independently H or C_1 - C_3 alkyl.

23. The compound of claim 22 represented by the following formula:



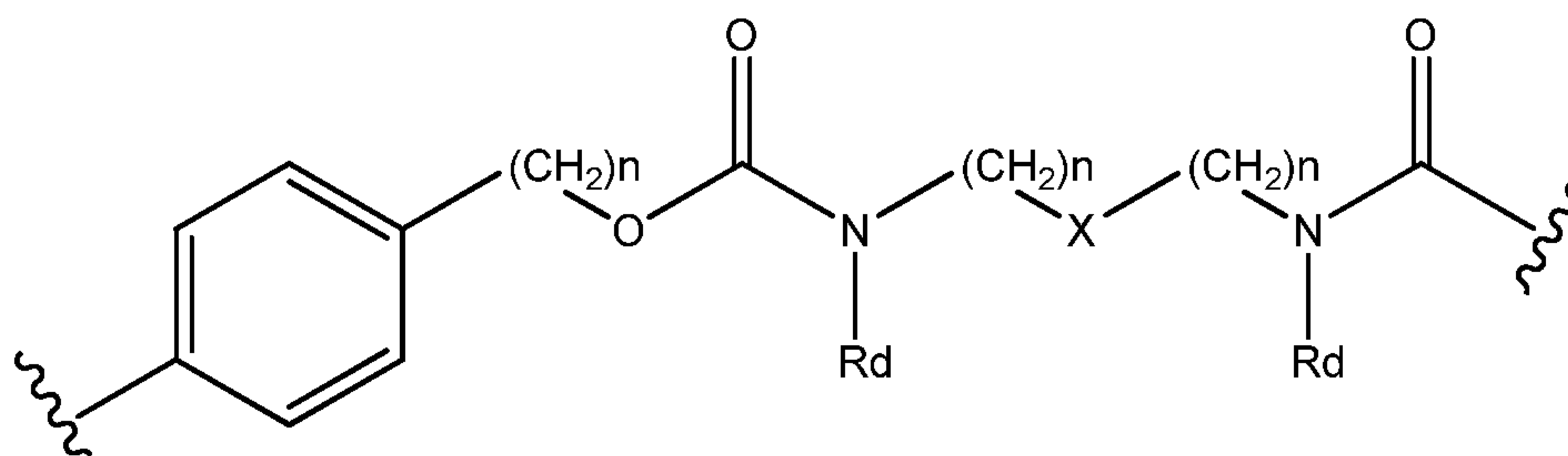
(I)(A)(LD3)

24. The compound of claim 18 or 21, wherein Str has the following formula:



wherein R^6 is selected from the group consisting of C_1 - C_{10} alkylene, C_3 - C_8 cycloalkyl, O -(C_1 - C_8 alkylene), and C_1 - C_{10} alkylene- $C(O)N(R^a)$ - C_2 - C_6 alkylene, where each alkylene may be substituted by one to five substituents selected from the group consisting of halo, trifluoromethyl, difluoromethyl, amino, alkylamino, cyano, sulfonyl, sulfonamide, sulfoxide, hydroxy, alkoxy, ester, carboxylic acid, alkylthio, aryl, arylalkyl, C_3 - C_8 cycloalkyl, C_4 - C_7 heterocycloalkyl and heteroaryl each R^a is independently H or C_1 - C_6 alkyl;

Sp is $—Ar—R^b—$, wherein Ar is aryl or heteroaryl, R^b is (C_1 - C_{10} alkylene) O - or Sp is the following formula



wherein

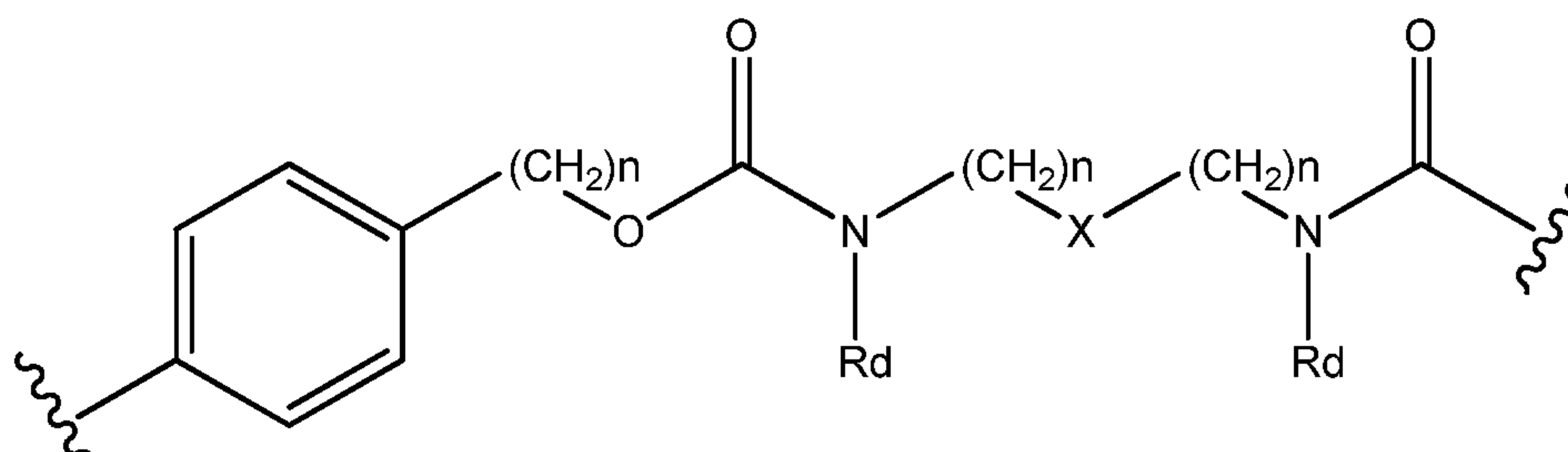
each n is independently 1-6;

X is N, CH_2 or a bond; and

each R^d is independently H or C_1 - C_3 alkyl.

25. The compound of claim 24, wherein R^6 is C_1 - C_{10} alkylene,

Sp is the following formula



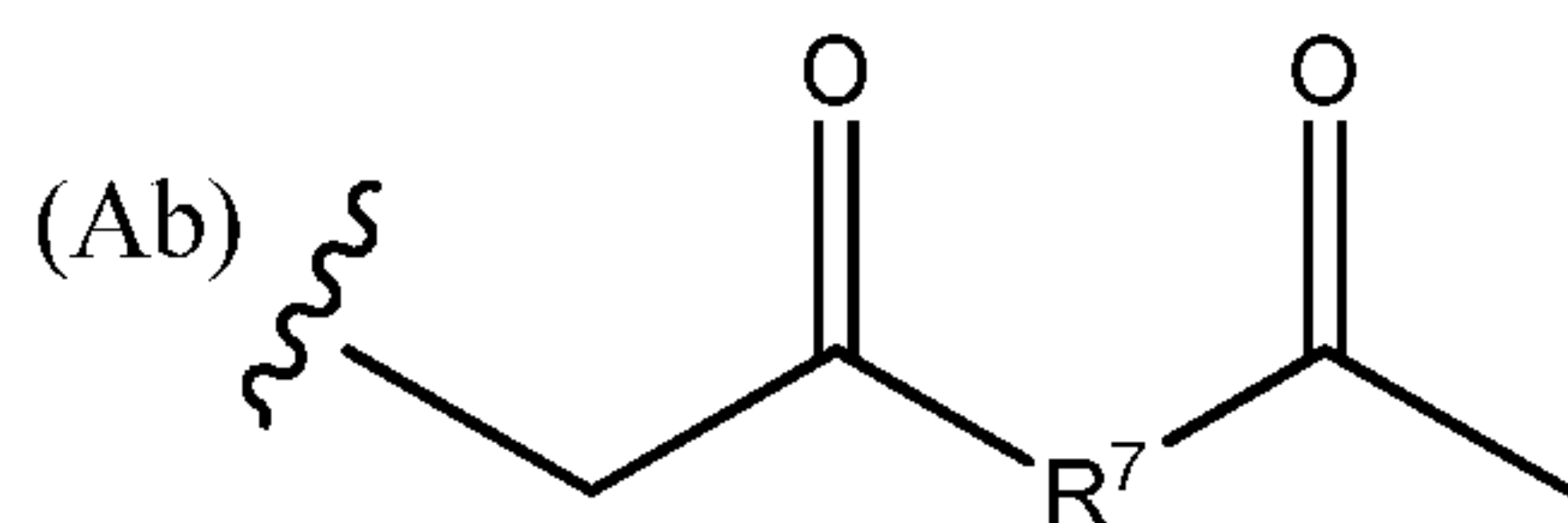
wherein

each n is independently 1-6;

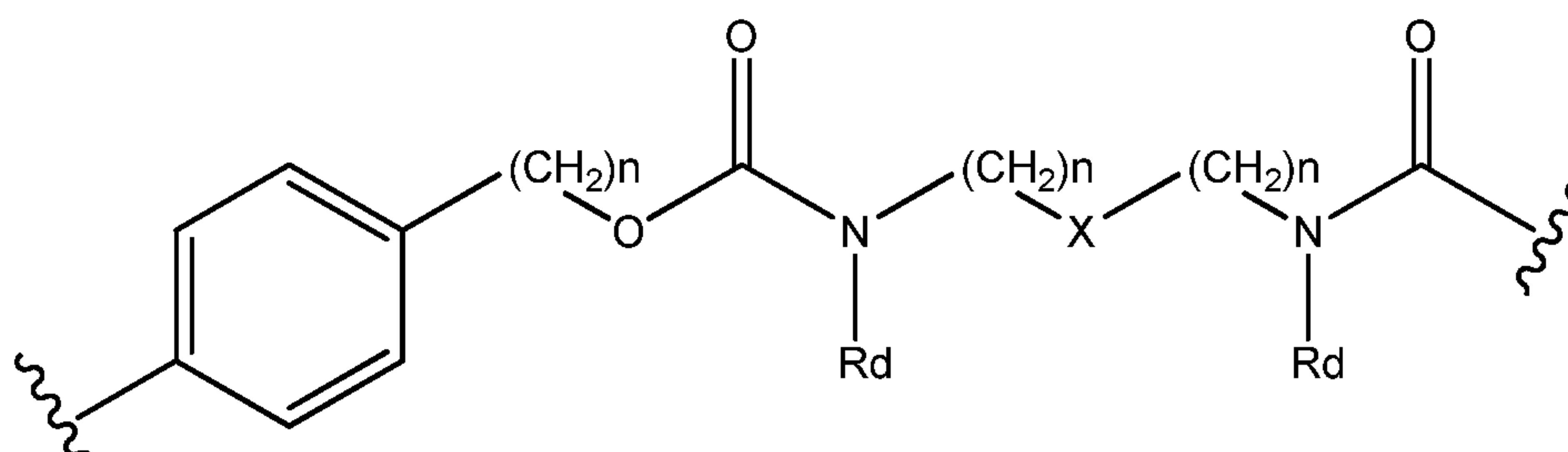
X is N, CH₂ or a bond; and
each R^d is independently H or C₁-C₃alkyl.

26. The compound of claim 18 and 21, where R₆ is -(CH₂)₅;

27. The compound of claim 18 or 21, wherein Str has the formula:

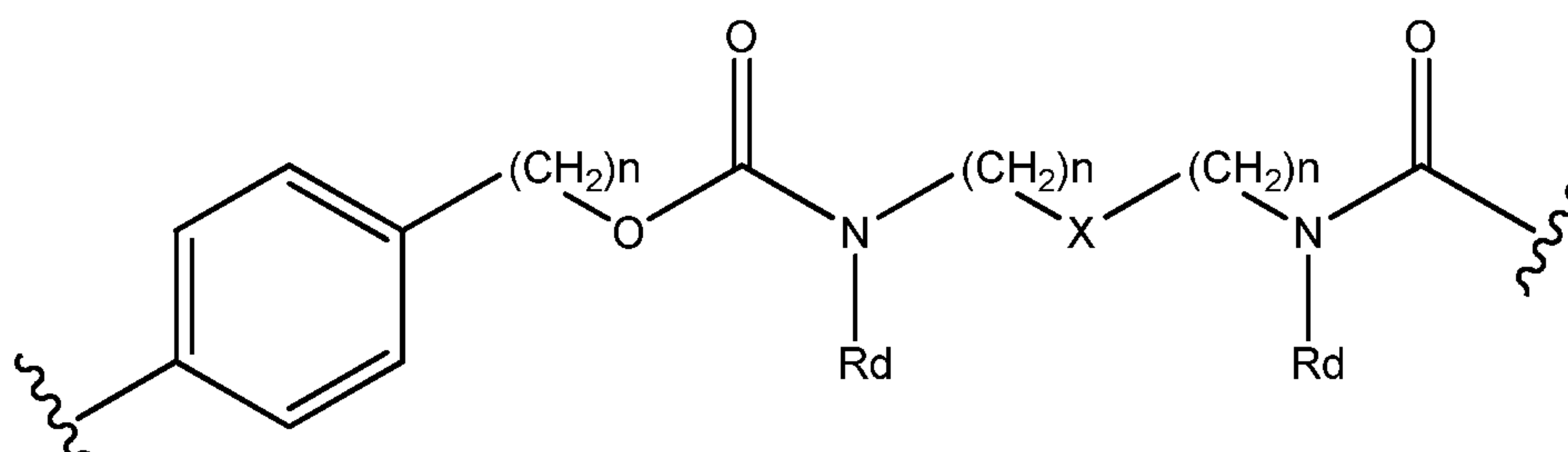


wherein R⁷ is selected from C₁-C₁₀alkylene, C₁-C₁₀alkylene-O, N(R^c)-(C₂-C₆alkylene)-N(R^c) and N(R^c)-(C₂-C₆alkylene); where each R^c is independently H or C₁-C₆ alkyl; Sp is —Ar—R^b—, wherein Ar is aryl or heteroaryl, R^b is (C₁-C₁₀ alkylene)O- or Sp is the following formula



wherein
each n is independently 1-6;
X is N, CH₂ or a bond; and
each R^d is independently H or C₁-C₃alkyl..

28. The compound of claim 23, wherein R⁶ is C₁-C₁₀ alkylene,
Sp is the following formula



wherein
each n is independently 1-6;

X is N, CH₂ or a bond; and
each R^d is independently H or C₁-C₃alkyl.

29. The antibody-drug conjugate according to any one of claims 1-17, wherein p is 2.
30. The antibody-drug conjugate of any one of claims 1-17, wherein the antibody binds to one or more of polypeptides selected from the group consisting of:
- CLL1;
 - BMPR1B;
 - E16;
 - STEAP1;
 - 0772P;
 - MPF;
 - NaPi2b;
 - Sema 5b;
 - PSCA hlg;
 - ETBR;
 - MSG783;
 - STEAP2;
 - TrpM4;
 - CRIPTO;
 - CD21;
 - CD79b;
 - FcRH2;
 - HER2;
 - NCA;
 - MDP;
 - IL20R α ;
 - Brevican;
 - EphB2R;
 - ASLG659;
 - PSCA;
 - GEDA;
 - BAFF-R;
 - CD22;
 - CD79a;
 - CXCR5;

HLA-DOB;
P2X5 ;
CD72;
LY64;
FcRH1;
IRTA2;
TENB2;
PMEL17;
TMEFF1;
GDNF-Ra1;
Ly6E;
TMEM46;
Ly6G6D;
LGR5;
RET;
LY6K;
GPR19;
GPR54;
ASPHD1;
Tyrosinase;
TMEM118;
GPR172A;
MUC16 and
CD33.

31. A method of treating a disease in a human in need thereof, comprising administering to said human an effective amount of an Antibody-drug conjugate of claim 1.
32. A pharmaceutical composition comprising a compound of claim 1 and a pharmaceutically acceptable carrier thereof.
33. The antibody-drug conjugate of claim 30, wherein the antibody binds to one or more of polypeptides selected from the group consisting of:
- CLL1;
STEAP1;
NaPi2b;
STEAP2;

TrpM4;
CRIPTO;
CD21;
CD79b;
FcRH2;
HER2;
CD22;
CD79a;
CD72;
LY64;
Ly6E;
MUC16; and
CD33.

34. The antibody-drug conjugate of any one of claims 1-17, wherein the antibody binds to CD33.

35. The antibody-drug conjugate of claim 34, wherein the anti-CD33 antibody comprise an HVR-L1 comprising the amino acid sequence of SEQ ID NO:11, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:12, an HVR-L3 comprising the amino acid sequence of SEQ ID NO:13, an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 14, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:15, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 16.

36. The antibody-drug conjugate of claim 34, wherein said anti-CD33 antibody comprises a VL domain comprising the amino acid sequence of SEQ ID NO:17 and a VH domain comprising the amino acid sequence of SEQ ID NO:18.

37. The antibody-drug conjugate of claim 34, wherein said anti-CD33 antibody comprises an amino acid sequence of SEQ ID NO:19 and an amino acid sequence of SEQ ID NO: 20.

38. The antibody-drug conjugate of claim 34, wherein said anti-CD33 antibody comprises an amino acid sequence that has at least 95% sequence identity with amino acid sequence of SEQ ID NO:19 and that comprises an amino acid sequence that has at least 95%sequence identity with an amino acid sequence of SEQ ID NO: 20.

39. The antibody-drug conjugate of any one of claims 1-17, wherein the antibody binds to NaPi2b.

40. The antibody-drug conjugate of claim 39, wherein the NaPi2b antibody comprise an HVR-L1 comprising the amino acid sequence of SEQ ID NO:1, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:2, an HVR-L3 comprising the amino acid sequence of SEQ ID NO:3, an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 4, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:5, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 6.

41. The antibody-drug conjugate of claim 39, wherein said NaPi2b antibody comprises a VL domain comprising the amino acid sequence of SEQ ID NO:7 and a VH domain comprising the amino acid sequence of SEQ ID NO:8.

42. The antibody-drug conjugate of claim 39, wherein said NaPi2b antibody comprises an amino acid sequence of SEQ ID NO:9 and an amino acid sequence of SEQ ID NO: 10.

43. The antibody-drug conjugate of claim 39, wherein said NaPi2b antibody comprises an amino acid sequence that has at least 95% sequence identity with amino acid sequence of SEQ ID NO:9 and that comprises an amino acid sequence that has at least 95%sequence identity with an amino acid sequence of SEQ ID NO: 10.

44. The antibody-drug conjugate of any one of claims 1-22, wherein the antibody binds to CD-22.

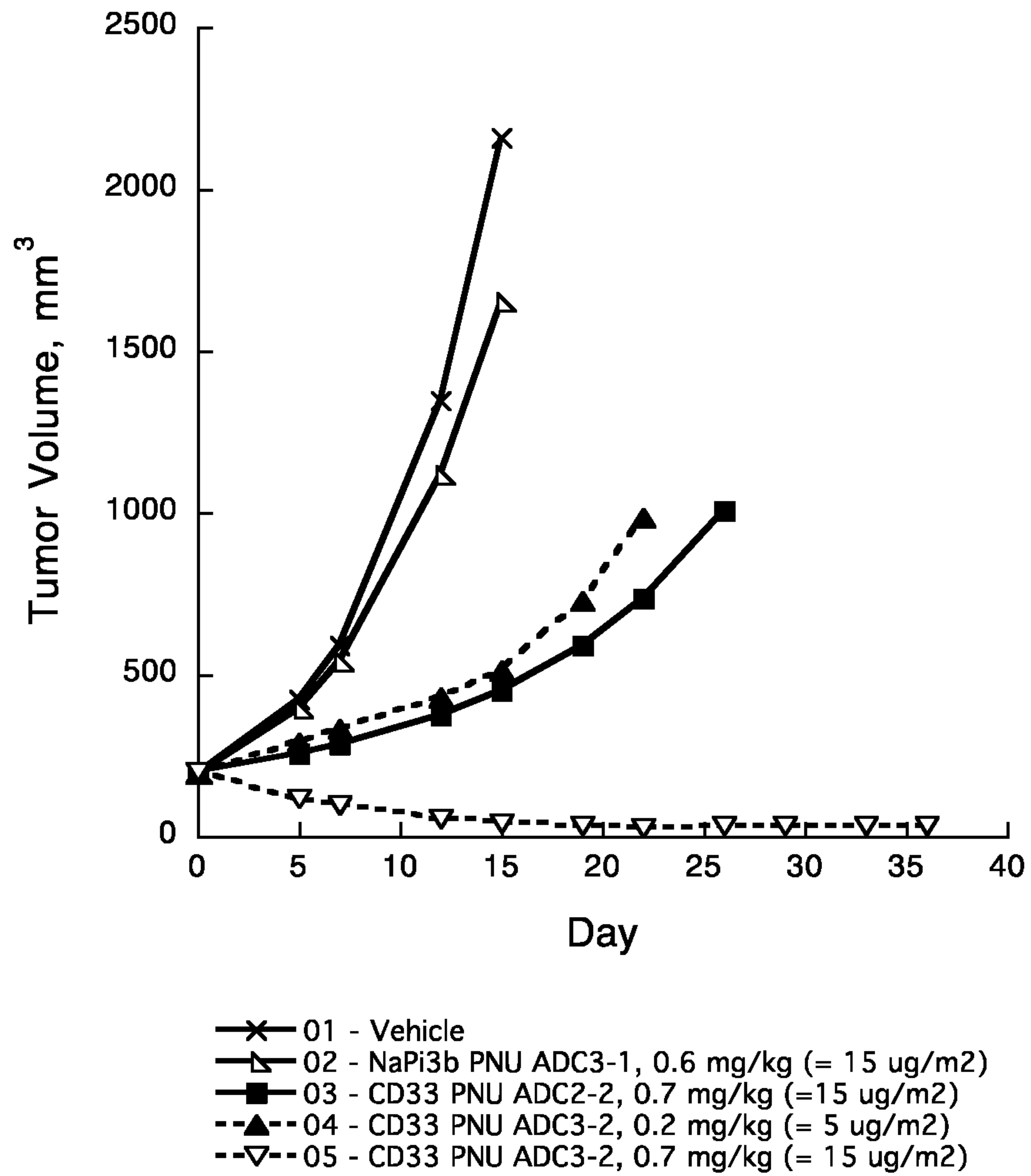
45. The antibody-drug conjugate of claim 44, wherein the CD-22 antibody comprise an HVR-L1 comprising the amino acid sequence of SEQ ID NO:41, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:42, an HVR-L3 comprising the amino acid sequence of SEQ ID NO:43, an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 44, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:45, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 46.

46. The antibody-drug conjugate of claim 44, wherein said CD-22 antibody comprises a VL domain comprising the amino acid sequence of SEQ ID NO:47 and a VH domain comprising the amino acid sequence of SEQ ID NO:48.

47. The antibody-drug conjugate of claim 44, wherein said CD-22 antibody comprises an amino acid sequence of SEQ ID NO:49 and an amino acid sequence of SEQ ID NO: 50.

48. The antibody-drug conjugate of claim 44, wherein said CD-22 antibody comprises an amino acid sequence that has at least 95% sequence identity with amino acid sequence of SEQ ID NO:49 and that comprises an amino acid sequence that has at least 95% sequence identity with an amino acid sequence of SEQ ID NO: 50.

Figure 1 (HL-60)



2/2

Figure 2 (HL-60)

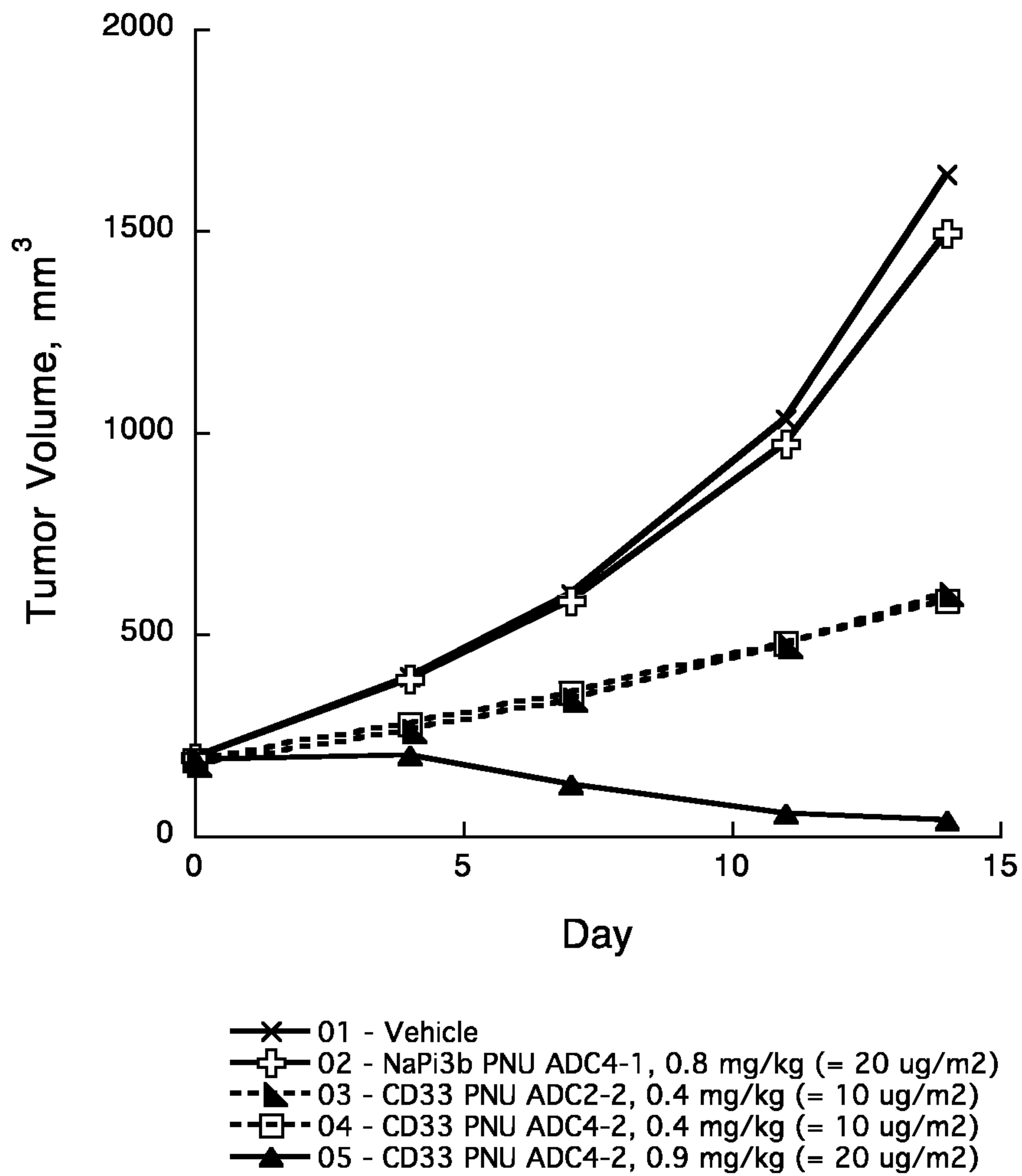
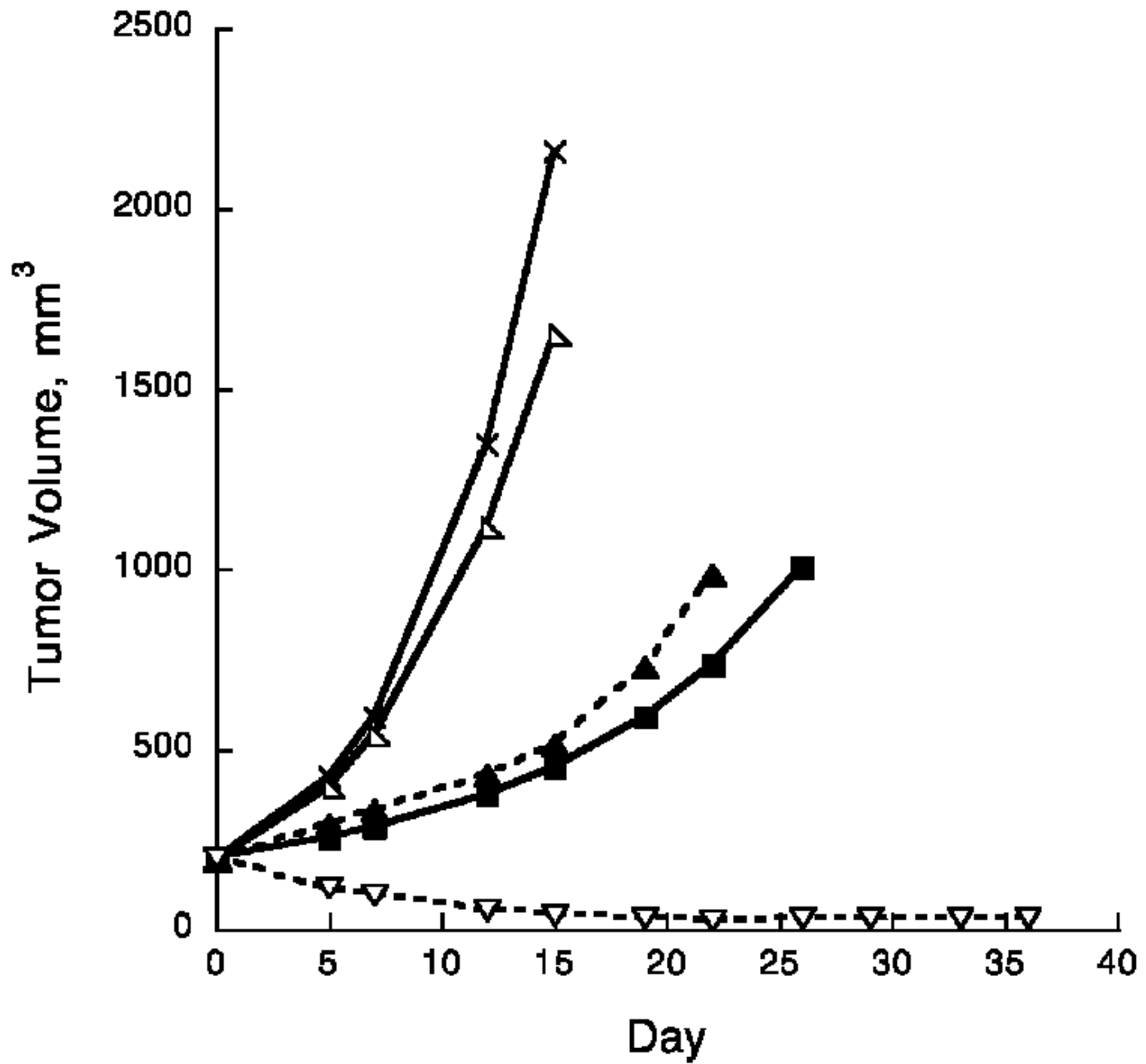


Figure 1 (HL-60)



- x— 01 - Vehicle
- △— 02 - NaPi3b PNU ADC3-1, 0.6 mg/kg (= 15 ug/m2)
- 03 - CD33 PNU ADC2-2, 0.7 mg/kg (=15 ug/m2)
- -▲- - 04 - CD33 PNU ADC3-2, 0.2 mg/kg (= 5 ug/m2)
- -▽- - 05 - CD33 PNU ADC3-2, 0.7 mg/kg (= 15 ug/m2)