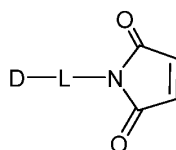


CLAIMS

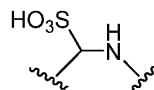
WE CLAIM:

1. A method of preparing a cell-binding agent-cytotoxic agent conjugate comprising the steps of:

(a) reacting an imine-moiety in an imine-containing cytotoxic agent represented by the following formula:



or a pharmaceutically acceptable salt thereof, with sulfur dioxide, a bisulfite salt or a metabisulfite salt in an aqueous solution at a pH of 1.9 to 5.0 to form a modified cytotoxic agent comprising a modified imine moiety represented by the following formula:



or a pharmaceutically acceptable salt thereof; and

(b) reacting the modified cytotoxic agent with a cell-binding agent to form the cell-binding agent-cytotoxic agent conjugate,

wherein D is an imine-containing benzodiazepine compound; and L is a linker.

2. The method of claim 1, wherein D is an imine-containing indolinobenzodiazepine

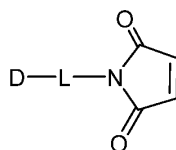
3. The method of claim 1 or 2, wherein the reaction of step (a) is carried out at a pH of 2.9 to 4.0.

4. The method of claim 1 or 2, wherein the reaction of step (a) is carried out at a pH of 2.9 to 3.7.

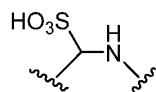
5. The method of claim 1 or 2, wherein the reaction of step (a) is carried out at a pH of 3.1 to 3.5.

6. The method of claim 1 or 2, wherein the reaction of step (a) is carried out at a pH of 3.2 to 3.4.

7. The method of claim 1 or 2, wherein the reaction of step (a) is carried out at a pH of 3.3.
8. The method of any one of claims 1-7, wherein the reaction of step (a) is carried out in the presence of a buffer solution.
9. The method of claim 8, wherein the buffer solution is a citrate buffer, an acetate buffer, a succinate buffer, and a phosphate buffer.
10. The method of claim 8, wherein the buffer solution is a succinate buffer.
11. The method of any one of claim 1-7, wherein the reaction of step (a) is carried out in the absence of a buffer solution.
12. A method of preparing a cell-binding agent-cytotoxic agent conjugate comprising the steps of:
- (a) reacting an imine-moiety in an imine-containing cytotoxic agent represented by the following formula:



or a pharmaceutically acceptable salt thereof, with sulfur dioxide, a bisulfite salt or a metabisulfite salt in an aqueous solution in the absence of a buffer to form a modified cytotoxic agent comprising a modified imine moiety represented by the following formula:



or a pharmaceutically acceptable salt thereof; and

- (b) reacting the modified cytotoxic agent with a cell-binding agent to form the cell-binding agent-cytotoxic agent conjugate,

wherein D is an imine-containing benzodiazepine compound; and L is a linker.

13. The method of any one of claims 1-12, wherein 0.5 to 5 equivalents of the bisulfite salt or 0.25 to 2.5 equivalents of the metabisulfite salt is reacted with 1 equivalent of the imine-containing cytotoxic agent.

14. The method of claim 13, wherein 0.8 to 2.0 equivalents of the bisulfite salt or 0.4 to 1.0 equivalents of the metabisulfite salt is reacted with 1 equivalent of the imine-containing cytotoxic agent.
15. The method of claim 13, wherein 1.1 to 1.6 equivalents of the bisulfite salt or 0.55 to 0.8 equivalents of the metabisulfite salt is reacted with 1 equivalent of the imine-containing cytotoxic agent.
16. The method of claim 13, wherein 1.4 equivalents of the bisulfite salt or 0.7 equivalent of the metabisulfite salt is reacted with 1 equivalent of the imine-containing cytotoxic agent.
17. The method of claim 1 or 2, wherein the reaction of step (a) is carried out at a pH of 2.9 to 3.7 and 1.0 to 1.8 equivalents of the bisulfite salt or 0.5 to 0.9 equivalent of the metabisulfite salt is reacted with 1 equivalent of the imine-containing cytotoxic agent.
18. The method of claim 1 or 2, wherein the reaction of step (a) is carried out at a pH of 3.1 to 3.5 and 1.1 to 1.6 equivalents of the bisulfite salt or 0.55 to 0.8 equivalent of the metabisulfite salt is reacted with 1 equivalent of the imine-containing cytotoxic agent.
19. The method of claim 1 or 2, wherein the reaction of step (a) is carried out at a pH of 3.3 and 1.4 equivalents of the bisulfite salt or 0.7 equivalent of the metabisulfite salt is reacted with 1 equivalent of the imine-containing cytotoxic agent.
20. The method of any one of claims 1-19, wherein the reaction of step (a) is carried out in a mixture of an organic solvent and water.
21. The method of claim 20, wherein the reaction of step (a) is carried out in a mixture of dimethylacetamide (DMA) and water.
22. The method of claim 20, wherein the reaction of step (a) is carried out in a mixture of DMA and water, wherein the volume ratio of DMA and water is 1:1.
23. The method of any one of claims 1-22, wherein the reaction of step (a) is carried out for 1 minute to 48 hours.
24. The method of claim 23, wherein the reaction of step (a) is carried out for 1 hour to 10 hours.

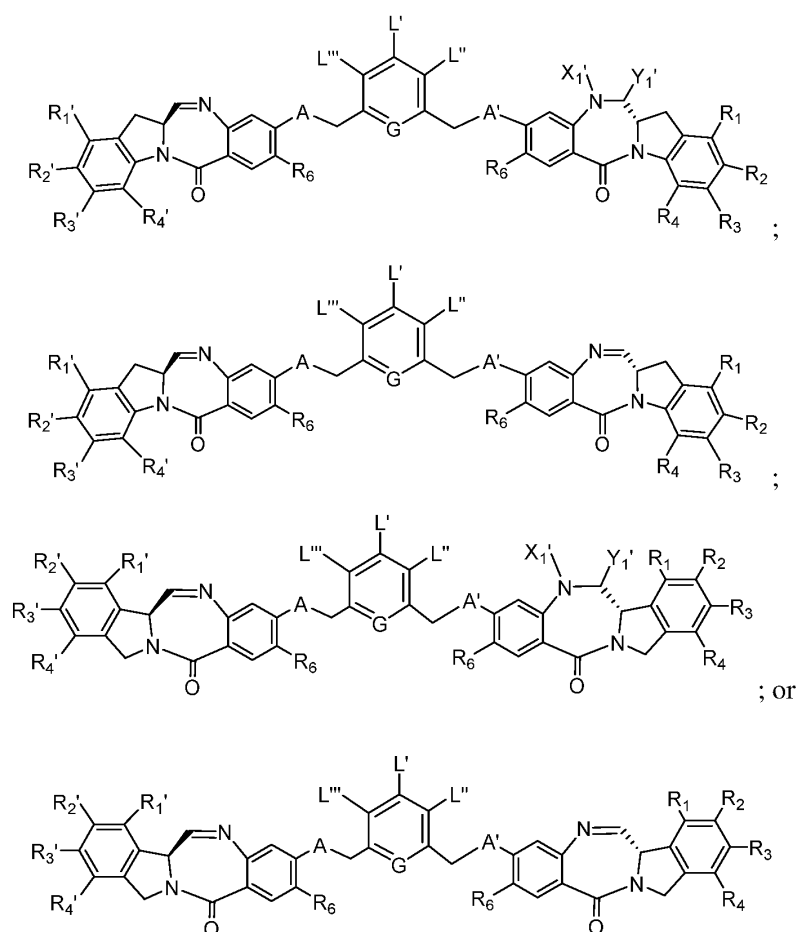
25. The method of claim 24, wherein the reaction of step (a) is carried out for 1 hour to 4 hours.
26. The method of any one of claims 1-25, wherein in step (a), the imine-containing cytotoxic agent is reacted with sodium bisulfite.
27. The method of any one of claims 1-25, wherein in step (a), the imine-containing cytotoxic agent is reacted with sodium metabisulfite.
28. The method of any one of claims 1-27, wherein the modified cytotoxic agent is not purified before reacting with the cell-binding agent in step (b).
29. The method of any one of claims 1-27, wherein the modified cytotoxic agent is purified before reacting with the cell-binding agent in step (b).
30. The method of any one of claims 1-29, wherein the reaction of step (b) is carried out at a pH of 4 to 9.
31. The method of claim 30, wherein the reaction of step (b) is carried out at a pH of 5 to 8.5.
32. The method of claim 30, wherein the reaction of step (b) is carried out at a pH of 5.5 to 6.5.
33. The method of any one of claims 1-32, wherein the conjugate is purified by tangential flow filtration to yield a purified conjugate.
34. The method of claim 33, wherein the purified conjugate is formulated in a formulation buffer comprising a bisulfite salt.
35. The method of claim 34, wherein the formulation buffer comprises 10 to 200 μM of sodium bisulfite.
36. The method of claim 33, wherein the formulation buffer comprises 40 to 80 μM of sodium bisulfite.
37. The method of claim 33, wherein the formulation buffer comprises 50 μM of sodium bisulfite.

38. The method of any one of claims 34-37, wherein the formulation buffer is at a pH of 4 to 5.

39. The method of claim 38, wherein the formulation buffer is at a pH of 4.2.

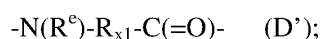
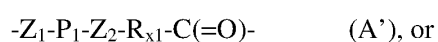
40. The method of any one of claims 1-39, wherein D is an imine-containing indolinobenzodiazepine compound.

41. The method of any one of claims 1-40, wherein D is represented by the following formula:



or a pharmaceutically acceptable salt thereof, wherein:

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



and the other two are each independently selected from -H, an optionally

substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, 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 having from 1 to 10 carbon atoms;

R^e is $-H$, a linear, branched or cyclic alkyl, alkenyl or alkynyl having 1 to 10 carbon atoms 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 having 1 to 10 carbon atoms;

R , for each occurrence, is independently selected from the group consisting of $-H$, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, 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 having from 1 to 10 carbon atoms, 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 having from 1 to 10 carbon atoms, 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 having from 1 to 10 carbon atoms, 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 having from 1 to 10 carbon atoms, a polyethylene glycol unit - $(CH_2CH_2O)_n-R^c$, halogen, guanidinium [$-NH(C=NH)NH_2$], -OR, -NR'R'', -NO₂, -NCO, -NR'COR'', -SR, -SOR', -SO₂R', -SO₃H, -OSO₃H, -SO₂NR'R'', cyano, an azido, -COR', -OCOR', and -OCONR'R'';

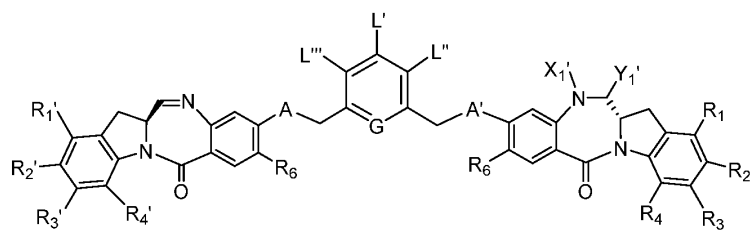
R_6 is -H, -R, -OR, -SR, -NR'R'', -NO₂, or halogen;

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-, -NR₅ and -CRR'N(R₅)-; and

R_5 for each occurrence is independently -H or an optionally substituted linear or branched alkyl having 1 to 10 carbon atoms.

42. The method of claim 41, wherein D is represented by the following formula:



or a pharmaceutically acceptable salt thereof

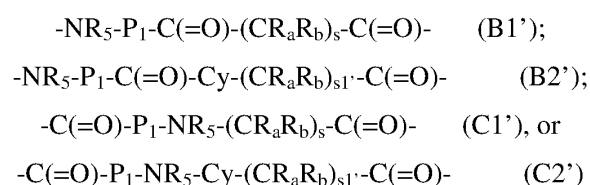
43. The method of claim 41 or 42, wherein one of L' , L'' and L''' is represented by formula (A') or (D'), and the others are -H, an linear or branched alkyl having from 1 to 6 carbon atoms, halogen, -OH, (C₁-C₆)alkoxy, or -NO₂.

44. The method of claim 43, wherein L' is represented by formula (A'); and L'' and L''' are both -H.

45. The method of claim 43, L' is represented by formula (D'); and L'' and L''' are both -H.

46. The method of any one of claims 41-45, wherein R_{x1} is a linear, branched or cyclic alkyl having 1 to 6 carbon atoms optionally substituted with halogen, -OH, -SO₃H, (C₁-C₃)alkyl, (C₁-C₃)alkoxy, halo(C₁-C₃)alkyl, or a charged substituent or an ionizable group Q.

47. The method of any one of claims 43-46, wherein L' is represented by the following formula:



wherein:

R_a and R_b, for each occurrence, are each independently -H, (C₁-C₃)alkyl or a charged substituent or an ionizable group Q;

s is an integer from 1 to 6;

s1' is 0 or an integer from 1 to 6; and

Cy is a cyclic alkyl having 5 or 6 ring carbon atoms optionally substituted with halogen, -OH, (C₁-C₃)alkyl, (C₁-C₃)alkoxy, or halo(C₁-C₃)alkyl.

48. The method of claim 47, wherein R_a and R_b are both H; Cy in formulas (B2') and (C2') is cyclohexane; and R₅ is H or Me.

49. The method of claim 47 or 48, wherein s1' is 0 or 1.

50. The method of any one of claims 41-49, wherein the charged substituent or an ionizable group Q is i) -SO₃H, -Z'-SO₃H, -OPO₃H₂, -Z'-OPO₃H₂, -PO₃H₂, -Z'-PO₃H₂, -CO₂H, -Z'-CO₂H, -NR₁₁R₁₂, or -Z'-NR₁₁R₁₂, or a pharmaceutically acceptable salt thereof; or, ii) -N⁺R₁₄R₁₅R₁₆X⁻ or -Z'-N⁺R₁₄R₁₅R₁₆X⁻; Z' is an optionally substituted alkylene, an optionally substituted cycloalkylene or an optionally substituted phenylene; R₁₄ to R₁₆ are

each independently an optionally substituted alkyl; and X⁻ is a pharmaceutically acceptable anion.

51. The method of claim 50, wherein Q is -SO₃H or a pharmaceutically acceptable salt thereof.

52. The method of any one of claims 41-51, wherein P₁ is a peptide containing 2 to 10 amino acid residues.

53. The method of claim 52, wherein P₁ is a peptide containing 2 to 5 amino acid residues.

54. The method of claim 53, wherein P₁ is Gly-Gly-Gly, Ala-Val, Val-Cit, Val-Lys, Phe-Lys, Lys-Lys, Ala-Lys, Phe-Cit, Leu-Cit, Ile-Cit, Trp, Cit, Phe-Ala, Phe-N⁹-tosyl-Arg, Phe-N⁹-nitro-Arg, Phe-Phe-Lys, D-Phe-Phe-Lys, Gly-Phe-Lys, Leu-Ala-Leu, Ile-Ala-Leu, Val-Ala-Val, Ala-Leu-Ala-Leu (SEQ ID NO: 1), β-Ala-Leu-Ala-Leu (SEQ ID NO: 2), Gly-Phe-Leu-Gly (SEQ ID NO: 3), Val-Arg, Arg-Arg, Val-D-Cit, Val-D-Lys, Val-D-Arg, D-Val-Cit, D-Val-Lys, D-Val-Arg, D-Val-D-Cit, D-Val-D-Lys, D-Val-D-Arg, D-Arg-D-Arg, Ala-Ala, Ala-D-Ala, D-Ala-Ala, D-Ala-D-Ala, Ala-Met, Gln-Val, Asn-Ala, Gln-Phe and Gln-Ala.

55. The method of claim 54, wherein P₁ is Gly-Gly-Gly, Ala-Val, Ala-Ala, Ala-D-Ala, D-Ala-Ala, or D-Ala-D-Ala.

56. The method of any one of claims 41-55, wherein X₁' is selected from the group consisting of -H, -OH, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, and phenyl; and Y₁' is selected from the group consisting of -H, an oxo group, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms.

57. The method of claim 56, wherein X₁' is -H, -OH, (C₁-C₃)alkyl, halo(C₁-C₃)alkyl, or phenyl; and Y₁' is -H, an oxo group, (C₁-C₃)alkyl or halo(C₁-C₃)alkyl..

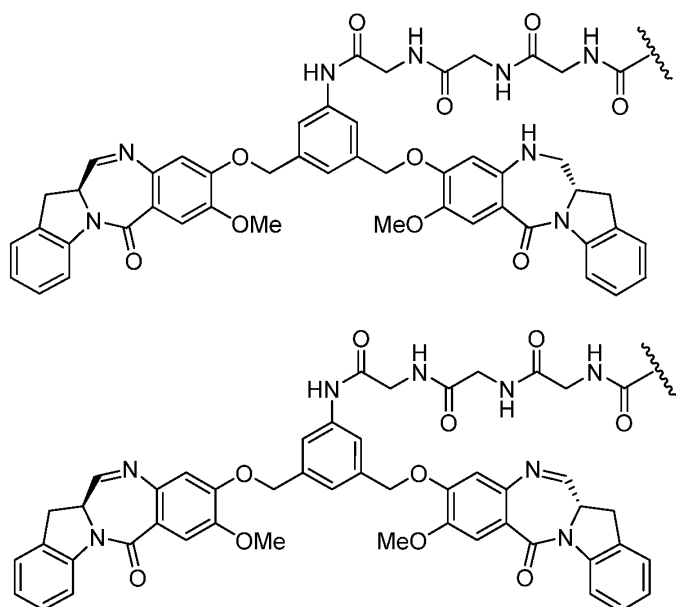
58. The method of claim 56, wherein X₁' is -H, -OH or -Me; and Y₁' is -H or oxo.

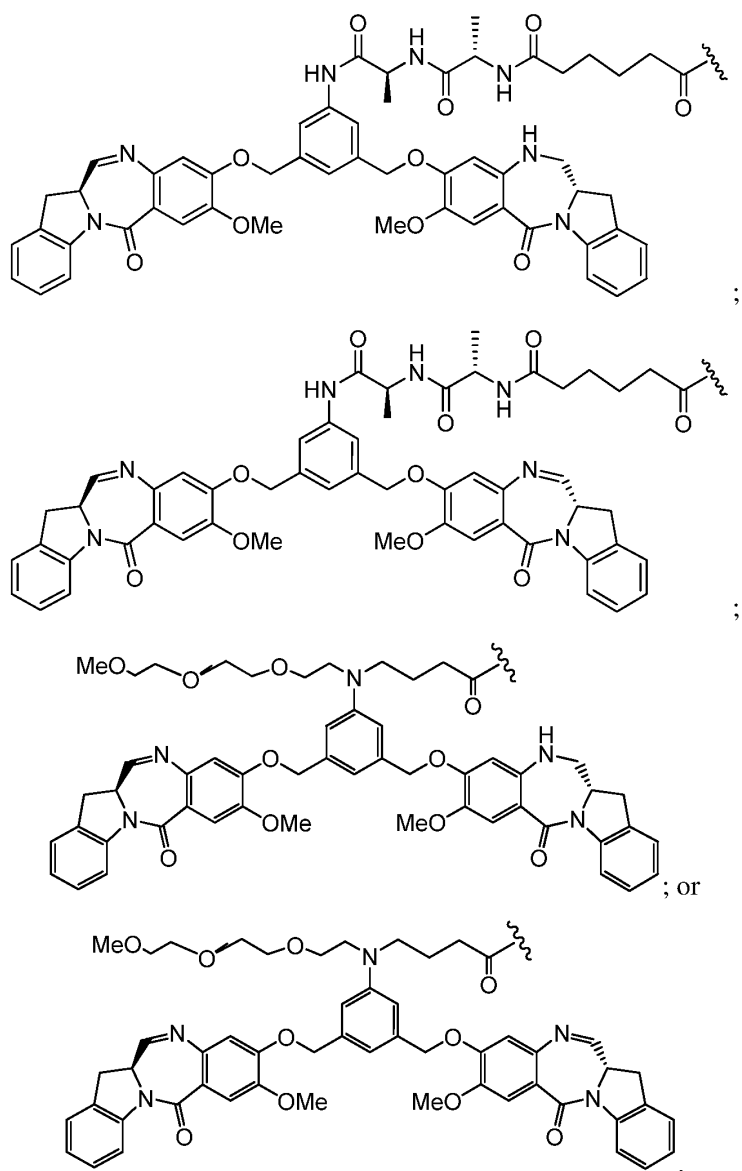
59. The method of claim 56, wherein X₁' is -H; and Y₁' is -H.

60. The method of any one of claims 41-59, wherein A and A' are the same or different, and are selected from -O- and -S-.

61. The method of claim 60, wherein A and A' are -O-.

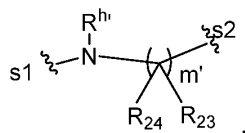
62. The method of any one of claims 41-61, wherein R₆ is -OMe.
63. The method of any one of claims 41-62, wherein R₁, R₂, R₃, R₄, R₁', R₂', R₃' and R₄' are independently -H, halogen, -NO₂, -OH, (C₁-C₃)alkyl, halo(C₁-C₃)alkyl or (C₁-C₃)alkoxy.
64. The method of claim 63, wherein R₁, R₂, R₃, R₄, R₁', R₂', R₃' and R₄' are all -H.
65. The method of any one of claims 41-64, wherein R, R', R'' and R₅ are each independently -H or (C₁-C₃)alkyl.
66. The method of any one of claims 41-55, wherein:
R₁, R₂, R₃, R₄, R₁', R₂', R₃' and R₄' are all -H;
R₆ is -OMe;
X₁' and Y₁' are both -H; and
A and A' are -O-.
67. The method of claim 41, wherein D is represented by the following structural formula:





or a pharmaceutically acceptable salt thereof.

68. The method of any one of claims 1-67, wherein -L- is represented by the following structural formula:



or a pharmaceutically acceptable salt thereof, wherein:

s1 is the site covalently linked to D; s2 is the site covalently linked to the maleimide group;

R_{23} and R_{24} , for each occurrence, are independently H or an optionally substituted alkyl;

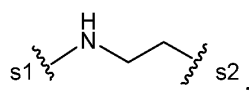
m' is an integer between 0 and 10; and

$R^{h'}$ is H or an optionally substituted alkyl.

69. The method of claim 68, wherein R_{23} and R_{24} are both H; and m' is an integer between 1 and 6.

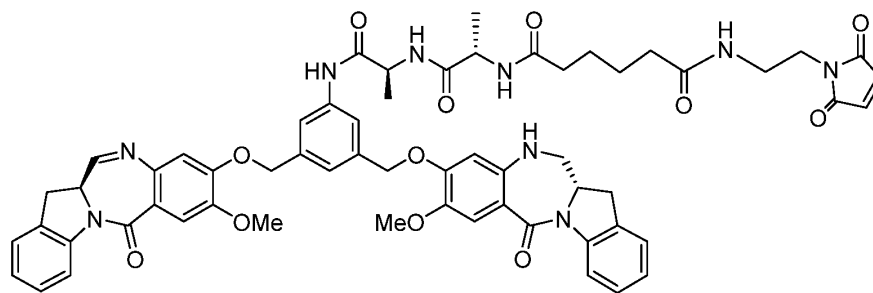
70. The method of claim 68 or 69, wherein $R^{h'}$ is H.

71. The method of claim 68, wherein L is represented by the following structural formula:

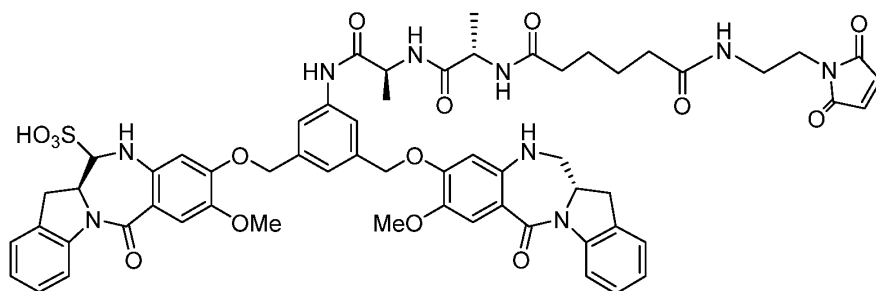


or a pharmaceutically acceptable salt thereof.

72. The method of any one of claims 1-40, wherein the imine-containing cytotoxic agent is represented by the following formula:

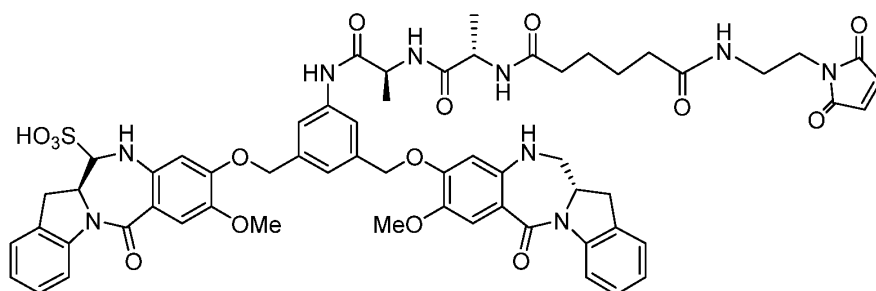


or a pharmaceutically acceptable salt thereof, and the modified cytotoxic agent is represented by the following formula:



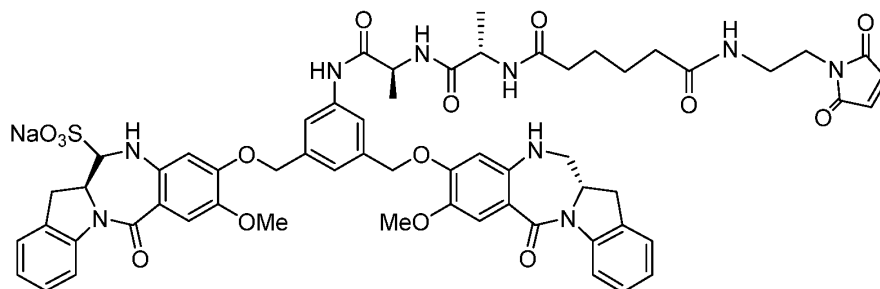
or a pharmaceutically acceptable salt thereof.

73. The method of claim 72, wherein the modified cytotoxic agent is represented by the following formula:

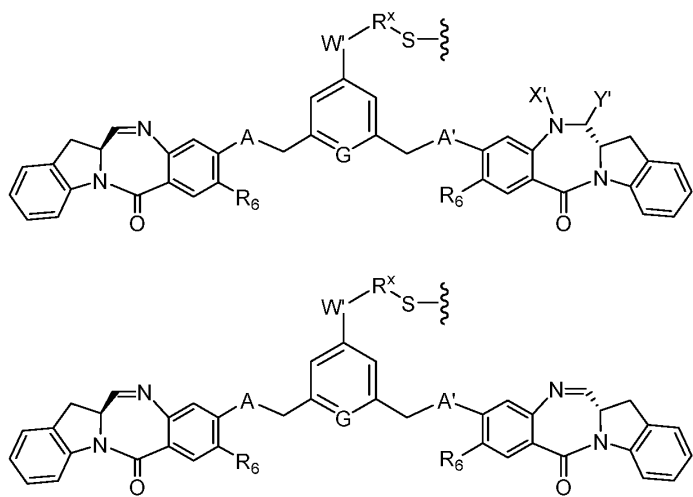


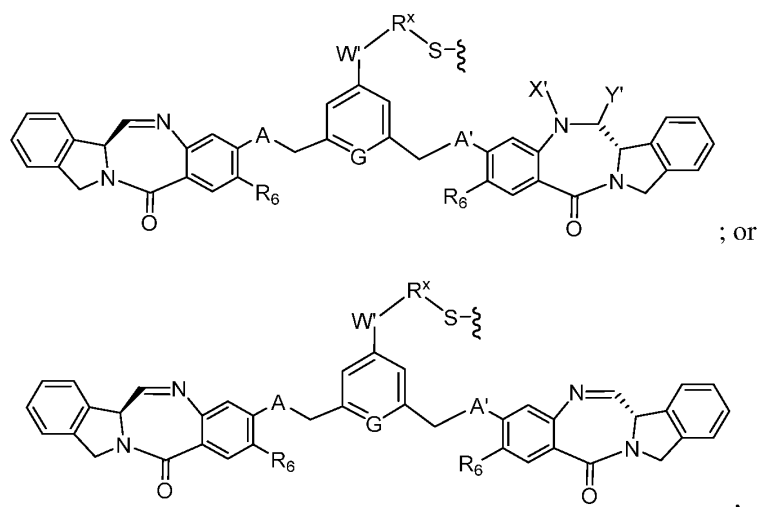
or a sodium or potassium salt thereof.

74. The method of claim 73, wherein the modified cytotoxic agent is represented by the following formula:



75. The method of any one of claims 1-40, wherein D is represented by the following formula:





or a pharmaceutically acceptable salt thereof, wherein:

X' is selected from the group consisting of -H, -OH, a substituted or unsubstituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, 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 having from 1 to 10 carbon atoms;

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 -CH₂-S-, -CH₂NR^e-;

R^x is absent or selected from a linear, branched or cyclic alkyl having 1 to 10 carbon atoms;

R^e is -H, a linear, branched or cyclic alkyl, alkenyl or alkynyl having 1 to 10 carbon atoms or -(CH₂-CH₂-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.*, -NHR¹⁰¹) or tertiary amino (-NR¹⁰¹R¹⁰²) group or a 5- or 6-membered nitrogen containing heterocycle, wherein R¹⁰¹ and R¹⁰² are each independently a linear, branched, or cyclic alkyl, alkenyl or alkynyl having 1 to 10 carbon atoms;

n is an integer from 1 to 24;

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

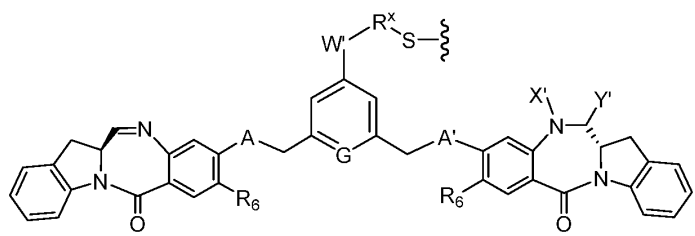
R₆ is -H, -R, -OR, -SR, -NR'R'', -NO₂, or halogen; and

R is -H, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl

having from 1 to 10 carbon atoms or a PEG group $-(\text{CH}_2\text{CH}_2\text{O})_n-\text{R}^c$, wherein n is an integer from 1 to 24, and R^c is a linear or branched alkyl having 1 to 4 carbon atoms;

R' and R'' are each independently selected from $-\text{H}$, $-\text{OH}$, $-\text{OR}$, $-\text{NRR}^g$, $-\text{COR}$, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, an optionally substituted aryl having from 6 to 18 carbon atoms, an optionally substituted 3- to 18-membered heterocyclic ring having 1 to 6 heteroatoms selected from O, S, N and P, a PEG group $-(\text{CH}_2\text{CH}_2\text{O})_n-\text{R}^c$, and R^g is $-\text{H}$, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms or a PEG group $-(\text{CH}_2\text{CH}_2\text{O})_n-\text{R}^c$.

76. The method of claim 75, wherein D is represented by the following structural formula:



or a pharmaceutically acceptable salt thereof.

77. The method of claim 75 or 76, wherein:

X' and Y' are both $-\text{H}$;

A and A' are both $-\text{O}-$;

R_6 is $-\text{OMe}$;

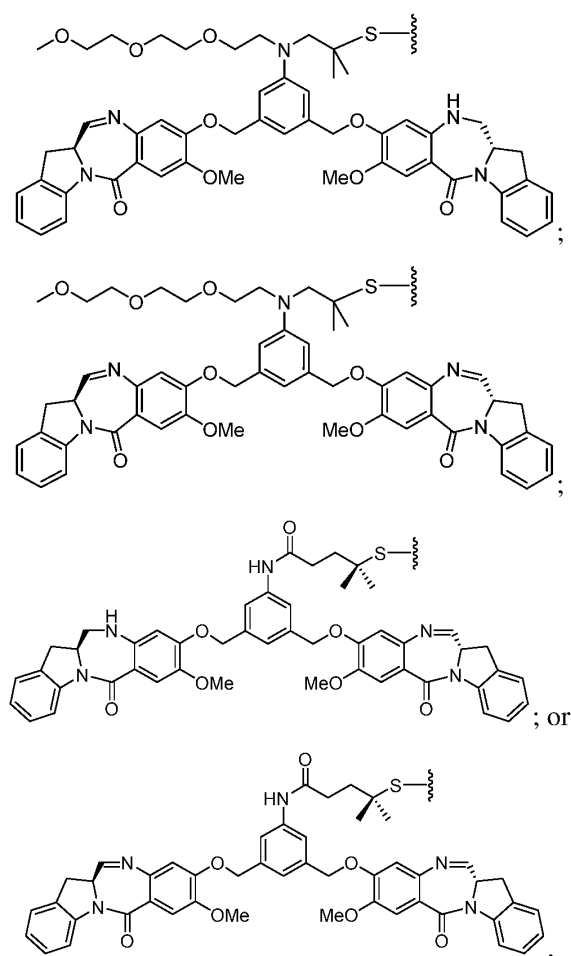
W' is $-\text{N}(\text{R}^c)-$ or $-\text{N}(\text{R}^c)-\text{C}(=\text{O})-$;

R^c is $-\text{H}$, a linear or branched alkyl having 1 to 4 carbon atoms or $-(\text{CH}_2-\text{CH}_2-\text{O})_n-\text{R}^k$, wherein R^k is a $-\text{H}$, a linear or branched alkyl having 1 to 4 carbon atoms;

n is an integer from 2 to 6; and

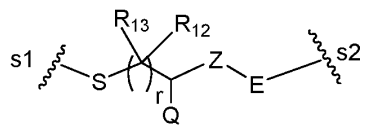
R^x is a linear or branched alkyl having 1 to 6 carbon atoms.

78. The method of claim 75, wherein D is represented by the following structural formula:



or a pharmaceutically acceptable salt thereof.

79. The method of any one of claims 75-78, wherein L is represented by the following structural formula:



or a pharmaceutically acceptable salt thereof, wherein:

s1 is the site covalently linked to D, and s2 is the site covalently linked to the maleimide group;

E is $-(CR_{10}R_{11})_{q-}$, cycloalkyl, or cycloalkylalkyl;

Z is absent, $-SO_2NR_9-$, $-NR_9SO_2-$, $-C(=O)-NR_9-$, $-NR_9-C(=O)-$, $-C(=O)-O-$,

-O-C(=O)-, -C(=O)-NR₉-(CH₂CH₂O)_p-, -NR₉-C(=O)-(CH₂CH₂O)_p-,
 -(OCH₂CH₂)_p-C(=O)NR₉-, or -(OCH₂CH₂)_p-NR₉-C(=O)-;

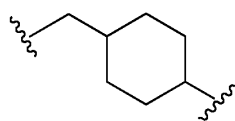
p is an integer from 1 to 24;

Q is H, a charged substituent, or an ionizable group;

R₉, R₁₀, R₁₁, R₁₂, and R₁₃, for each occurrence, are independently H or an optionally substituted alkyl; and,

q and r, for each occurrence, are independently an integer between 0 and 10.

80. The method of claim 79, wherein E is -(CR₁₀R₁₁)_q-.



81. The method of claim 79, wherein E is

82. The method of any one of claims 79-81, wherein Z is -C(=O)-NR₉- or -NR₉-C(=O)-.

83. The method of claim 82, wherein R₉ is -H.

84. The method of any one of claims 79-83, wherein Q is:

i) H;

ii) -SO₃H, -Z'-SO₃H, -OPO₃H₂, -Z'-OPO₃H₂, -PO₃H₂, -Z'-PO₃H₂, -CO₂H, -Z'-CO₂H, -NR₁₁R₁₂, or -Z'-NR₁₄R₁₅, or a pharmaceutically acceptable salt thereof; or,

iii) -N⁺R₁₄R₁₅R₁₆X⁻ or -Z'-N⁺R₁₄R₁₅R₁₆X⁻;

Z' is an optionally substituted alkylene, an optionally substituted cycloalkylene, or an optionally substituted phenylene;

R₁₄, R₁₅ and R₁₆ are each independently an optionally substituted alkyl; and,

X⁻ is a pharmaceutically acceptable anion.

85. The method of claim 84, wherein Q is H or -SO₃H or a pharmaceutically acceptable salt thereof.

86. The method of any one of claims 78-85, wherein R₉, R₁₀, R₁₁, R₁₂, and R₁₃ are all H; and q and r are each independently an integer between 1 and 6.

87. The method of claim 79, wherein:

R₁₂ and R₁₃, for each occurrence, are each independently H or (C₁-C₃)alkyl;

Q is H or -SO₃H or a pharmaceutically acceptable salt thereof

Z is $-\text{C}(=\text{O})-\text{NR}_9-$ or $-\text{NR}_9-\text{C}(=\text{O})-$;

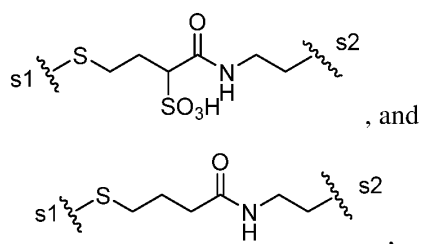
R_9 is H or (C_1-C_3) alkyl;

E is $-(\text{CR}_{10}\text{R}_{11})_q-$.

R_{10} and R_{11} , for each occurrence, are independently H or (C_1-C_3) alkyl; and

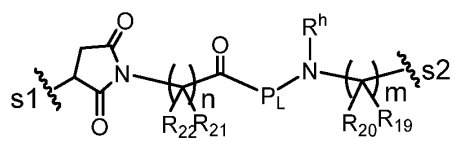
q and r are each an integer from 1 to 5.

88. The method of claim 79, wherein L is represented by any one of the following structural formulae:



or a pharmaceutically acceptable salt thereof.

89. The method of any one of claims 75-79, wherein L is represented by the following structural formula:



or a pharmaceutically acceptable salt thereof, wherein:

s1 is the site covalently linked to D; s2 is the site covalently linked to the maleimide group;

R_{19} , R_{20} , R_{21} and R_{22} , for each occurrence, are independently H or an optionally substituted alkyl;

m and n are each independently an integer between 0 and 10;

R^h is H or an optionally substituted alkyl;

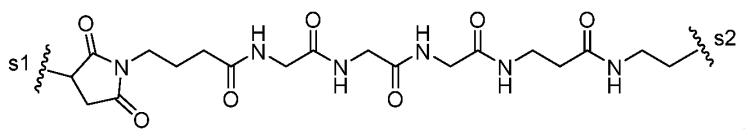
P_L is an optionally substituted alkylene, $-(\text{CH}_2-\text{CH}_2-\text{O})_j-$ (wherein the oxygen atom is connected to the $-(\text{C}=\text{O})-$ group connected to P), an amino acid residue or a peptide containing 2 to 20 amino acid residues; and

j is an integer from 1 to 24.

90. The method of claim 89, wherein R_{19} , R_{20} , R_{21} and R_{22} are each H; and m and n are each independently an integer between 1 and 6.

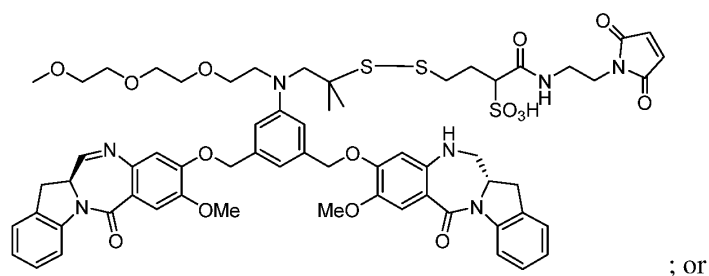
91. The method of claim 89 or 90, wherein P_L is an amino acid residue or a peptide containing 2 to 10 amino acid residues.
92. The method of claim 91, wherein P_L is a peptide containing 2 to 5 amino acid residues.
93. The method of claim 92, wherein P_L is selected from the group consisting of: Ala-Val, Val-Ala, Val-Cit, Cit-Val, Val-Lys, Phe-Lys, Lys-Lys, Ala-Lys, Phe-Cit, Leu-Cit, Ile-Cit, Trp-Cit, Phe-Ala, Phe-N⁹-tosyl-Arg, Phe-N⁹-nitro-Arg, Phe-Phe-Lys, D-Phe-Phe-Lys, Gly-Phe-Lys, Leu-Ala-Leu, Ile-Ala-Leu, Val-Ala-Val, Ala-Leu-Ala-Leu (SEQ ID NO: 1), β -Ala-Leu-Ala-Leu (SEQ ID NO: 2), Gly-Phe-Leu-Gly (SEQ ID NO: 3), Val-Arg, Arg-Arg, Val-D-Cit, Val-D-Lys, Val-D-Arg, D-Val-Cit, D-Val-Lys, D-Val-Arg, D-Val-D-Cit, D-Val-D-Lys, D-Val-D-Arg, D-Arg-D-Arg, Ala-Ala, Ala-D-Ala, D-Ala-Ala, D-Ala-D-Ala., Ala-Met, Met-Ala, Gln-Val, Asn-Ala, Gln-Phe, Gln-Ala, Gly-Gly-Gly, Ala-Ala-Ala, D-Ala-Ala-Ala, Ala-D-Ala-Ala, Ala-Ala-D-Ala, Ala-Val-Cit, Ala-Val-Ala, and β -Ala-Gly-Gly-Gly.
94. The method of claim 93, wherein P_L is Gly-Gly-Gly, Ala-Ala-Ala, D-Ala-Ala-Ala, Ala-D-Ala-Ala, Ala-Val-Ala, or β -Ala-Gly-Gly-Gly.

95. The method of claim 89, wherein L is represented by the following structural formula:

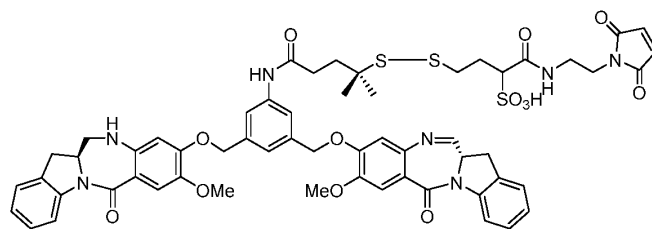


or a pharmaceutically acceptable salt thereof.

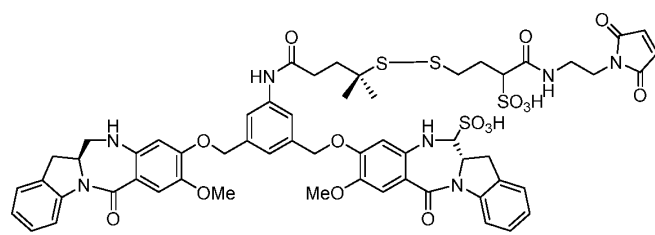
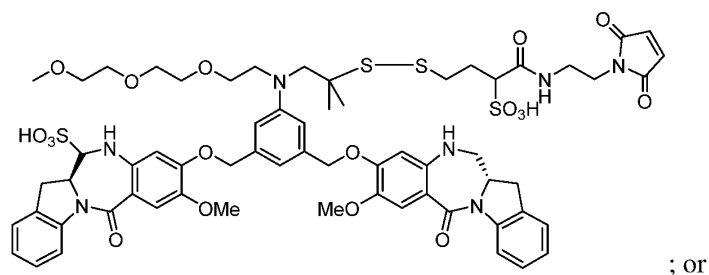
96. The method of any one of claims 1-40, wherein the imine-containing cytotoxic agent is represented by the following formula:



; or

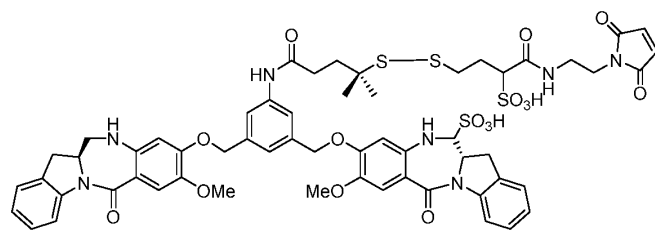
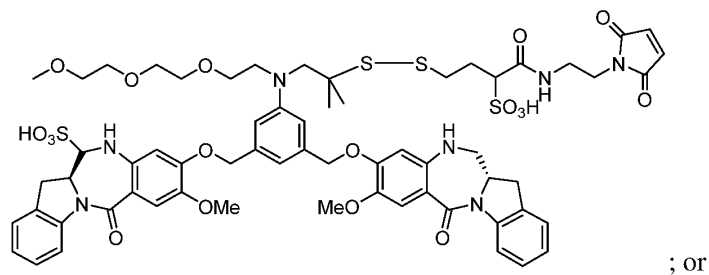


or a pharmaceutically acceptable salt thereof, and the modified cytotoxic agent is represented by the following formula:



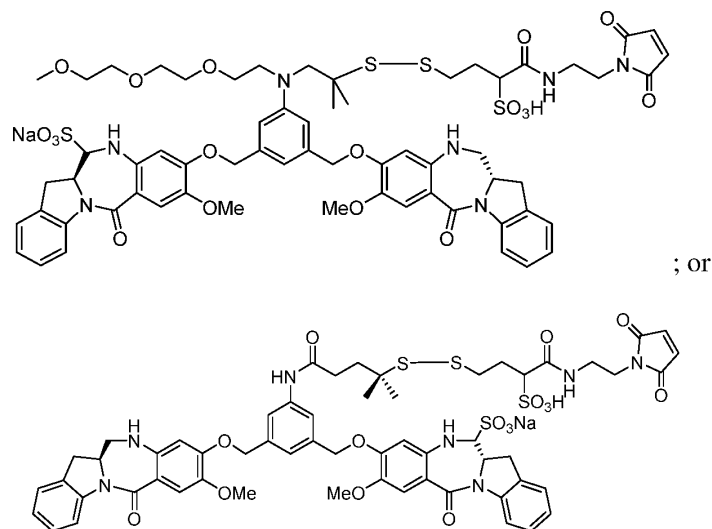
or a pharmaceutically acceptable salt thereof.

97. The method of claim 96, wherein the modified cytotoxic agent is represented by the following formula:



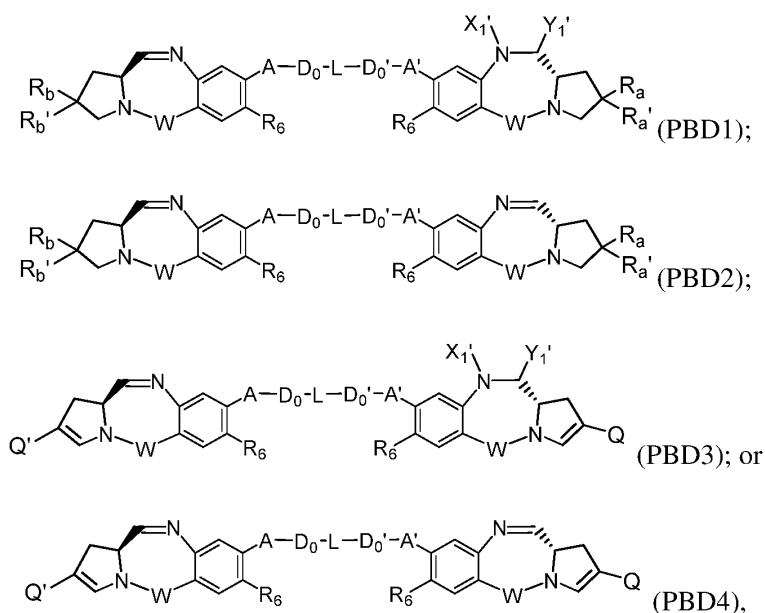
or a sodium or potassium salt thereof.

98. The method of claim 97, wherein the modified cytotoxic agent is represented by the following formula:



99. The method of any one of claims 1-39, wherein D is an imine-containing pyrrolobenzodiazepine (PBD) compound.

100. The method of claim 99, wherein D is represented by the following formula:



or a pharmaceutically acceptable salt thereof, wherein:

W is selected from C=O, C=S, CH₂, BH, SO and SO₂;

X₁' is selected from -H, an amine-protecting group, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, a polyethylene glycol unit -(CH₂CH₂O)_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;

Y₁' is selected from -H, an oxo group, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, 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;

R₆ is -H, -R, -OR, -SR, -NR'R'', -NO₂, or halogen;

A and A' are the same or different, and are independently selected from -O-, oxo (-C(=O)-), -CRR'O-, -CRR'-, -S-, -CRR'S-, -NR₅ and -CRR'N(R₅)-,

R, for each occurrence, is independently selected from the group consisting of -H, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, a polyethylene glycol unit -(CH₂CH₂O)_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, -NR₂, -COR, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, a polyethylene glycol unit -(CH₂CH₂O)_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 a substituted or unsubstituted linear or branched alkyl having 1 to 4 carbon atoms, or the linking group with the reactive group bonded thereto;

n is an integer from 1 to 24;

R_5 for each occurrence is independently -H or an optionally substituted linear or branched alkyl having 1 to 10 carbon atoms;

D_0 and D_0' are the same or different, and are independently absent or selected from the group consisting of an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having 1 to 10 carbon atoms, an amino acid, a peptide bearing 2 to 6 amino acids, and a polyethylene glycol unit $(-OCH_2CH_2)_n$;

L is absent, a linker, a polyethylene glycol unit $(-OCH_2CH_2)_n$, an optionally substituted linear, branched or cyclic alkyl or alkenyl having 1 to 10 carbon atoms, an optionally substituted phenyl group, an optionally substituted 3 to 18-membered heterocyclic ring or a 5- to 18-membered heteroaryl ring having 1 to 6 heteroatoms independently selected from O, S, N and P;

R_a , R_a' , R_b and R_b' are the same or different, and are independently selected from the group consisting of -H, halide, or an optionally substituted branched, linear or cyclic alkyl having 1 to 10 carbon atoms; or R_a and R_a' and/or R_b and R_b' together form a double bond containing group $=B$ and $=B'$ respectively;

$=B$ and $=B'$ are the same or different and independently selected from an optionally substituted branched or linear alkenyl or a carbonyl group;

Q is Q_1 -Ar- Q_2 ;

Q' is Q_1' -Ar'- Q_2' ;

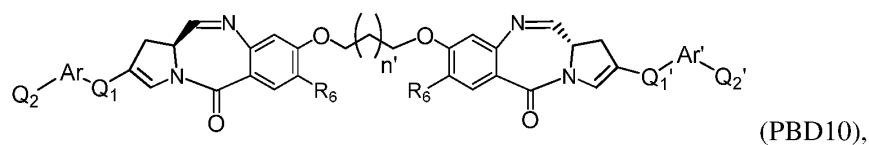
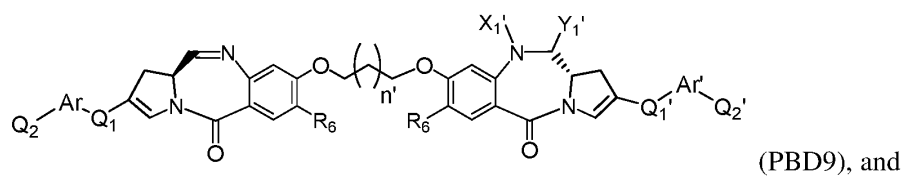
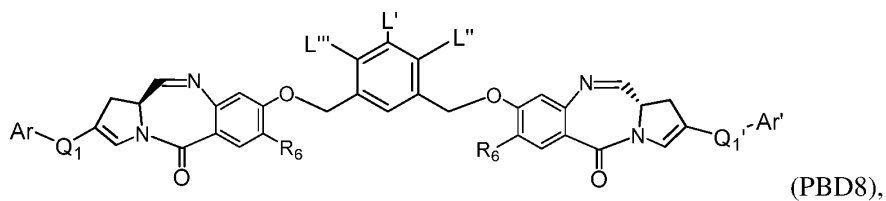
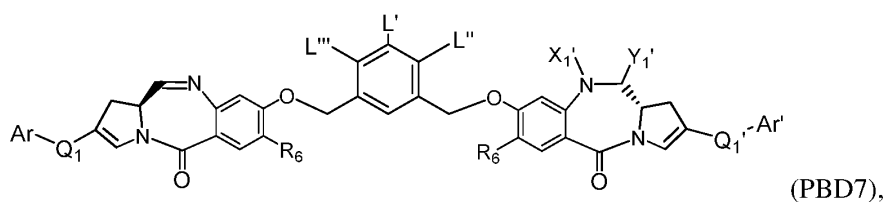
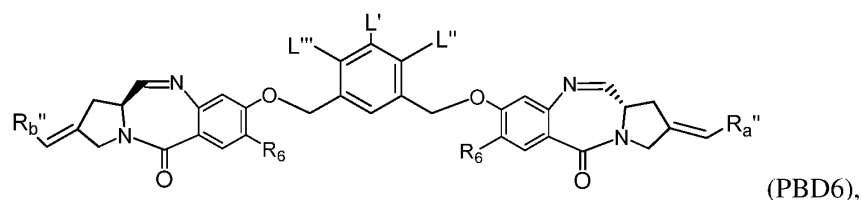
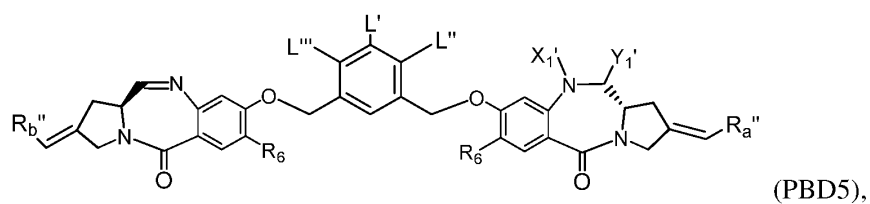
Q_1 and Q_1' are each independently absent, a linear, branched or cyclic alkyl from 1 to 6 carbon atoms or a $-CH=CH$ unit;

Ar and Ar' are each independently absent, or represent an aryl group;

Q_2 and Q_2' are each independently selected from -H, a linker, a substituted or unsubstituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, a polyethylene glycol unit $-R^{c'}-(OCH_2CH_2)_n-R^{c'}$, or a substituent selected from a halogen, guanidinium $[-NH(C=NH)NH_2]$, -R, -OR, -NR'R'', -NO₂, -NCO, -NR'COR'', NR'(C=O)OR'', -SR, a sulfoxide represented by -SOR', a sulfone represented by -SO₂R', a sulfonate -SO₃M, a sulfate -OSO₃M, a sulfonamide represented by SO₂NR'R'', cyano, an azido, -COR', -OCOR' or -OCONR'R''; and

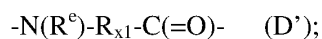
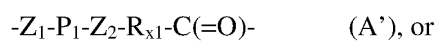
$R^{c'}$ is absent or selected from linear or branched alkyl, alkenyl or alkynyl having 1 to 5 carbon atoms.

101. The method of claim 100, wherein D is selected from one of the following:



or a pharmaceutically acceptable salt thereof, wherein:

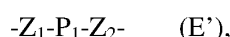
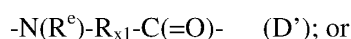
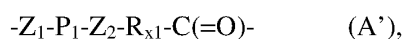
one of L', L'', and L''' in formula (PBD5), (PBD6), (PBD7) or (PBD8) is represented by the following formula:



and the other two are each independently selected from -H, an optionally

substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, 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 Q_2 and Q_2' in formula (PBD9) or (PBD10) is represented by the following formula:



and the other one is selected from $-H$, $-R$, $-OR$, $-NR'R''$, $-NO_2$, $-NR'(C=O)OR''$, $-SR$, or $-NO_2$;

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 having from 1 to 10 carbon atoms;

R^e is $-H$, a linear, branched or cyclic alkyl, alkenyl or alkynyl having 1 to 10 carbon atoms 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 having 1 to 10 carbon atoms;

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

R_5 for each occurrence is independently $-H$ or an optionally substituted linear or branched alkyl having 1 to 10 carbon atoms.

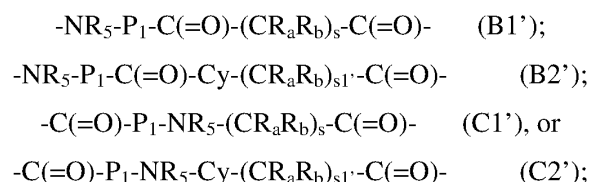
R_a'' and R_b'' are the same or different, and are selected from $-H$ and $-Me$; and n' is selected from 0, 1, 2 and 3.

102. The method of claim 101, wherein one of L' , L'' and L''' in formula (PBD5), (PBD6), (PBD7) or (PBD8) is represented by formula (A') or (D') and the other two are $-H$, an linear or branched alkyl having from 1 to 6 carbon atoms, halogen, $-OH$, (C_1-C_6) alkoxy or $-NO_2$; or one of Q_2 and Q_2' in formula (PBD9) or (PBD10) is represented by formula (A'),

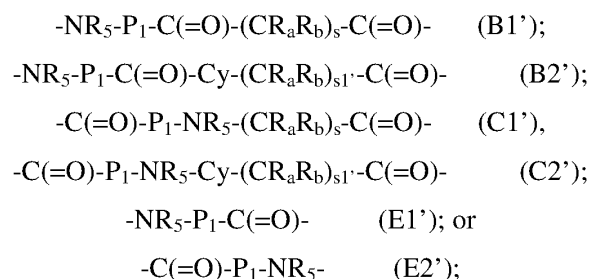
(D') or (E'); and the other is -H, an linear or branched alkyl having from 1 to 6 carbon atoms, halogen, -OH, (C₁-C₆)alkoxy or -NO₂.

103. The method of claim 102, wherein:

L' and L''' are both -H; and L' in formula (PBD5), (PBD6), (PBD7) or (PBD8) is represented by the following formula:



one of Q₂ and Q₂' in formula (PBD9) or (PBD10) is represented by the following formula:



wherein:

R_a and R_b, for each occurrence, are each independently -H, (C₁-C₃)alkyl or a charged substituent or an ionizable group Q;

s is an integer from 1 to 6;

s1' is 0 or an integer from 1 to 6; and

Cy is a cyclic alkyl having 5 or 6 ring carbon atoms optionally substituted with halogen, -OH, (C₁-C₃)alkyl, (C₁-C₃)alkoxy, or halo(C₁-C₃)alkyl.

104. The method of claim 103, wherein R_a and R_b are both H; Cy in formulas (B2') and (C2') is cyclohexane; R₅ is H or Me; and s1' is 0 or 1.

105. The method of any one of claims 101-104, wherein Q is -SO₃H or a pharmaceutically acceptable salt thereof.

106. The method of any one of claims 101-105, wherein P₁ is a peptide containing 2 to 5 amino acid residues.

107. The method of claim 106, wherein P₁ is Gly-Gly-Gly, Ala-Val, Val-Cit, Val-Lys, Phe-Lys, Lys-Lys, Ala-Lys, Phe-Cit, Leu-Cit, Ile-Cit, Trp, Cit, Phe-Ala, Phe-N⁹-tosyl-Arg, Phe-N⁹-nitro-Arg, Phe-Phe-Lys, D-Phe-Phe-Lys, Gly-Phe-Lys, Leu-Ala-Leu, Ile-Ala-Leu, Val-Ala-Val, Ala-Leu-Ala-Leu (SEQ ID NO: 1), β-Ala-Leu-Ala-Leu (SEQ ID NO: 2), Gly-Phe-Leu-Gly (SEQ ID NO: 3), Val-Arg, Arg-Arg, Val-D-Cit, Val-D-Lys, Val-D-Arg, D-Val-Cit, D-Val-Lys, D-Val-Arg, D-Val-D-Cit, D-Val-D-Lys, D-Val-D-Arg, D-Arg-D-Arg, Ala-Ala, Ala-D-Ala, D-Ala-Ala, D-Ala-D-Ala, Ala-Met, Gln-Val, Asn-Ala, Gln-Phe and Gln-Ala.

108. The method of claim 107, wherein P₁ is Gly-Gly-Gly, Ala-Val, Ala-Ala, Ala-D-Ala, D-Ala-Ala, or D-Ala-D-Ala.

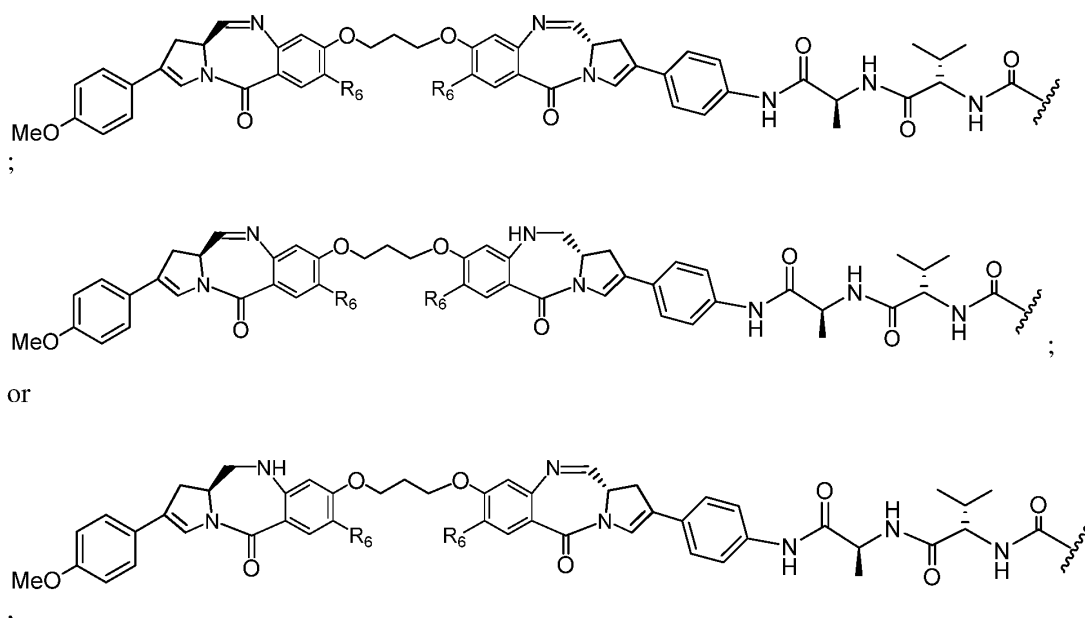
109. The method of any one of claims 100-108, wherein:

R₆ is -OMe;

X₁' and Y₁' are both -H; and

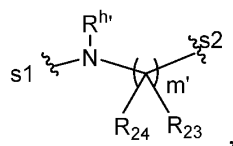
A and A' are -O-.

110. The method of claim 100, wherein D is represented by the following structural formula:



or a pharmaceutically acceptable salt thereof.

111. The method of any one of claims 100-110, wherein -L- is represented by the following structural formula:



or a pharmaceutically acceptable salt thereof, wherein:

s1 is the site covalently linked to D; s2 is the site covalently linked to the maleimide group;

R₂₃ and R₂₄, for each occurrence, are independently H or an optionally substituted alkyl;

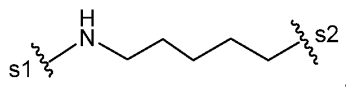
m' is an integer between 0 and 10; and

R^h is H or an optionally substituted alkyl.

112. The method of claim 111, wherein R₂₃ and R₂₄ are both H; and m' is an integer between 1 and 6.

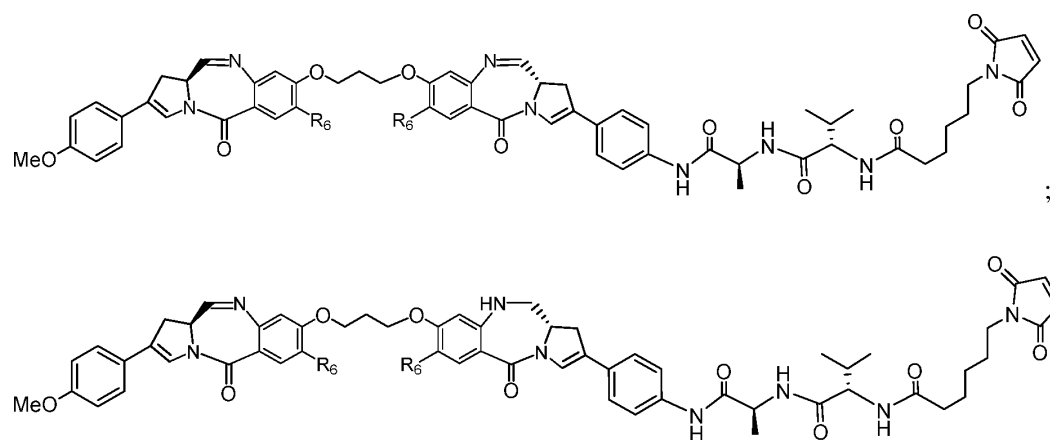
113. The method of claim 111 or 112, wherein R^h is H.

114. The method of claim 113, wherein L is represented by the following structural formula:

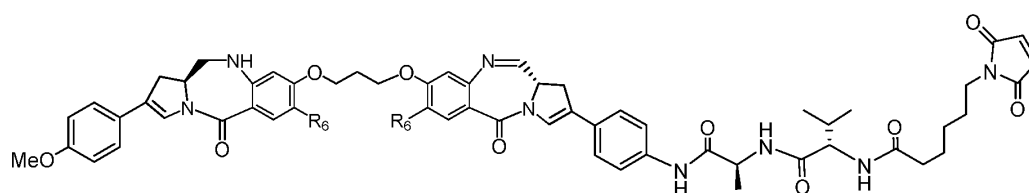


or a pharmaceutically acceptable salt thereof.

115. The method of any one of claims 100-114, wherein the imine-containing cytotoxic agent is represented by the following formula:

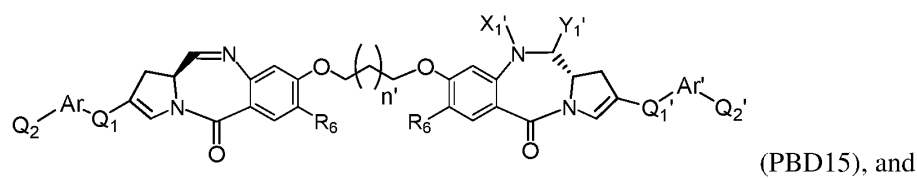
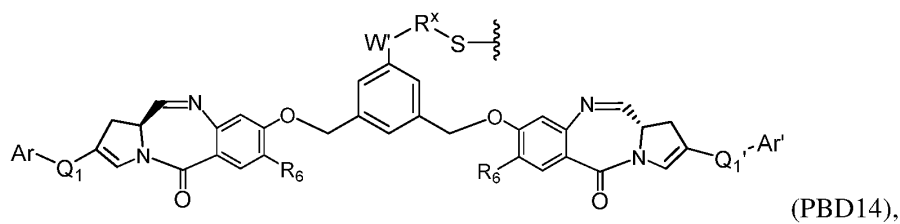
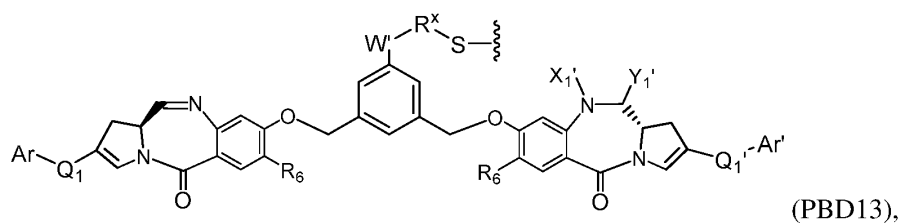
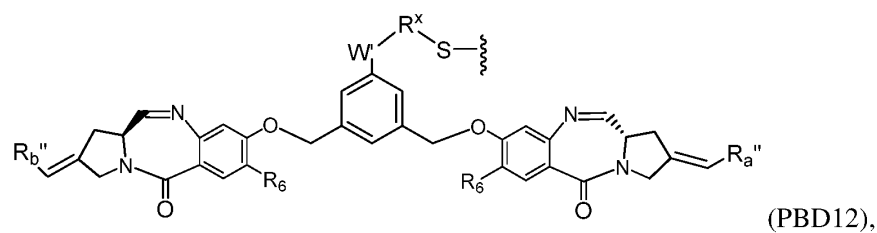
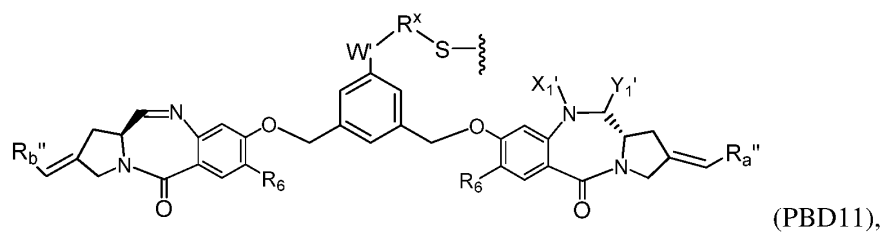


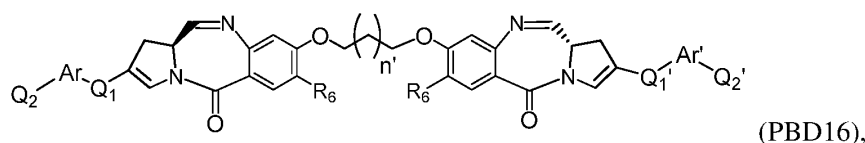
;



or a pharmaceutically acceptable salt thereof.

116. The method of claims 100, wherein D is represented by the following formula:





or a pharmaceutically acceptable salt thereof, wherein:

X_1' is selected from the group consisting of -H, -OH, a substituted or unsubstituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, phenyl, and an amine-protecting group;

Y_1' is selected from the group consisting of -H, an oxo group, a substituted or unsubstituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms;

one of Q_2 and Q_2' in formula (PBD15) or (PBD16) is $-W'-R^x-S-$; and the other is selected from -H, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, a polyethylene glycol unit $-(CH_2CH_2O)_n-R^c$, halogen, guanidinium $[-NH(C=NH)NH_2]$, -OR, -NR'R'', -NO₂, -NR'COR'', -SR, -SOR', -SO₂R', -SO₃H, -OSO₃H, -SO₂NR'R'', cyano, an azido, -COR', -OCOR', and -OCONR'R'';

W' is absent, or selected from -O-, -N(R^e)-, -N(R^e)-C(=O)-, -N(C(=O)R^e)-, -S- or -CH₂-S-, -CH₂NR^e-;

R^x is absent or selected from a linear, branched or cyclic alkyl having 1 to 10 carbon atoms;

R^e is -H, a linear, branched or cyclic alkyl, alkenyl or alkynyl having 1 to 10 carbon atoms 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.*, -NHR¹⁰¹) or tertiary amino (-NR¹⁰¹R¹⁰²) 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 having 1 to 10 carbon atoms;

n is an integer from 1 to 24;

R_6 is -H, -R, -OR, -SR, -NR'R'', -NO₂, or halogen; and

R is -H, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms or a PEG group $-(CH_2CH_2O)_n-R^c$, wherein n is an integer from 1 to 24, and R^c is a linear or branched alkyl having 1 to 4 carbon atoms;

R' and R'' are each independently selected from -H, -OH, -OR, -NRR^g, -COR, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, an optionally substituted aryl having from 6 to 18 carbon atoms, an optionally

substituted 3- to 18-membered heterocyclic ring having 1 to 6 heteroatoms selected from O, S, N and P, a PEG group $-(CH_2CH_2O)_n-R^c$, and $R^{g'}$ is -H, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms or a PEG group $-(CH_2CH_2O)_n-R^c$.

117. The method of claim 116, wherein

X_1' and Y_1' are both -H;

R_6 is -OMe;

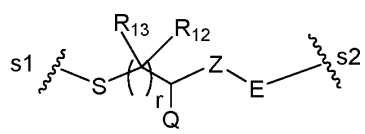
W' is $-N(R^e)-$ or $-N(R^e)-C(=O)-$;

R^e is -H, a linear or branched alkyl having 1 to 4 carbon atoms or $-(CH_2-CH_2-O)_n-R^k$, wherein R^k is a -H, a linear or branched alkyl having 1 to 4 carbon atoms;

n is an integer from 2 to 6; and

R^x is a linear or branched alkyl having 1 to 6 carbon atoms.

118. The method of claim 116 or 117, wherein L is represented by the following structural formula:



or a pharmaceutically acceptable salt thereof, wherein:

s_1 is the site covalently linked to D, and s_2 is the site covalently linked to the maleimide group;

E is $-(CR_{10}R_{11})_q-$, cycloalkyl, or cycloalkylalkyl;

Z is absent, $-SO_2NR_9-$, $-NR_9SO_2-$, $-C(=O)-NR_9-$, $-NR_9-C(=O)-$, $-C(=O)-O-$, $-O-C(=O)-$, $-C(=O)-NR_9-(CH_2CH_2O)_p-$, $-NR_9-C(=O)-(CH_2CH_2O)_p-$, $-(OCH_2CH_2)_p-C(=O)NR_9-$, or $-(OCH_2CH_2)_p-NR_9-C(=O)-$;

p is an integer from 1 to 24;

Q is H, a charged substituent, or an ionizable group;

R_9 , R_{10} , R_{11} , R_{12} , and R_{13} , for each occurrence, are independently H or an optionally substituted alkyl; and,

q and r , for each occurrence, are independently an integer between 0 and 10.

119. The method of claim 118, wherein E is $-(CR_{10}R_{11})_q-$.

120. The method of claim 118 or 119, wherein Z is $-C(=O)-NR_9-$ or $-NR_9-C(=O)-$.

121. The method of claim 120, wherein R_9 is $-H$.

122. The method of any one of claims 118-121, wherein Q is H or $-SO_3H$ or a pharmaceutically acceptable salt thereof.

123. The method of any one of claims 118-122, wherein R_9 , R_{10} , R_{11} , R_{12} , and R_{13} are all H ; and q and r are each independently an integer between 1 and 6.

124. The method of claim 118, wherein:

R_{12} and R_{13} , for each occurrence, are each independently H or $(C_1-C_3)alkyl$;

Q is H or $-SO_3H$ or a pharmaceutically acceptable salt thereof

Z is $-C(=O)-NR_9-$ or $-NR_9-C(=O)-$;

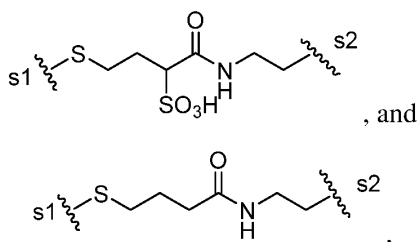
R_9 is H or $(C_1-C_3)alkyl$;

E is $-(CR_{10}R_{11})_q-$.

R_{10} and R_{11} , for each occurrence, are independently H or $(C_1-C_3)alkyl$; and

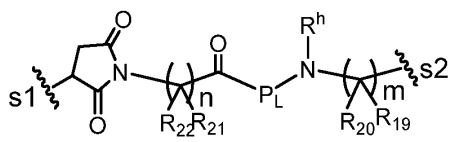
q and r are each an integer from 1 to 5.

125. The method of claim 118, wherein L is represented by any one of the following structural formulae:



or a pharmaceutically acceptable salt thereof.

126. The method of claim 116 or 117, wherein L is represented by the following structural formula:



or a pharmaceutically acceptable salt thereof, wherein:

$s1$ is the site covalently linked to D ; $s2$ is the site covalently linked to the maleimide group;

R_{19} , R_{20} , R_{21} and R_{22} , for each occurrence, are independently H or an optionally substituted alkyl;

m and n are each independently an integer between 0 and 10;

R^h is H or an optionally substituted alkyl;

P_L is an optionally substituted alkylene, $-(CH_2-CH_2-O)_j-$ (wherein the oxygen atom is connected to the $-(C=O)-$ group connected to P), an amino acid residue or a peptide containing 2 to 20 amino acid residues; and

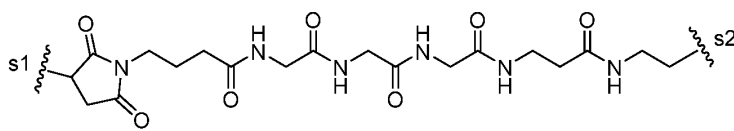
j is an integer from 1 to 24.

127. The method of claim 126, wherein R_{19} , R_{20} , R_{21} and R_{22} are each H; and m and n are each independently an integer between 1 and 6.

128. The method of claim 126 or 127, wherein P_L is a peptide containing 2 to 5 amino acid residues.

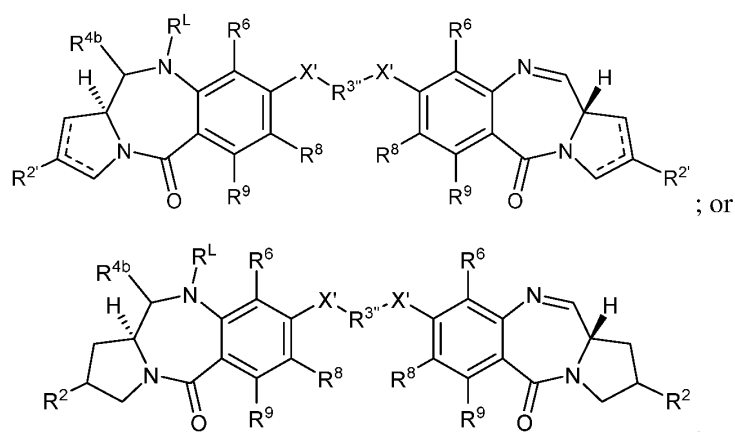
129. The method of claim 128, wherein P_L is selected from the group consisting of: Ala-Val, Val-Ala, Val-Cit, Cit-Val, Val-Lys, Phe-Lys, Lys-Lys, Ala-Lys, Phe-Cit, Leu-Cit, Ile-Cit, Trp-Cit, Phe-Ala, Phe- N^9 -tosyl-Arg, Phe- N^9 -nitro-Arg, Phe-Phe-Lys, D-Phe-Phe-Lys, Gly-Phe-Lys, Leu-Ala-Leu, Ile-Ala-Leu, Val-Ala-Val, Ala-Leu-Ala-Leu (SEQ ID NO: 1), β -Ala-Leu-Ala-Leu (SEQ ID NO: 2), Gly-Phe-Leu-Gly (SEQ ID NO: 3), Val-Arg, Arg-Arg, Val-D-Cit, Val-D-Lys, Val-D-Arg, D-Val-Cit, D-Val-Lys, D-Val-Arg, D-Val-D-Cit, D-Val-D-Lys, D-Val-D-Arg, D-Arg-D-Arg, Ala-Ala, Ala-D-Ala, D-Ala-Ala, D-Ala-D-Ala., Ala-Met, Met-Ala, Gln-Val, Asn-Ala, Gln-Phe, Gln-Ala, Gly-Gly-Gly, Ala-Ala-Ala, D-Ala-Ala-Ala, Ala-D-Ala-Ala, Ala-Ala-D-Ala, Ala-Val-Cit, Ala-Val-Ala, and β -Ala-Gly-Gly-Gly.

130. The method of claim 126, wherein L is represented by the following structural formula:



or a pharmaceutically acceptable salt thereof.

131. The method of any one of claims 1-39, wherein the imine-containing cytotoxic agent is represented by the following formula:



or a pharmaceutically acceptable salt thereof, wherein:

the dotted lines indicate the optional presence of a double bond;

$R^{3''}$ is a C_{3-12} alkylene group,

each X' , for each occurrence, is independently -O-, -S- or -N(H)-

each R^2 is independently selected from -H, -OH, -CN, $-R^{1'}$, $-OR^{1'}$, $-O-SO_2-R^{1'}$, $-CO_2R^{1'}$, $-COR^{1'}$, or halo, or both R^2 taken together, are =O, =CH₂, =CH- R^a , or =C(R^a)₂;

each $R^{2'}$ is independently selected from -H, -OH, -CN, $-R^{1'}$, $-OR^{1'}$, $-O-SO_2-R^{1'}$, $-CO_2R^{1'}$, $-COR^{1'}$ or halo;

R^{4b} is a leaving group selected from $-OR^{6'}$, $-OCOR^{4'}$, $-OCOOR^{4'}$, $-OCONR^{4'}R^{5'}$, $-NR^{4'}R^{5'}$, $-NR^{4'}COR^{5'}$, $-NR^{4'}NR^{4'}R^{5'}$, an optionally substituted 5- or 6-membered nitrogen-containing heterocycle (*e.g.*, piperidine, tetrahydropyrrole, pyrazole, morpholine), a guanidinium represented by $-NR^{4'}(C=NH)NR^{4'}R^{5'}$, an amino acid, or a peptide represented by $-NR^{6'}COP'$, wherein P' is an amino acid or a polypeptide containing between 2 to 20 amino acid units, $-SR^{6'}$, $-SOR^{4'}$, $-SO_2M$, $-SO_3M$, $-OSO_3M$, halogen, cyano and an azido;

R^L is linker bearing a maleimide moiety that can form a covalent bond with a cell binding agent (CBA);

R^6 and R^9 are independently selected from -H, $-R^{1'}$, -OH, $-OR^{1'}$, -SH, $-SR^{1'}$, $-NH_2$, $-NHR^{1'}$, $-NR^{1'}R^{3'}$, $-NO_2$, Me_3Sn and halo; and,

$R^{1'}$ and $R^{3'}$ are each independently selected from optionally substituted C_{1-12} alkyl, C_{3-20} heterocyclyl or C_{5-20} aryl groups, and optionally in relation to the group $-NR^{1'}R^{3'}$, $R^{1'}$ and $R^{3'}$ together with the nitrogen atom to which they are attached form an optionally substituted 4-, 5-, 6- or 7-membered heterocyclic ring; or any pair of adjacent groups from R^6 to R^9 together form a group $-O-(CH_2)_p-O-$, where p is 1 or 2;

$R^{4'}$ and $R^{5'}$ are each independently selected from -H, -OH, -OR^{6'}, -NHR^{6'}, -NR^{6'}₂, -COR^{6'}, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, a polyethylene glycol unit -(CH₂CH₂O)_n-R^b, or an optionally substituted 3-18-membered heterocyclic ring having 1 to 6 heteroatoms independently selected from O, S, N or P;

$R^{6'}$, for each occurrence, is independently selected from the group consisting of -H, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, a polyethylene glycol unit -(CH₂CH₂O)_n-R^b, 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, or sulfur, and an optionally substituted 3- to 18-membered heterocyclic ring containing 1 to 6 heteroatoms independently selected from O, S, N or P;

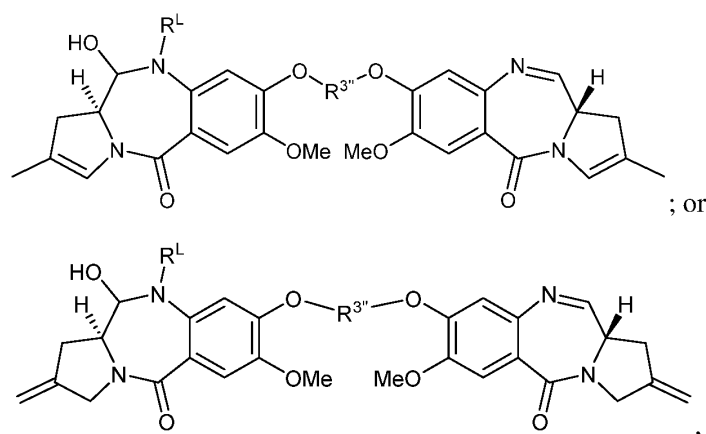
R^a is independently selected from -R^{1'}, -CO₂R^{1'}, -COR^{1'}, -CHO, -CO₂H, or halo;

R^b is -H or a substituted or unsubstituted linear or branched alkyl having 1 to 4 carbon atoms;

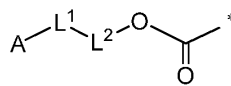
M is H or a pharmaceutically acceptable cation; and

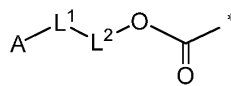
n is an integer from 1 to 24.

132. The method of claim 131, wherein the imine-containing cytotoxic agent is represented by the following formula:



or a pharmaceutically acceptable salt thereof, wherein $R^{3''}$ is a C₃₋₅alkylene.

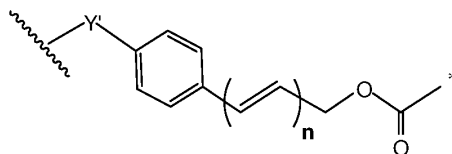


133. The method of claim 131 or 132, R^L is , wherein L^1 is a cleavable linker, A is a connecting group bearing a maleimide capable of connecting L^1 to the cell binding agent, L^2 is a covalent bond or together with -OC(=O)- forms a self-immolative linker.

134. The method of claim 133, wherein L^1 is a peptide containing 2 to 5 amino acid residues.

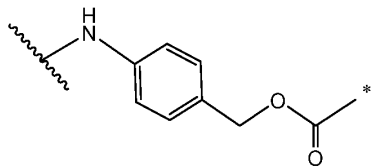
135. The method of claim 134, wherein L^1 is selected from the group consisting of Phe-Lys, Val-Ala, Val-Lys, Ala-Lys, Val-Cit, Phe-Cit, Leu-Cit, Ile-Cit, Phe-Arg, Trp-Cit, Lys-Lys, Phe-Ala, Phe-N9-tosyl-Arg, Phe-N9-nitro-Arg, Val-Arg, Arg-Val, Arg-Arg, Val-D-Cit, Val-D-Lys, Val-D-Arg, D-Val-Cit, D-Val-Lys, D-Val-Arg, D-Val-D-Cit, D-Val-D-Lys, D-Val-D-Arg, D-Arg-D-Arg, Gly-Gly-Gly, Phe-Phe-Lys, D-Phe-Phe-Lys, Gly-Phe-Lys, Leu-Ala-Leu, Ile-Ala-Leu, Val-Ala-Leu, Ala-Leu-Ala-Leu (SEQ ID NO:1), β -Ala-Leu-Ala-Leu (SEQ ID NO:2) and Gly-Phe-Leu-Gly (SEQ ID NO:3).

136. The method of any one of claims 131-135, wherein -C(=O)O- and L^2 together form the group:

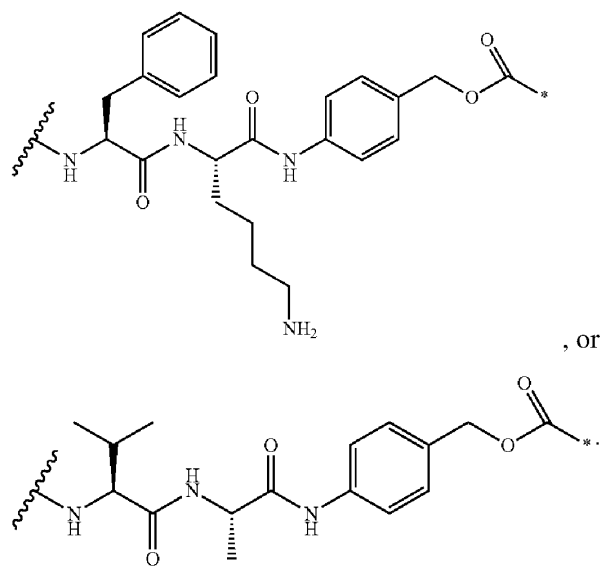


where the asterisk indicates the point of attachment to the N10 position, the wavy line indicates the point of attachment to the linker L^1 , Y' is -NH-, -O-, -C(=O)NH- or -C(=O)O-, and n is 0 to 3.

137. The method of claim 136, wherein -C(=O)O- and L^2 together form the group:

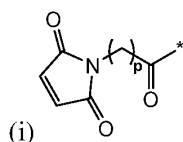


138. The method of claim 136, wherein L^1 and L^2 together with -OC(=O)- comprise a group selected from:

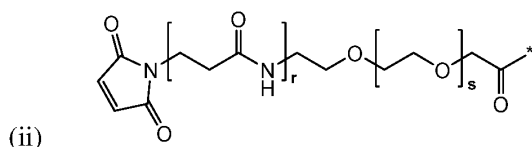


where the asterisk indicates the point of attachment to the N10 position, and the wavy line indicates the point of attachment to the remaining portion of the linker L^1 or the point of attachment to A.

139. The method of any one of claims 131-138, wherein A is represented by one of the following:



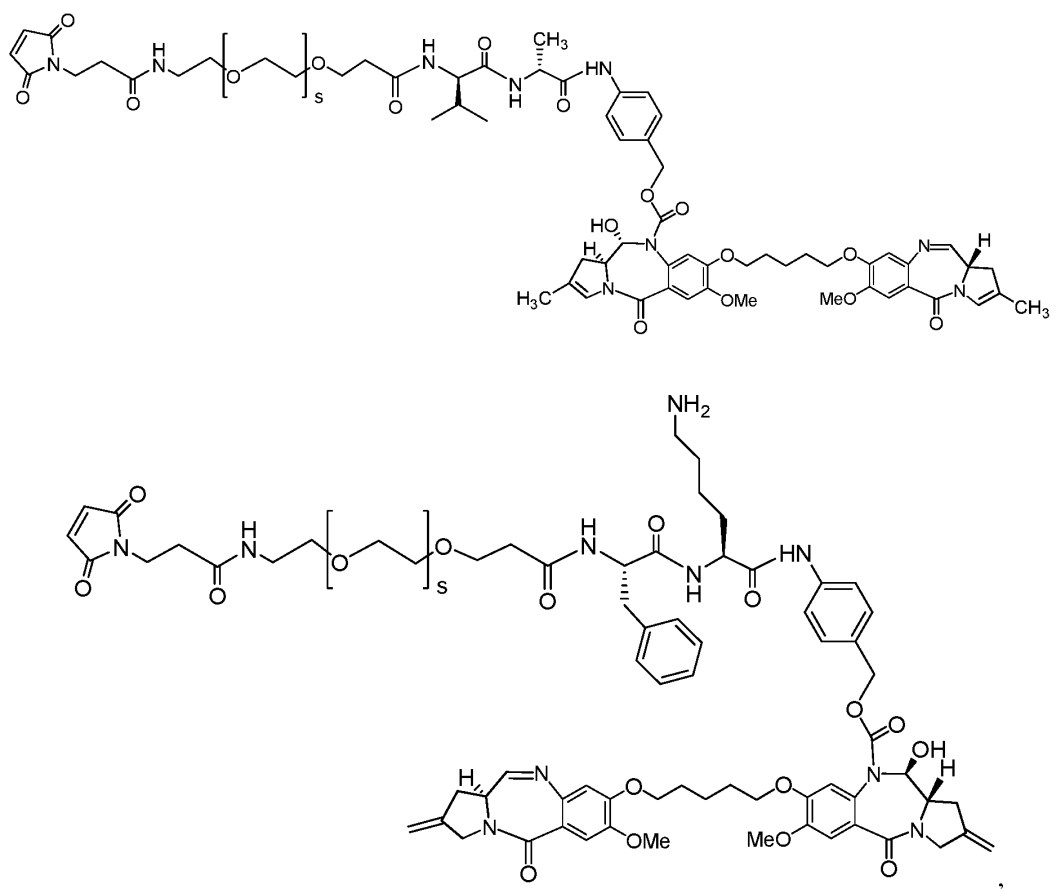
, where the asterisk indicates the point of attachment to L^1 , and p is 1 to 6;



, where the asterisk indicates the point of attachment to L^1 , r is 0 or 1, and s is 0 to 30.

140. The method of claim 139, wherein p is 4 to 6; r is 1; and s is 1 to 10.

141. The method of claim 131, wherein the imine-containing cytotoxic agent is represented by the following formula:



or a pharmaceutically acceptable salt thereof, wherein s is 2 to 8.

142. The method of claim 141, wherein s is 7.

143. The method of any one of claims 1-142, wherein the cell-binding agent is an antibody or antibody fragment thereof.

144. The method of any one of claims 1-142, wherein the cell-binding agent is an antibody having an engineered cysteine residue.

145. The method of claim 144, wherein the engineered cysteine residue is at the EU/OU numbering position 442 of one or both of the antibody heavy chains.

146. The method of claim 145, wherein the engineered cysteine residue is on both of the heavy chains.

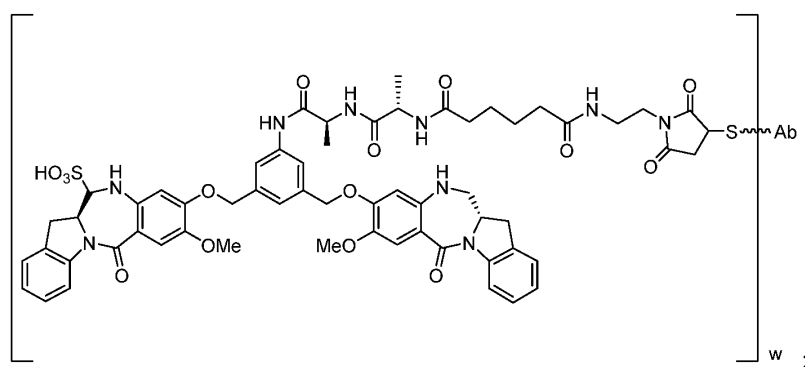
147. The method of claim 145, wherein the engineered cysteine residue is on one of the heavy chains.

148. The method of any one of claims 143-147, wherein the antibody is a monoclonal antibody, a chimeric antibody, a humanized antibody, a resurfaced antibody, or a human antibody.

149. The method of claim 148, wherein the antibody is an anti-CD123 antibody.

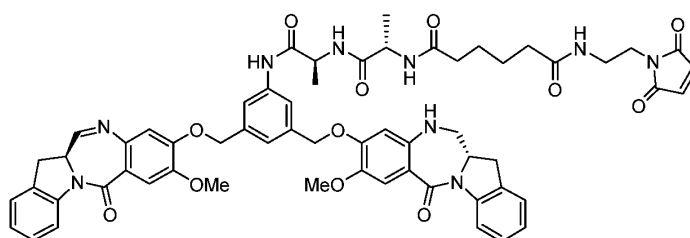
150. The method of claim 149, wherein the anti-CD123 antibody comprises an immunoglobulin heavy chain region having the amino acid sequence of SEQ ID NO:25 and an immunoglobulin light chain region having the amino acid sequence of SEQ ID NO:26.

151. The method of any one of claims 1-39, wherein the method is for preparing a cell-binding agent-cytotoxic agent conjugate represented by the following formula:

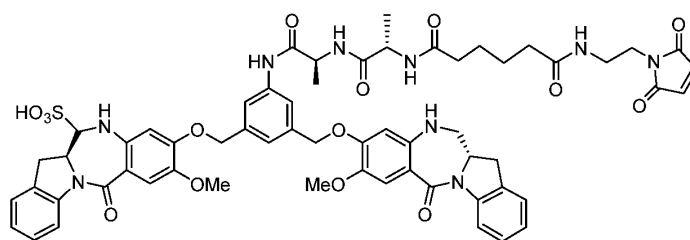


or a pharmaceutically acceptable salt thereof, and the method comprises the steps of:

(a) reacting an imine-moiety in an imine-containing cytotoxic agent represented by the following formula:



or a pharmaceutically acceptable salt thereof, with sulfur dioxide, a bisulfite salt or a metabisulfite salt in an aqueous solution at a pH of 3.1 to 3.5 to form a modified cytotoxic agent represented by the following formula:



or a pharmaceutically acceptable salt thereof; and

(b) reacting the modified cytotoxic agent or a pharmaceutically acceptable salt thereof with the cell-binding agent Ab to form the cell-binding agent-cytotoxic agent conjugate, wherein:

Ab is an anti-CD123 antibody comprising an immunoglobulin heavy chain region having the amino acid sequence of SEQ ID NO:25 and an immunoglobulin light chain region having the amino acid sequence of SEQ ID NO:26; and

w is 1 or 2.

152. The method of claim 151, wherein the reaction of step (a) is carried out at a pH of 3.2 to 3.4.

153. The method of claim 151, wherein the reaction of step (a) is carried out at a pH of 3.3.

154. The method of any one of claims 151-153, wherein the reaction of step (a) is carried out in the presence of a buffer solution.

155. The method of claim 154, wherein the buffer solution is a citrate buffer, an acetate buffer, a succinate buffer or a phosphate buffer.

156. The method of claim 155, wherein the buffer is a succinate buffer.

157. The method of any one of claims 151-156, wherein 1.1 to 1.6 equivalents of the bisulfite salt or 0.55 to 0.8 equivalents of the metabisulfite salt is reacted with 1 equivalent of the imine-containing cytotoxic agent.

158. The method of claim 157, wherein 1.3 to 1.5 equivalents of the bisulfite salt or 0.65 to 0.75 equivalents of the metabisulfite salt is reacted with 1 equivalent of the imine-containing cytotoxic agent.

159. The method of claim 157, wherein 1.4 equivalents of the bisulfite salt or 0.7 equivalents of the metabisulfite salt is reacted with 1 equivalent of the imine-containing cytotoxic agent.

160. The method of any one of claims 151-159, wherein the reaction of step (a) is carried out at a pH of 3.2 to 3.4 and 1.3 to 1.5 equivalents of the sodium bisulfite salt is reacted with 1 equivalent of the imine-containing cytotoxic agent.

161. The method of any one of claims 151-160, wherein the reaction of step (a) is carried out in a mixture of dimethylacetamide (DMA) and water.

162. The method of any one of claims 151-161, wherein the reaction of step (a) is carried out at room temperature.

163. The method of any one of claims 151-162, wherein the modified cytotoxic agent is not purified before reacting with the cell-binding agent in step (b).

164. The method of any one of claims 151-163, wherein the reaction of step (b) is carried out at a pH of 5.5 to 6.5.

165. The method of any one of claims 151-164, wherein the conjugate is purified by tangential flow filtration to yield purified conjugate.

166. The method of claim 165, wherein the purified conjugate is formulated in a formulation buffer comprising 40 to 80 μM of sodium bisulfite having a pH of 4 to 5.

167. The method of claim 166, wherein the formulation buffer comprises 50 μM of sodium bisulfite having a pH of 4.2.