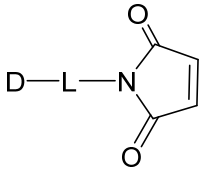
CLAIMS

1. A method of preparing a cell-binding agent-cytotoxic agent conjugate comprising a cell-binding agent (CysCBA) having one or more unpaired cysteine residues covalently linked to a cytotoxic agent, wherein the method comprises the steps of:

(a) reacting a reducing agent with a capped cell-binding agent (cCysCBA) having one or more unpaired cysteine residues capped with a capping agent to form a reduced cell-binding agent, wherein the capping agent is removed to form a free thiol (-SH) group in the reduced cell-binding agent;

(b) reacting the reduced cell-binding agent with a selective oxidizing agent to form the CysCBA, wherein the free thiol group in the unpaired cysteine residue is not oxidized; and

(c) reacting the CysCBA with a compound of formula (I):



(I)

or a pharmaceutically acceptable salt thereof, thereby forming the conjugate, wherein the reduced cell-binding agent in step (a) is used in step (b) without purification; and the CysCBA in step (b) is used in step (c) without purification, wherein:

D is a cytotoxic agent; and

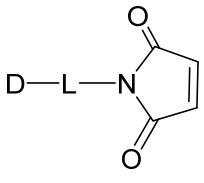
L is a linker.

2. A continuous method of preparing a cell-binding agent-cytotoxic agent conjugate (ADC) comprising a cell-binding agent (CysCBA) having one or more unpaired cysteine residues covalently linked to a cytotoxic agent, wherein the method comprises the steps of:

(a) reacting a reducing agent with a capped cell-binding agent (cCysCBA) having one or more unpaired cysteine residues capped with a capping agent to form a reduced cell-binding agent, wherein the capping agent is removed to form a free thiol (-SH) group in the reduced cell-binding agent;

(b) reacting the reduced cell-binding agent with a selective oxidizing agent to form the CysCBA, wherein the free thiol group in the unpaired cysteine residue is not oxidized; and

(c) reacting the CysCBA with a compound of formula (I):



(I)

or a pharmaceutically acceptable salt thereof, thereby forming the conjugate,

wherein the reduced cell-binding agent in step (a) is used in step (b) without purification; the CysCBA step (b) is used in step (c) without purification, and any one or more steps selected from steps (a) to (c) are performed continuously, wherein:

D is a cytotoxic agent; and

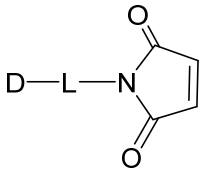
L is a linker.

3. A continuous method of preparing a cell-binding agent-cytotoxic agent conjugate (ADC) comprising a cell-binding agent (CysCBA) having one or more unpaired cysteine residues covalently linked to a cytotoxic agent, wherein the method comprises the steps of:

(a) reacting a reducing agent with a capped cell-binding agent (cCysCBA) having one or more unpaired cysteine residues capped with a capping agent to form a reduced cell-binding agent, wherein the capping agent is removed to form a free thiol (-SH) group in the reduced cell-binding agent;

(b) reacting the reduced cell-binding agent with a selective oxidizing agent to form the CysCBA, wherein the free thiol group in the unpaired cysteine residue is not oxidized; and

(c) reacting the CysCBA with a compound of formula (I):



(I)

or a pharmaceutically acceptable salt thereof, thereby forming the conjugate; and

(d) removing unconjugated compound of formula (I);

wherein the reduced cell-binding agent in step (a) is used in step (b) without purification; the CysCBA step (b) is used in step (c) without purification, any one or more steps selected from steps (a) to (d) are performed continuously; and wherein:

D is a cytotoxic agent; and

L is a linker.

4. The method of any one of claims 1-3, wherein the reducing agent is tris(2-carboxyethyl)phosphine hydrochloride (TCEP), trishydroxypropyl phosphine (THPP), tris (2-cyanoethyl)phosphine, dithiothreitol (DTT), dicyclohexylphosphino)benzenesulfonic acid, bis(p-sulfonatophenyl)phenylphosphine, (diphenylphosphino)benzenesulfonic acid, (diphenylphosphino)benzoic acid, 2-(diphenylphosphino)-N,N,N-trimethylbenzylammonium triflate, (diphenylphosphino)ethylamine, 2-(diisopropylphosphino)ethylamine, or 3-(diphenylphosphino)propylamine (DPPA).

5. The method of any one of claims 1-4, wherein the oxidizing agent is dehydroascorbic acid (DHAA), copper sulfate, oxygen or air.

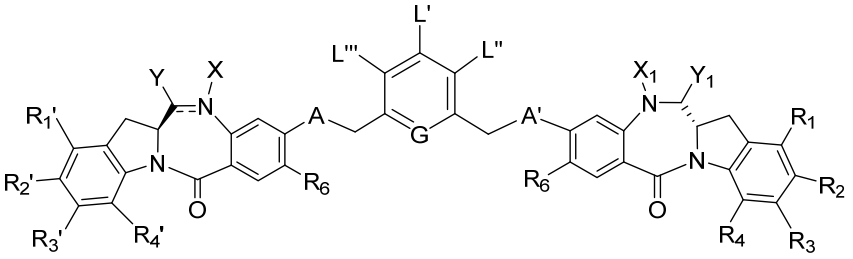
6. The method of any one of claims 1-3, wherein the reducing agent is TCEP and the reaction of step (a) is carried out at a pH between 6.0 and 8.0.

7. The method of any one of claims 1-3, wherein the reducing agent is DPPA and the reaction of step (a) is carried out at a pH between 6.0 and 8.0.

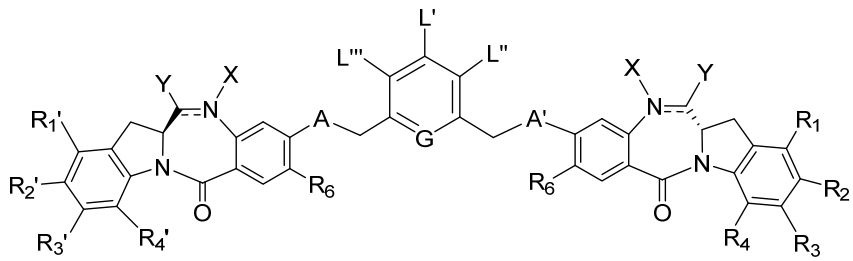
8. The method of any one of claims 1-7, wherein the molar ratio of the reducing agent in step (a) to the oxidizing agent in step (b) is between 2:1 and 1:2.

9. The method of any one of claims 1-8, wherein D is a benzodiazepine compound or a maytansinoid compound.

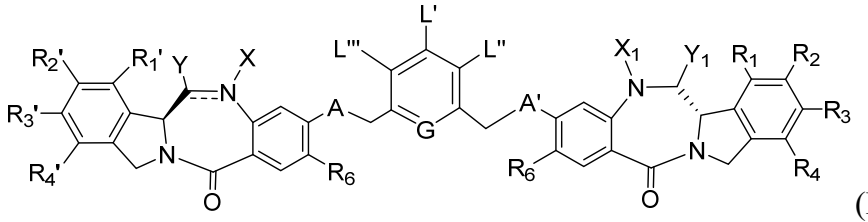
10. The method of any one of claims 1-8, wherein D is represented by the following formula:



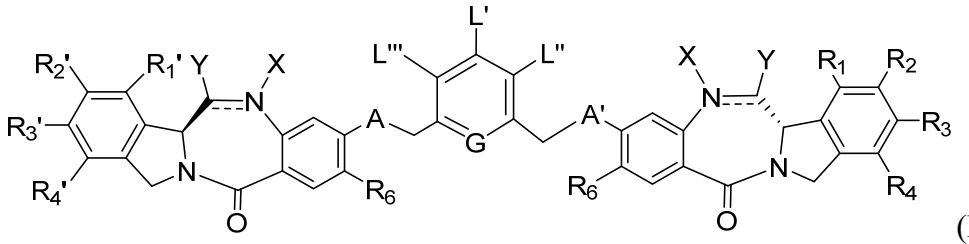
(D-IA);



(D-IB);



(D-IC); or



(D-ID);

or a pharmaceutically acceptable salt thereof, wherein:

the double line == between N and C represents a single bond or a double bond, provided that when it is a double bond, X is absent and Y is -H or a (C_1-C_4)alkyl; and when it is a single bond, X is -H or an amine protecting moiety, Y is -OH or -SO_3H or a pharmaceutically acceptable salt thereof;

one of L', L'', and L''' is represented by the following formula:

$$-Z_1\text{-}P_1\text{-}Z_2\text{-}R_{x1}\text{-}C(=O)- \quad (A'), \text{ or}$$

$$-N(R^e)\text{-}R_{x1}\text{-}C(=O)- \quad (D');$$

and the other two are each independently selected from -H, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl, a polyethylene glycol unit -$(CH_2CH_2O)_n$-R^c, halogen, guanidinium [-$NH(C=NH)NH_2$], -OR, -NR'R'', -NO_2, -NR'COR'', -SR, -SOR', -SO_2R', -SO_3H, -OSO_3H, -SO_2NR'R'', cyano, an azido, -COR', -OCOR', and -OCONR'R'';

one of the Z_1 and Z_2 is -C(=O)-, and the other is -NR_5-;

P_1 is an amino acid residue or a peptide containing between 2 to 20 amino acid residues;

R_{x1} is an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl;

R^e is -H, a linear, branched or cyclic alkyl, alkenyl or alkynyl or -$(CH_2$-CH_2-$O)_n$-R^k, wherein R^k is a -H, a linear, branched cyclic alkyl having 1 to 6 carbon atoms, optionally bearing a secondary amino (*e.g.*, -NHR^{101}) or tertiary amino (-$NR^{101}R^{102}$) group or a 5- or 6-membered nitrogen containing heterocycle, wherein R^{101} and R^{102} are each independently a linear, branched, or cyclic alkyl, alkenyl or alkynyl;

R, for each occurrence, is independently selected from the group consisting of -H, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl, a polyethylene

glycol unit -$(CH_2CH_2O)_n$-R^c, an optionally substituted aryl having 6 to 18 carbon atoms, an optionally substituted 5- to 18-membered heteroaryl ring containing one or more heteroatoms independently selected from nitrogen, oxygen, and sulfur, or an optionally substituted 3- to 18-membered heterocyclic ring containing 1 to 6 heteroatoms independently selected from O, S, N and P;

R' and R'' are each independently selected from -H, -OH, -OR, -NHR, -NR_2, -COR, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl, a polyethylene glycol unit -$(CH_2CH_2O)_n$-R^c, and an optionally substituted 3- to 18-membered heterocyclic ring having 1 to 6 heteroatoms independently selected from O, S, N and P;

R^c is -H or an optionally substituted linear or branched alkyl having 1 to 4 carbon atoms;

n is an integer from 1 to 24;

X_1 is selected from -H, an amine-protecting group, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl, a polyethylene glycol unit -$(CH_2CH_2O)_n$-R^c, an optionally substituted aryl having 6 to 18 carbon atoms, an optionally substituted 5- to 18-membered heteroaryl ring containing one or more heteroatoms independently selected from nitrogen, oxygen, and sulfur, and an optionally substituted 3- to 18-membered heterocyclic ring containing 1 to 6 heteroatoms independently selected from O, S, N and P;

Y_1 is selected from -H, an oxo group, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl, an optionally substituted 6- to 18-membered aryl, an optionally substituted 5- to 18-membered heteroaryl ring containing one or more heteroatoms independently selected from nitrogen, oxygen, and sulfur, an optionally substituted 3- to 18-membered heterocyclic ring having 1 to 6 heteroatoms;

R_1, R_2, R_3, R_4, R_1', R_2', R_3' and R_4' are each independently selected from the group consisting of -H, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl, a polyethylene glycol unit -$(CH_2CH_2O)_n$-R^c, halogen, guanidinium [-NH(C=NH)NH_2], -OR, -NR'R'', -NO_2, -NCO, -NR'COR'', -SR, -SOR', -SO_2R', -SO_3^-H, -OSO_3H, -SO_2NR'R'', cyano, an azido, -COR', -OCOR', and -OCONR'R'';

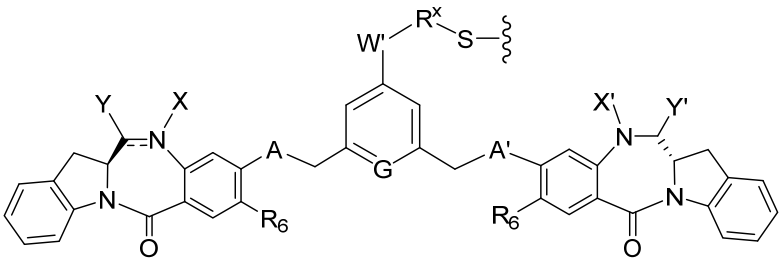
R_6 is -H, -R, -OR, -SR, -NR'R'', -NO_2, or halogen;

G is -CH- or –N-;

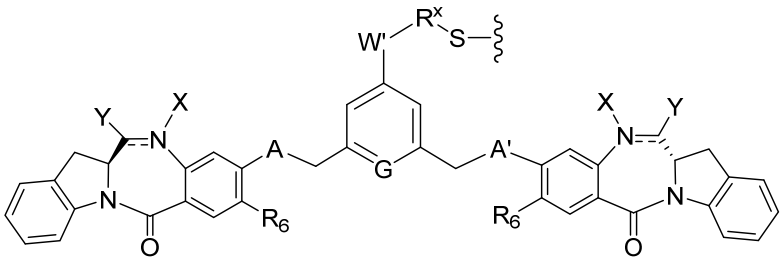
A and A' are the same or different, and are independently selected from -O-, oxo (-C(=O)-), -CRR'O-, -CRR'-, -S-, -CRR'S-, -NR_5 and -CRR'N(R_5)-; and

R_5 for each occurrence is independently -H or an optionally substituted linear or branched alkyl.

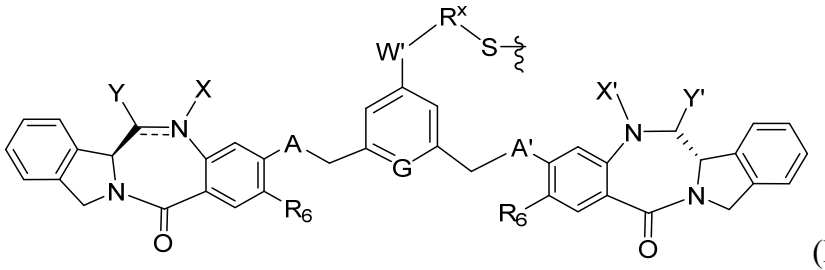
11. The method of any one of claims 1-8, wherein D is represented by the following formula:



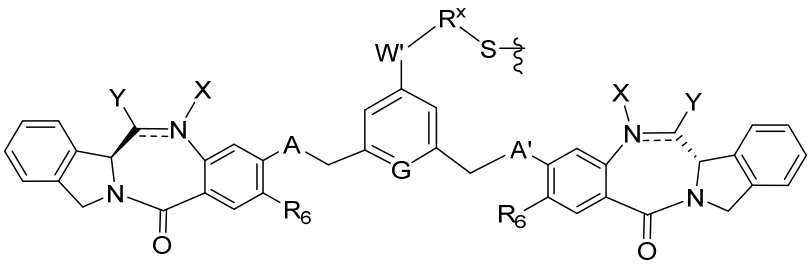
(D-IIA);



(D-IIB);



(D-IIC); or



(D-IID),

or a pharmaceutically acceptable salt thereof, wherein:

the double line == between N and C represents a single bond or a double bond, provided that when it is a double bond, X is absent and Y is -H or a (C_1-C_4)alkyl; and when it is a single bond, X is -H or an amine protecting moiety, Y is -OH or -SO_3H or a pharmaceutically acceptable salt thereof;

X' is selected from the group consisting of -H, -OH, a substituted or unsubstituted linear, branched or cyclic alkyl, alkenyl or alkynyl, phenyl, and an amine-protecting group;

Y' is selected from the group consisting of -H, an oxo group, a substituted or unsubstituted linear, branched or cyclic alkyl, alkenyl or alkynyl;

A and A' are selected from -O- and -S-;

W' is absent, or selected from -O-, -N(R^e)-, -N(R^e)-C(=O)-, -N(C(=O)R^e)-, -S- or -CH_2-S-, -CH_2NR^e-;

R^x is absent or selected from a linear, branched or cyclic alkyl;

R^e is -H, a linear, branched or cyclic alkyl, alkenyl or alkynyl or -$(CH_2$-CH_2-$O)_n$-R^k, wherein R^k is a -H, a linear, branched cyclic alkyl having 1 to 6 carbon atoms, optionally bearing a secondary amino (*e.g.*, -NHR^{101}) or tertiary amino (-$NR^{101}R^{102}$) group or a 5- or 6-membered nitrogen containing heterocycle, wherein R^{101} and R^{102} are each independently a linear, branched, or cyclic alkyl, alkenyl or alkynyl;

n is an integer from 1 to 24;

G is selected from -CH- or -N-;

R_6 is -H, -R, -OR, -SR, -NR'R'', -NO_2, or halogen; and

R, for each occurrence, is independently selected from the group consisting of -H, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl, a polyethylene glycol unit -$(CH_2CH_2O)_n$-R^c, an optionally substituted aryl having 6 to 18 carbon atoms, an optionally substituted 5- to 18-membered heteroaryl ring containing one or more heteroatoms independently selected from nitrogen, oxygen, and sulfur, or an optionally substituted 3- to 18-membered heterocyclic ring containing 1 to 6 heteroatoms independently selected from O, S, N and P;

R' and R'' are each independently selected from -H, -OH, -OR, -NHR, -NR_2, -COR, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl, a polyethylene glycol unit -$(CH_2CH_2O)_n$-R^c, and an optionally substituted 3- to 18-membered heterocyclic ring having 1 to 6 heteroatoms independently selected from O, S, N and P;

R^c is -H or an optionally substituted linear or branched alkyl having 1 to 4 carbon atoms.

12. The method of any one of claims 1-8, wherein the compound of formula (I) is presented by the following formula:

$$L_2'—A—NH—CR^{1A}R^{2A}—S-L_1—D \quad \text{(I-a)},$$

or a pharmaceutically acceptable salt thereof, wherein:

L_2' is a spacer bearing a maleimide moiety;

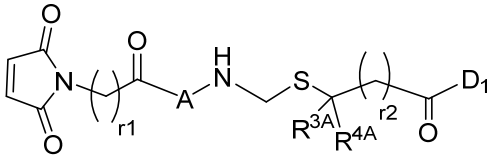
A is an amino acid or a peptide comprising 2 to 20 amino acids;

R^{1A} and R^{2A} are each independently H or a $C_{1\text{-}3}$alkyl;

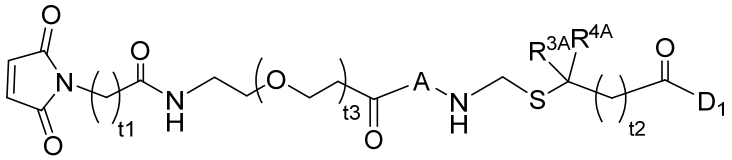
L_1 is a spacer; and

D-L_1-SH is a cytotoxic agent.

13. The method of claim 12, wherein the compound of formula (I) is represented by the following formula:



(I-b); or



(I-c);

or a pharmaceutically acceptable salt thereof, wherein:

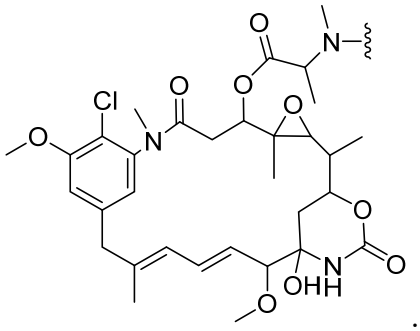
R^{3A} and R^{4A} are each independently H or Me;

r1 and t1 are each independently an integer from 1 to 6;

r2 and t2 are each independently an integer from 1 to 7;

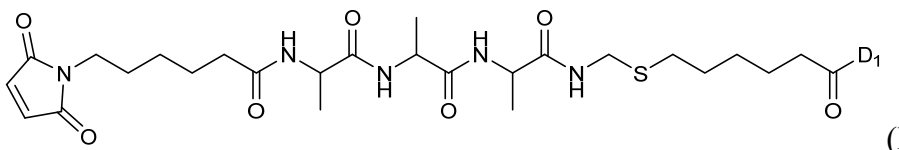
t3 is an integer from 1 to 12;

D_1 is represented by the following formula:

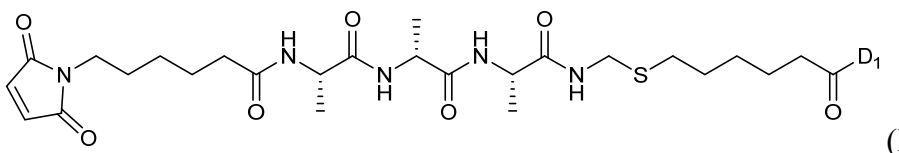


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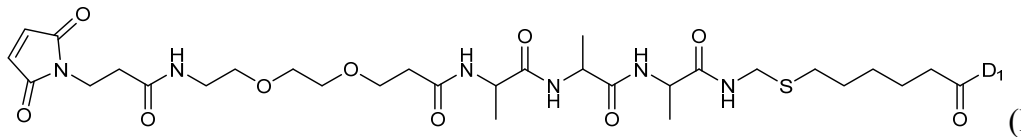
14. The method of claim 13, wherein the compound is represented by the following formula:

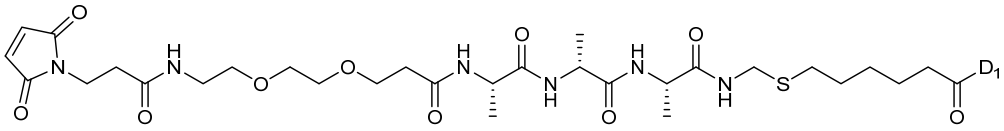


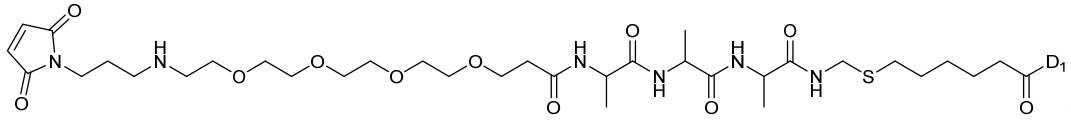
(I10);

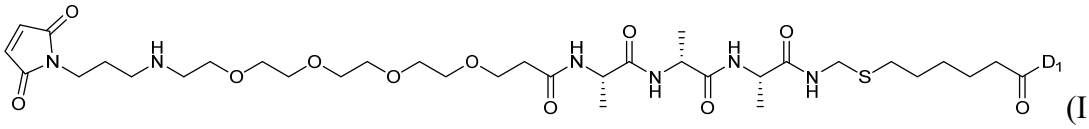


(I11);

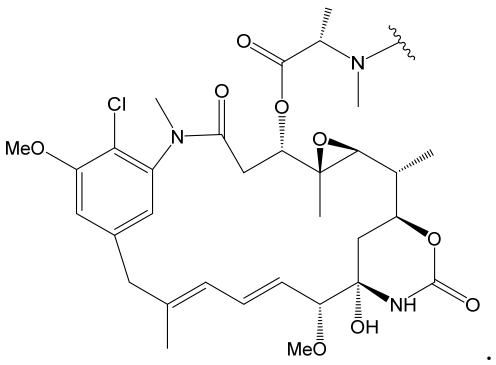

(I12);


(I13);

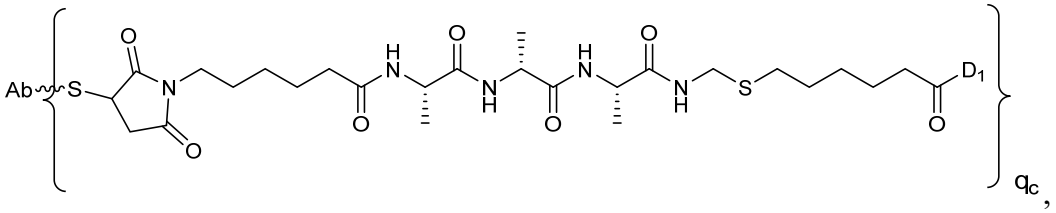

(I14); or


(I15),

wherein D_1 is represented by the following formula:

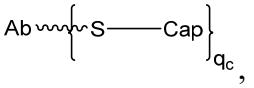

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15. A method of preparing an antibody-cytotoxic agent conjugate (ADC) represented by the following formula:


,

comprising the steps of:

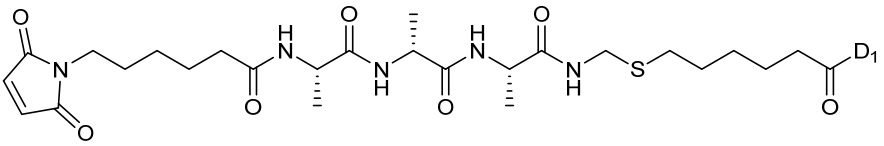
(a) reacting a reducing agent with a capped cysteine-engineered antibody (cCysAb) represented by the following formula:


,

to form a reduced antibody or antigen-binding fragment thereof, wherein the capping agent (Cap) is removed to form a free thiol (-SH) group in the reduced antibody or antigen-binding fragment thereof;

(b) reacting the reduced antibody or antigen-binding fragment thereof with a selective oxidizing agent to form the CysAb, wherein the free thiol group in the engineered cysteine residue is not oxidized; and

(c) reacting the CysAb with a compound represented by the following formula:



(I11),

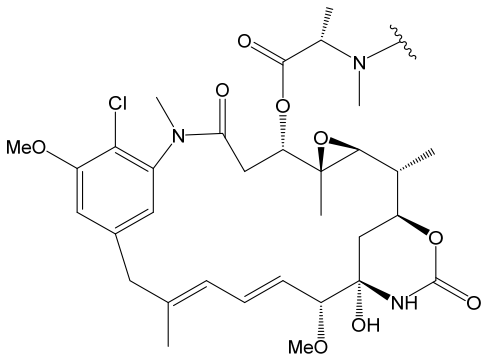
or a pharmaceutically acceptable salt thereof, thereby forming the ADC, wherein the reduced antibody or antigen-binding fragment thereof in step (a) is used in step (b) without purification, and the CysAb or antigen-binding fragment thereof of step (b) is used in step (c) without purification,

wherein:

Ab〰S— is a cysteine-engineered antibody (CysAb) or antigen-binding fragment thereof covalently linked to the cytotoxic agent through the thiol group located on the engineered cysteine residue; and wherein the CysAb or antigen-binding fragment thereof is a humanized anti-ADAM9 antibody or ADAM9-binding fragment thereof comprising a CDR_H1 domain, a CDR_H2 domain, and a CDR_H3 domain and a CDR_L1 domain, a CDR_L2 domain, and a CDR_L3 domain having the sequences of SEQ ID NOs: 24, 25, and 26 and SEQ ID NOs: 21, 22, 23, respectively;

q_c is 1 or 2;

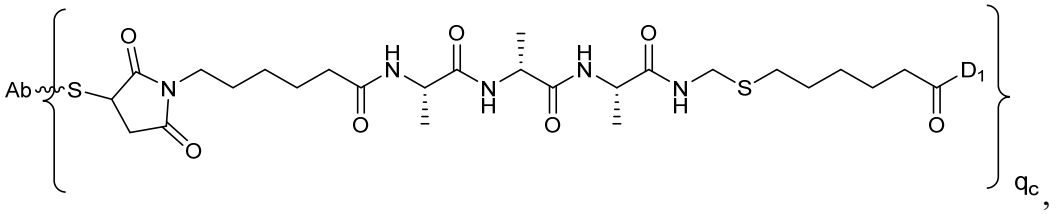
D_1 is represented by the following formula:



, and

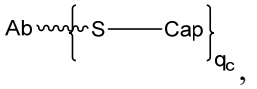
Cap is a capping agent.

16. A continuous method of preparing an antibody-cytotoxic agent conjugate (ADC) represented by the following formula:



comprising the steps of:

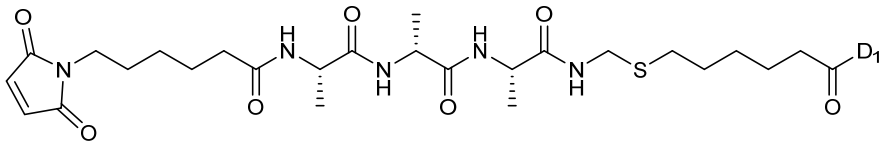
(a) reacting a reducing agent with a capped cysteine-engineered antibody (cCysAb) represented by the following formula:



to form a reduced antibody or antigen-binding fragment thereof, wherein the capping agent (Cap) is removed to form a free thiol (-SH) group in the reduced antibody or antigen-binding fragment thereof;

(b) reacting the reduced antibody or antigen-binding fragment thereof with a selective oxidizing agent to form the CysAb, wherein the free thiol group in the engineered cysteine residue is not oxidized; and

(c) reacting the CysAb with a compound represented by the following formula:



(I11),

or a pharmaceutically acceptable salt thereof, thereby forming the ADC, wherein the reduced antibody or antigen-binding fragment thereof in step (a) is used in step (b) without purification, and the CysAb or antigen-binding fragment thereof of step (b) is used in step (c) without purification, steps (a) to (c) are performed continuously, and

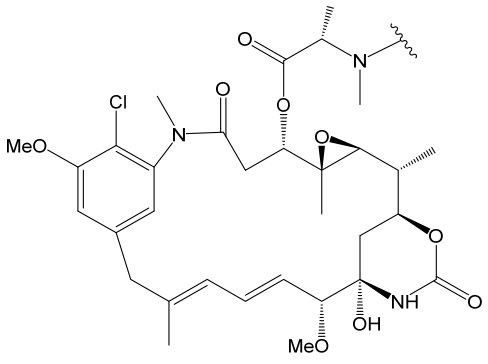
wherein:

 is a cysteine-engineered antibody (CysAb) or antigen-binding fragment thereof covalently linked to the cytotoxic agent through the thiol group located on the engineered cysteine residue; and wherein the CysAb or antigen-binding fragment thereof is a humanized anti-ADAM9 antibody or ADAM9-binding fragment thereof comprising a CDR_H1 domain, a

CDR_H2 domain, and a CDR_H3 domain and a CDR_L1 domain, a CDR_L2 domain, and a CDR_L3 domain having the sequences of SEQ ID NOs: 24, 25, and 26 and SEQ ID NOs: 21, 22, 23, respectively;

q_c is 1 or 2;

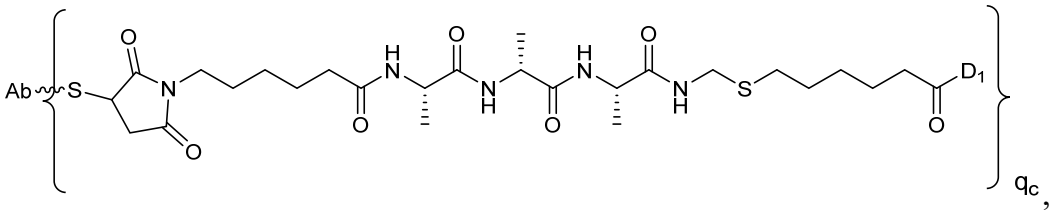
D_1 is represented by the following formula:



, and

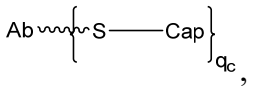
Cap is a capping agent.

17. A continuous method of preparing an antibody-cytotoxic agent conjugate (ADC) represented by the following formula:



comprising the steps of:

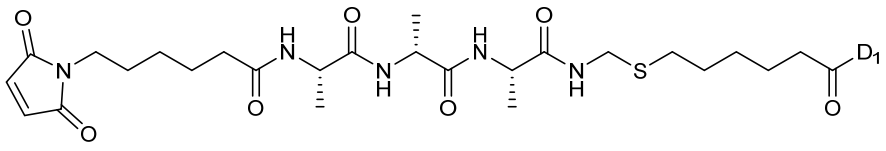
(a) reacting a reducing agent with a capped cysteine-engineered antibody (cCysAb) represented by the following formula:



to form a reduced antibody or antigen-binding fragment thereof, wherein the capping agent (Cap) is removed to form a free thiol (-SH) group in the reduced antibody or antigen-binding fragment thereof;

(b) reacting the reduced antibody or antigen-binding fragment thereof with a selective oxidizing agent to form the CysAb, wherein the free thiol group in the engineered cysteine residue is not oxidized;

(c) reacting the CysAb with a compound represented by the following formula:



(I11),

or a pharmaceutically acceptable salt thereof, thereby forming the ADC; and

(d) removing unconjugated compound of formula (I11);

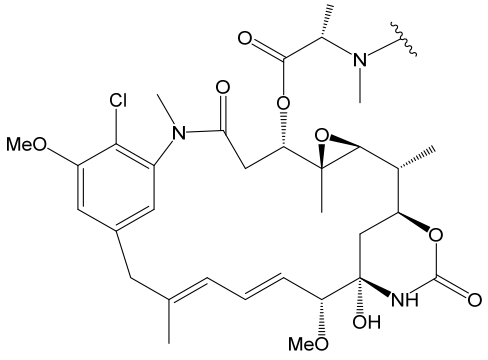
wherein the reduced antibody or antigen-binding fragment thereof in step (a) is used in step (b) without purification, and the CysAb or antigen-binding fragment thereof of step (b) is used in step (c) without purification, steps (a) to (d) are performed continuously, and

wherein:

Ab〰S— is a cysteine-engineered antibody (CysAb) or antigen-binding fragment thereof covalently linked to the cytotoxic agent through the thiol group located on the engineered cysteine residue; and wherein the CysAb or antigen-binding fragment thereof is a humanized anti-ADAM9 antibody or ADAM9-binding fragment thereof comprising a CDR_H1 domain, a CDR_H2 domain, and a CDR_H3 domain and a CDR_L1 domain, a CDR_L2 domain, and a CDR_L3 domain having the sequences of SEQ ID NOs: 24, 25, and 26 and SEQ ID NOs: 21, 22, 23, respectively;

q_c is 1 or 2;

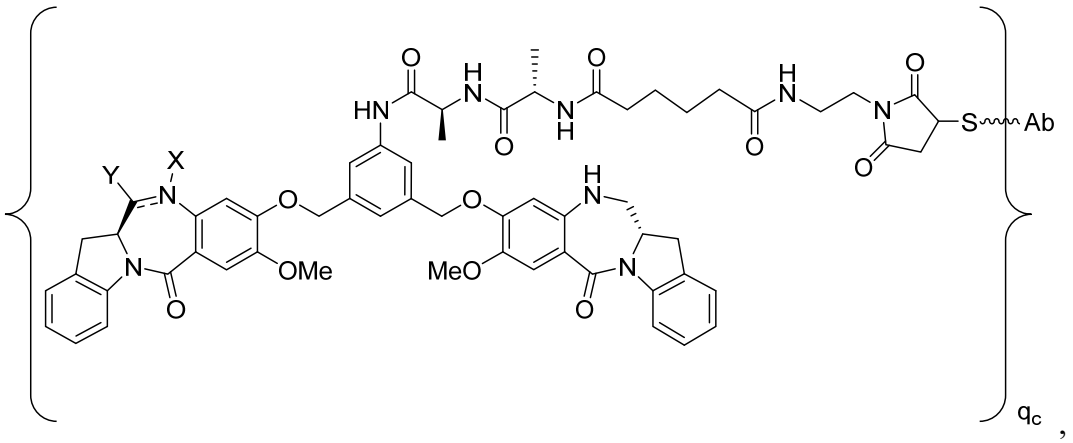
D_1 is represented by the following formula:



, and

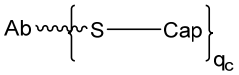
Cap is a capping agent.

18. A method of preparing an antibody-cytotoxic agent conjugate (ADC) represented by the following formula:



comprising the steps of:

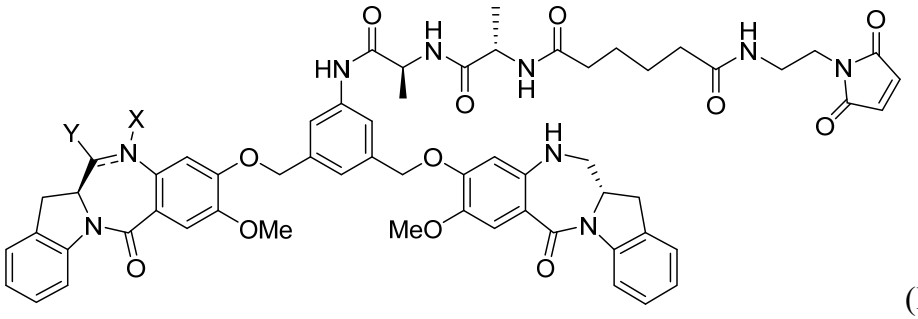
(a) reacting a reducing agent with a capped cysteine-engineered antibody (cCysAb) represented by the following formula:



to form a reduced antibody or antigen-binding fragment thereof, wherein the capping agent is removed to form a free thiol (-SH) group in the reduced antibody or antigen-binding fragment thereof;

(b) reacting the reduced antibody or antigen-binding fragment thereof with a selective oxidizing agent to form the CysAb, wherein the free thiol group in the engineered cysteine residue is not oxidized; and

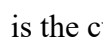
(c) reacting the CysAb with a compound represented by the following formula:



(I1a),

or a pharmaceutical acceptable salt thereof, thereby forming the ADC, wherein the reduced antibody or antigen-binding fragment thereof in step (a) is used in step (b) without purification, and the CysAb or antigen-binding fragment thereof of step (b) is used in step (c) without purification, wherein:

the double line == between N and C represents a single bond or a double bond, provided that when it is a double bond, X is absent and Y is -H, and when it is a single bond, X is -H, and Y is $-SO_3M$, and M is H^+ or a cation;

 is the cysteine-engineered antibody or antigen-binding fragment thereof covalently linked to the cytotoxic agent through the thiol group located on the engineered cysteine residue; wherein the cysteine-engineered antibody is an anti-CD123 antibody or antigen-binding fragment comprising:

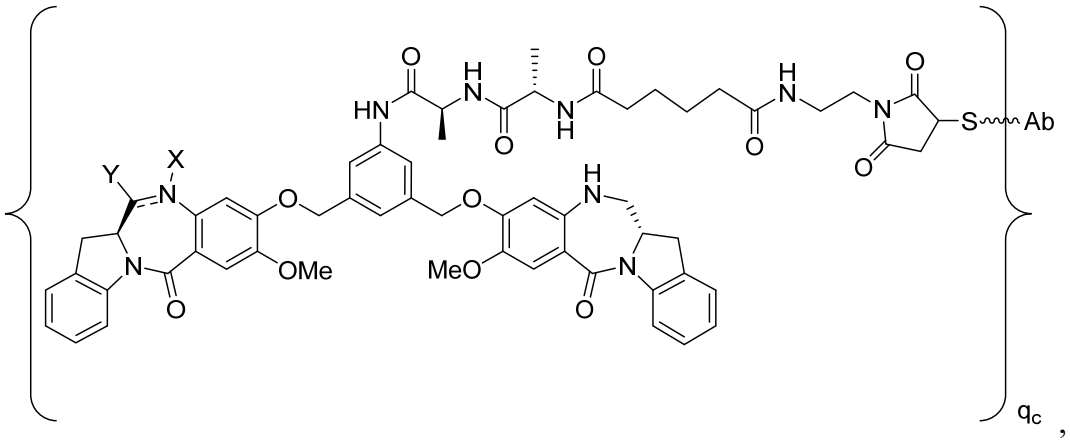
a) an immunoglobulin heavy chain variable region comprising a CDR_H1 having an amino acid sequence set forth in SEQ ID NO:4, a CDR_H2 having an amino acid sequence set forth in SEQ ID NO:5, and a CDR_H3 having an amino acid sequence set forth in SEQ ID NO:6; and

b) an immunoglobulin light chain variable region comprising a CDR_L1 having an amino acid sequence set forth in SEQ ID NO:1, a CDR_L2 having an amino acid sequence set forth in SEQ ID NO:2, and a CDR_L3 having an amino acid sequence set forth in SEQ ID NO:3;

q_c is 1 or 2; and

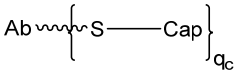
Cap is a capping agent.

19. A continuous method of preparing an antibody-cytotoxic agent conjugate (ADC) represented by the following formula:



comprising the steps of:

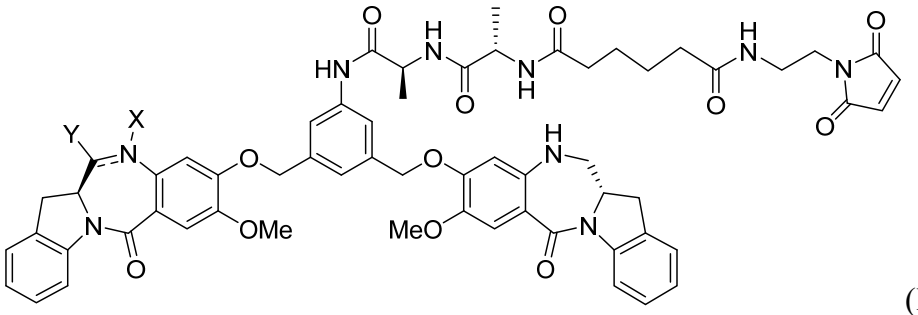
(a) reacting a reducing agent with a capped cysteine-engineered antibody (cCysAb) represented by the following formula:



to form a reduced antibody or antigen-binding fragment thereof, wherein the capping agent is removed to form a free thiol (-SH) group in the reduced antibody or antigen-binding fragment thereof;

(b) reacting the reduced antibody or antigen-binding fragment thereof with a selective oxidizing agent to form the CysAb, wherein the free thiol group in the engineered cysteine residue is not oxidized; and

(c) reacting the CysAb with a compound represented by the following formula:



(I1a),

or a pharmaceutical acceptable salt thereof, thereby forming the ADC, wherein the reduced antibody or antigen-binding fragment thereof in step (a) is used in step (b) without purification, and the CysAb or antigen-binding fragment thereof of step (b) is used in step (c) without purification; and steps (a) to (c) are performed continuously,

wherein:

the double line == between N and C represents a single bond or a double bond, provided that when it is a double bond, X is absent and Y is -H, and when it is a single bond, X is -H, and Y is $-SO_3M$, and M is H^+ or a cation;

Ab〰S— is the cysteine-engineered antibody or antigen-binding fragment thereof covalently linked to the cytotoxic agent through the thiol group located on the engineered cysteine residue; wherein the cysteine-engineered antibody is an anti-CD123 antibody or antigen-binding fragment comprising:

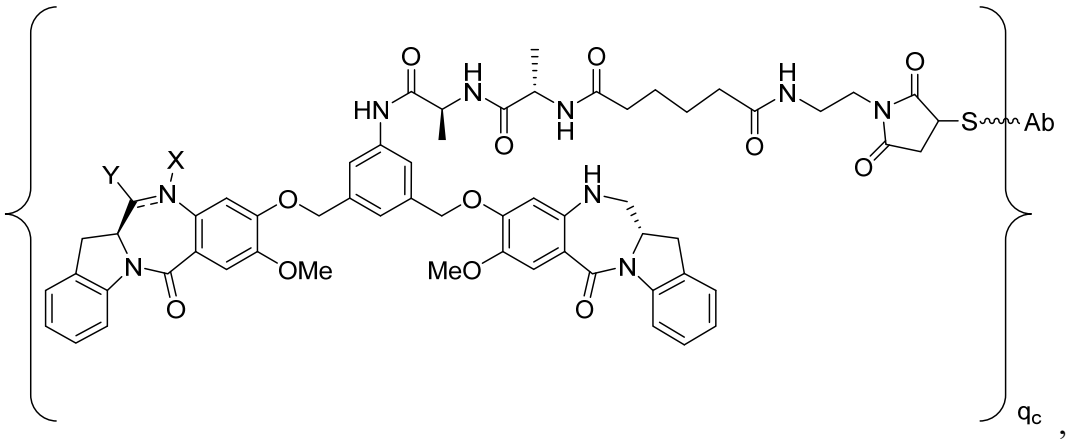
a) an immunoglobulin heavy chain variable region comprising a CDR_H1 having an amino acid sequence set forth in SEQ ID NO:4, a CDR_H2 having an amino acid sequence set forth in SEQ ID NO:5, and a CDR_H3 having an amino acid sequence set forth in SEQ ID NO:6; and

b) an immunoglobulin light chain variable region comprising a CDR_L1 having an amino acid sequence set forth in SEQ ID NO:1, a CDR_L2 having an amino acid sequence set forth in SEQ ID NO:2, and a CDR_L3 having an amino acid sequence set forth in SEQ ID NO:3;

q_c is 1 or 2; and

Cap is a capping agent.

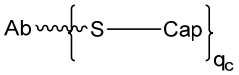
20. A continuous method of preparing an antibody-cytotoxic agent conjugate (ADC) represented by the following formula:



,

comprising the steps of:

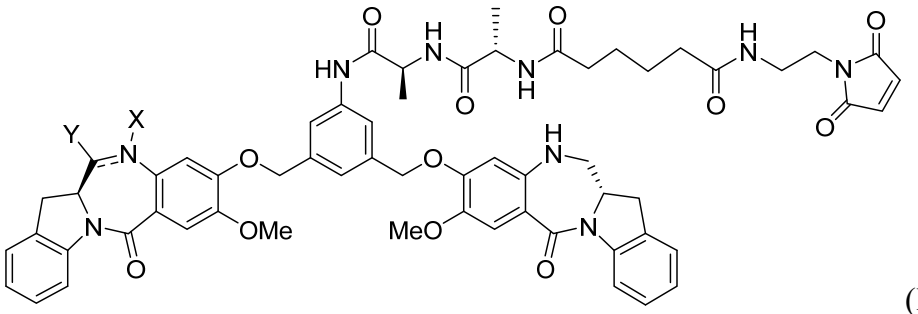
(a) reacting a reducing agent with a capped cysteine-engineered antibody (cCysAb) represented by the following formula:



to form a reduced antibody or antigen-binding fragment thereof, wherein the capping agent is removed to form a free thiol (-SH) group in the reduced antibody or antigen-binding fragment thereof;

(b) reacting the reduced antibody or antigen-binding fragment thereof with a selective oxidizing agent to form the CysAb, wherein the free thiol group in the engineered cysteine residue is not oxidized; and

(c) reacting the CysAb with a compound represented by the following formula:



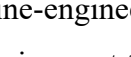
(I1a),

or a pharmaceutical acceptable salt thereof, thereby forming the ADC; and

(d) removing unconjugated compound of formula (I1a), wherein the reduced antibody or antigen-binding fragment thereof in step (a) is used in step (b) without purification, and the CysAb or antigen-binding fragment thereof of step (b) is used in step (c) without purification; and steps (a) to (d) are performed continuously,

wherein:

the double line == between N and C represents a single bond or a double bond, provided that when it is a double bond, X is absent and Y is -H, and when it is a single bond, X is -H, and Y is $-SO_3M$, and M is H^+ or a cation;

 is the cysteine-engineered antibody or antigen-binding fragment thereof covalently linked to the cytotoxic agent through the thiol group located on the engineered cysteine residue; wherein the cysteine-engineered antibody is an anti-CD123 antibody or antigen-binding fragment comprising:

a) an immunoglobulin heavy chain variable region comprising a CDR_H1 having an amino acid sequence set forth in SEQ ID NO:4, a CDR_H2 having an amino acid sequence set forth in SEQ ID NO:5, and a CDR_H3 having an amino acid sequence

set forth in SEQ ID NO:6; and

b) an immunoglobulin light chain variable region comprising a CDR_L1 having an amino acid sequence set forth in SEQ ID NO:1, a CDR_L2 having an amino acid sequence set forth in SEQ ID NO:2, and a CDR_L3 having an amino acid sequence set forth in SEQ ID NO:3;

q_c is 1 or 2; and

Cap is a capping agent.