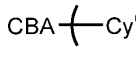
CLAIMS

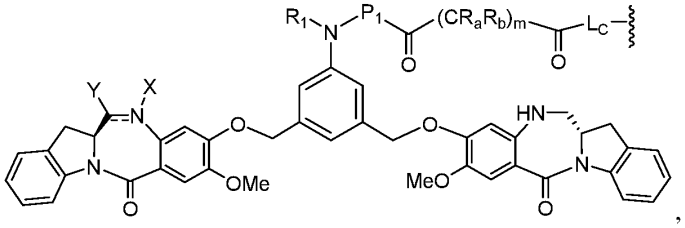
1. A pharmaceutical composition comprising an immunoconjugate and 0.1 mM to 20 mM methionine, wherein the immunoconjugate is represented by the following formula:



CBA is an antibody or antigen-binding fragment thereof;

W_C is 1 or 2; and

Cy^{Cys} is represented by the following formula:



or a pharmaceutically acceptable salt thereof, wherein:

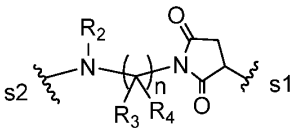
the double line == between N and C represents a single bond or a double bond, provided that when it is a double bond, X is absent and Y is -H or a $(C_1$-$C_4)$alkyl; and when it is a single bond, X is -H or an amine protecting moiety, Y is -OH or $-SO_3H$;

R_1 is -H or a $(C_1$-$C_3)$alkyl;

P_1 is an amino acid residue or a peptide containing 2 to 5 amino acid residues;

R_a and R_b, for each occurrence, are independently -H, $(C_1$-$C_3)$alkyl, or a charged substituent or an ionizable group Q;

m is an integer from 1 to 6;

L_C is represented by , s1 is the site covalently linked to CBA, and s2 is the site covalently linked to the -C(=O)- group on Cy^{Cys}; wherein:

R_2 is -H or a $(C_1$-$C_3)$alkyl

R_3 and R_4, for each occurrence, are independently -H or a $(C_1$-$C_3)$alkyl; and

n is an integer between 1 and 10.

2. The pharmaceutical composition of claim 1, wherein R_a and R_b are both H; and R_1 is H or Me.

3. The pharmaceutical composition of claim 1 or 2, wherein P is selected from Gly-Gly-Gly, Ala-Val, Val-Ala, Val-Cit, 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:21), β-Ala-Leu-Ala-Leu (SEQ ID NO:22), Gly-Phe-Leu-Gly (SEQ ID NO:23), 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, Ala-Ala, Ala-D-Ala, D-Ala-Ala, D-Ala-D-Ala, Ala-Met, and Met-Ala.

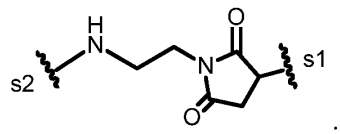
4. The pharmaceutical composition of claim 3, wherein P is Ala-Ala, Ala-D-Ala, D-Ala-Ala, or D-Ala-D-Ala.

5. The pharmaceutical composition of any one of claims 1-4, wherein Q is -SO_3H.

6. The pharmaceutical composition of any one of claims 1-4, wherein Q is H.

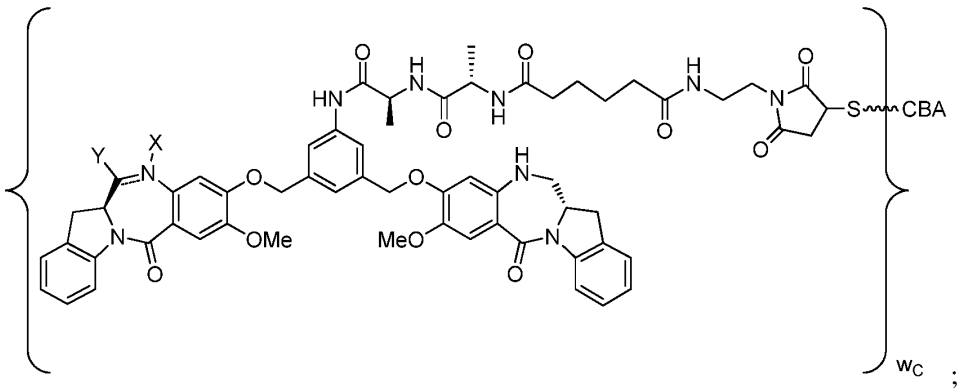
7. The pharmaceutical composition of any one of claims 1-6, wherein R_3 and R_4 are both H; and n is an integer from 1 to 6.

8. The pharmaceutical composition of any one of claims 1-7, wherein -L_C- is represented by the following formula:



.

9. The pharmaceutical composition of claim 1, wherein the immunoconjugate is represented by the following formula:



;

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, and when it is a single bond, X is -H, and Y is -OH or $-SO_3H$..

10. The pharmaceutical composition of any one of claims 1-9, wherein CBA is an antibody or an antigen-binding fragment thereof that (a) binds an epitope within amino acids 101 to 346 of human CD123 / IL3-Rα antigen, and (b) inhibits IL3-dependent proliferation in antigen-positive TF-1 cells.

11. The pharmaceutical composition of claim 10, wherein the antibody or antigen-binding fragment thereof comprises:

a) an immunoglobulin heavy chain variable region comprising a CDR1 having an amino acid sequence set forth in SEQ ID NO:4, a CDR2 having an amino acid sequence set forth in SEQ ID NO:5, and a CDR3 having an amino acid sequence set forth in SEQ ID NO:6; and

b) an immunoglobulin light chain variable region comprising a CDR1 having an amino acid sequence set forth in SEQ ID NO:1, a CDR2 having an amino acid sequence set forth in SEQ ID NO:2, and a CDR3 having an amino acid sequence set forth in SEQ ID NO:3.

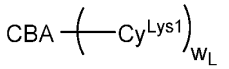
12. The pharmaceutical composition of claim 10, wherein the antibody or antigen-binding fragment thereof comprises a V_H sequence of SEQ ID NO:7 and a V_L sequence of SEQ ID NO:9.

13. The pharmaceutical composition of claim 10, wherein the antibody comprises:

a) an immunoglobulin heavy chain having the amino acid sequence set forth in SEQ ID NO:8; and

b) an immunoglobulin light chain having the amino acid sequence set forth in SEQ ID NO:10.

14. A pharmaceutical composition comprising an immunoconjugate and 0.1 mM to 20 mM methionine, wherein the immunoconjugate is represented by the following formula:

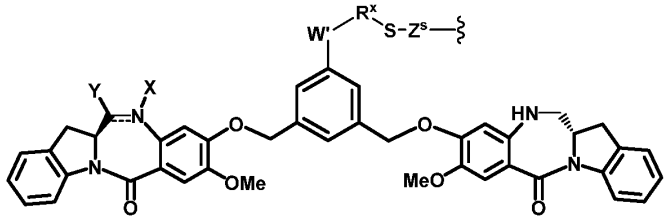


wherein:

CBA is an antibody or antigen-binding fragment thereof;

W_L is an integer from 1 to 20; and

Cy^{Lys1} is represented by the following formula:



;

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, and Y is -OH or -SO_3H;

R^x is independently a (C_1-C_6)alkyl;

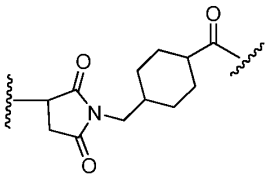
W' is -NR^e,

R^e is -$(CH_2$-CH_2-$O)_{n1}$-R^k;

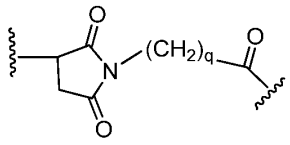
n1 is an integer from 2 to 6;

R^k is -H or -Me;

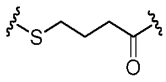
Z^s is selected from any one of the following formulas:



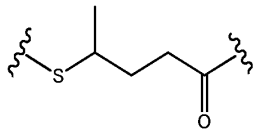
(b1);



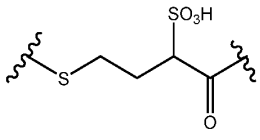
(b2);



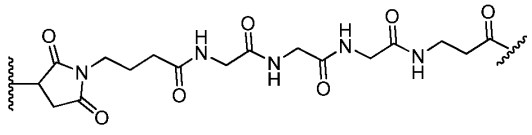
(b3);



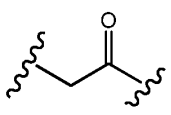
(b4);



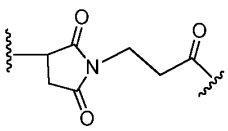
(b5),



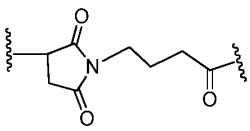
(b6),



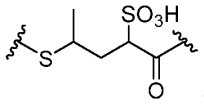
(b7);



(b8);



(b9); and



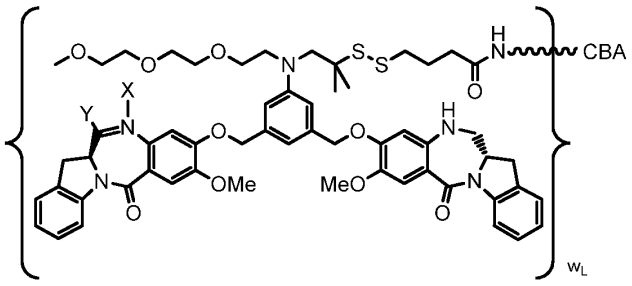
(b10),

or a pharmaceutically acceptable salt thereof, wherein q is an integer from 1 to 5.

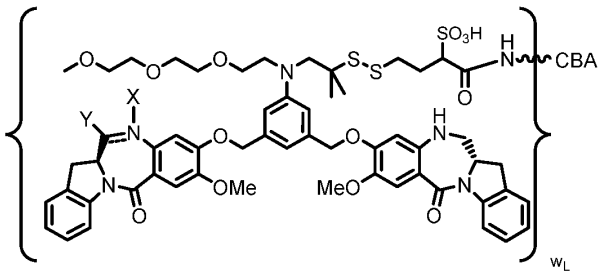
15. The pharmaceutical composition of claim 14, wherein R^x is independently -$(CH_2)_p$-(CR^fR^g)-, wherein R^f and R^g are each independently -H or a $(C_1$-$C_4)$alkyl; and p is 0, 1, 2 or 3.

16. The pharmaceutical composition of claim 15, wherein R^f and R^g are the same or different, and are selected from -H and -Me.

17. The pharmaceutical composition of claim 14, wherein the immunoconjugate is represented by:



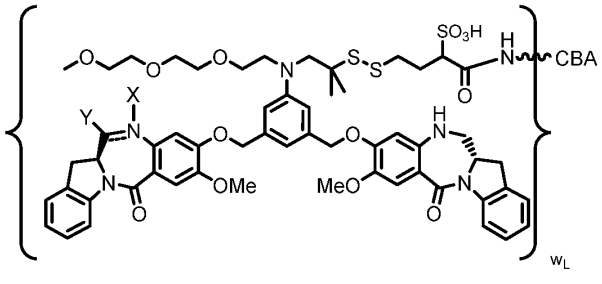
; or



;

or a pharmaceutically acceptable salt thereof, wherein W_L is an integer from 1 to 10; 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 -OH or -SO_3H.

18. The pharmaceutical composition of claim 17, wherein the immunoconjugate is represented by the following formula:



;

or a pharmaceutically acceptable salt thereof.

19. The pharmaceutical composition of any one of claims 14-18, wherein the antibody is an anti-CD33 antibody comprising an immunoglobulin heavy chain having the amino acid sequence set forth in SEQ ID NO:18 and an immunoglobulin light chain having the amino acid sequence set forth in SEQ ID NO:20.

20. The pharmaceutical composition of any one of claims 1-19, wherein the double line == between N and C represents a single bond, X is -H and Y is $-SO_3H$.

21. The pharmaceutical composition of any one of claims 1-20, wherein Y is $-SO_3H$ or the sodium or potassium salt thereof.

22. The pharmaceutical composition of any one of claims 1-21, wherein the pharmaceutical composition comprises 0.1 mM to 10 mM methionine.

23. The pharmaceutical composition of claim 22, wherein the pharmaceutical composition comprises 0.5 mM to 5 mM methionine.

24. The pharmaceutical composition of claim 22, wherein the pharmaceutical composition comprises 1.0 mM to 4.0 mM methionine.

25. The pharmaceutical composition of claim 24, wherein the pharmaceutical composition comprises 3.0 mM methionine.

26. The pharmaceutical composition of any one of claims 1-25, wherein when the pharmaceutical composition is exposed to light at room temperature for 6 hours or more, the immunoconjugate has less than 40%, less than 35%, 30%, less than 25%, less than 20% or less than 15% of methionine oxidation.

27. The pharmaceutical composition of any one of claims 1-26, wherein the pharmaceutical composition is in a container that protects the pharmaceutical composition from light exposure.

28. The pharmaceutical composition of any one of claims 1-27, wherein the pharmaceutical composition comprises 1 mg/mL to 10 mg/mL of the immunoconjugate.

29. The pharmaceutical composition of any one of claims 1-27, wherein the pharmaceutical composition comprises 1 mg/mL to 5 mg/mL of the immunoconjugate.

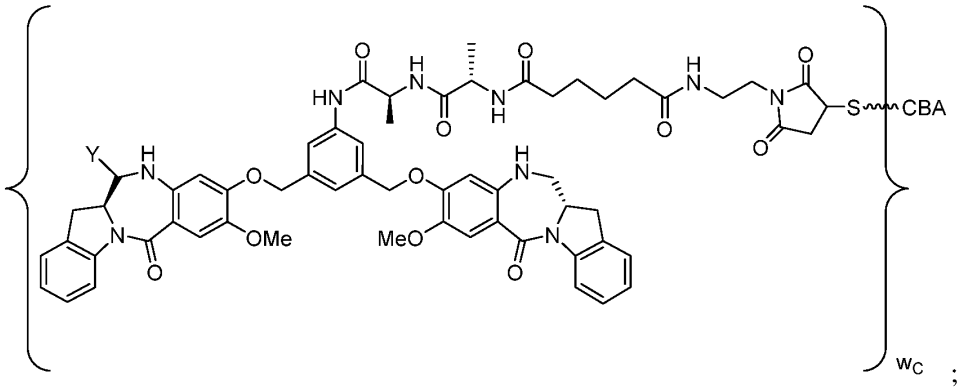
30. The pharmaceutical composition of any one of claims 1-29, further comprising sodium bisulfite.

31. The pharmaceutical composition of claim 30, wherein the pharmaceutical composition further comprises 10 μM to 100 μM, 20 μM to 90 μM, or 30 μM to 80 μM of sodium bisulfite.

32. The pharmaceutical composition of claim 30, wherein the pharmaceutical composition further comprises 40 μM to 60 μM of sodium bisulfite.

33. The pharmaceutical composition of claim 30, wherein the pharmaceutical composition further comprises 50 μM of sodium bisulfite.

34. A pharmaceutical composition comprising 1 mM to 4 mM of methionine and an immunoconjugate represented by the following formula:



;

or a pharmaceutically acceptable salt thereof, wherein:

Y is $-SO_3H$ or sodium salt thereof;

W_C is 2; and

CBA is an anti-CD123 antibody comprising: a) an immunoglobulin heavy chain having the amino acid sequence set forth in SEQ ID NO:8; and b) an immunoglobulin light chain having the amino acid sequence set forth in SEQ ID NO:10.

35. The pharmaceutical composition of claim 34, wherein the pharmaceutical composition comprises 3 mM methionine.

36. The pharmaceutical composition of claim 34 or 35, wherein when the pharmaceutical composition is exposed to light at room temperature for 6 hours, the immunoconjugate has less than 15% of methionine oxidation.

37. The pharmaceutical composition of any one of claims 34-36, wherein the pharmaceutical composition is in a container that protects the pharmaceutical composition from light exposure.

38. The pharmaceutical composition of any one of claims 34-37, wherein the pharmaceutical composition comprises 1 mg/mL to 5 mg/mL of the immunoconjugate.

39. The pharmaceutical composition of claim 38, wherein the pharmaceutical composition comprises 1 mg/mL to 3 mg/mL of the immunoconjugate.

40. The pharmaceutical composition of claim 38, wherein the pharmaceutical composition comprises 1.5 mg/mL to 2.5 mg/mL of the immunoconjugate.

41. The pharmaceutical composition of claim 38, wherein the pharmaceutical composition comprises 2 mg/mL of the immunoconjugate.

42. The pharmaceutical composition of any one of claims 34-41, further comprising sodium bisulfite.

43. The pharmaceutical composition of claim 42, wherein the pharmaceutical composition further comprises 10 μM to 100 μM, 20 μM to 90 μM, or 30 μM to 80 μM of sodium bisulfite.

44. The pharmaceutical composition of claim 43, wherein the pharmaceutical composition further comprises 40 μM to 60 μM of sodium bisulfite.

45. The pharmaceutical composition of claim 43, wherein the pharmaceutical composition further comprises 50 μM of sodium bisulfite.

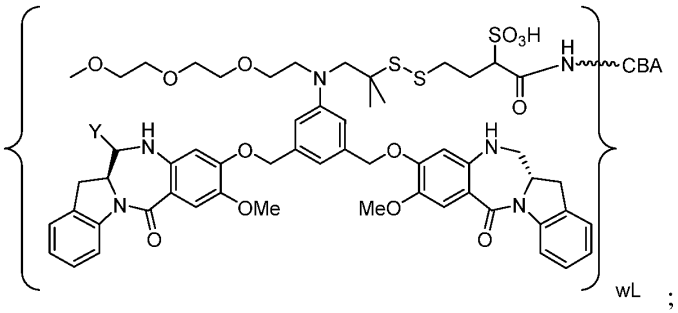
46. The pharmaceutical composition of any one of claims 34-45, wherein the pharmaceutical composition further comprises trehalose, polysorbate 20, and succinate.

47. The pharmaceutical composition of any one of claims 34-41, wherein the pharmaceutical composition further comprises 10 mM succinate, 50 μM sodium bisulfite, 7.2% (w/v) trehalose, and 0.01% (w/v) polysorbate 20.

48. The pharmaceutical composition of any one of claims 34-47, wherein the pharmaceutical composition has a pH of 4 to 4.5.

49. The pharmaceutical composition of claim 48, wherein the pharmaceutical composition has a pH of 4.2.

50. A pharmaceutical composition comprising 1 mM to 4 mM of methionine and an immunoconjugate represented by the following formula:



;

or a pharmaceutically acceptable salt thereof, wherein:

Y is $-SO_3H$ or sodium salt therof;

W_L is an integer from 1 to 10; and

CBA is an anti-CD33 antibody comprising an immunoglobulin heavy chain having the amino acid sequence set forth in SEQ ID NO:18 and an immunoglobulin light chain having the amino acid sequence set forth in SEQ ID NO:20.

51. The pharmaceutical composition of claim 50, wherein the pharmaceutical composition comprises 3 mM methionine.

52. The pharmaceutical composition of claim 50 or 51, wherein when the pharmaceutical composition is exposed to light at room temperature for 6 hours, the immunoconjugate has less than 15% of methionine oxidation.

53. The pharmaceutical composition of any one of claims 50-52, wherein the pharmaceutical composition is in a container that protects the pharmaceutical composition from light exposure.

54. The pharmaceutical composition of any one of claims 50-53, wherein the pharmaceutical composition comprises 1 mg/mL to 5 mg/mL of the immunoconjugate.

55. The pharmaceutical composition of claim 54, wherein the pharmaceutical composition comprises 1 mg/mL to 3 mg/mL of the immunoconjugate.

56. The pharmaceutical composition of claim 55, wherein the pharmaceutical composition comprises 1.5 mg/mL to 2.5 mg/mL of the immunoconjugate.

57. The pharmaceutical composition of claim 56, wherein the pharmaceutical composition comprises 2 mg/mL of the immunoconjugate.

58. The pharmaceutical composition of any one of claims 50-57, further comprising sodium bisulfite.

59. The pharmaceutical composition of claim 58, wherein the pharmaceutical composition further comprises 10 μM to 100 μM, 20 μM to 90 μM, or 30 μM to 80 μM of sodium bisulfite.

60. The pharmaceutical composition of claim 59, wherein the pharmaceutical composition further comprises 40 μM to 60 μM of sodium bisulfite.

61. The pharmaceutical composition of claim 59, wherein the pharmaceutical composition further comprises 50 μM of sodium bisulfite.

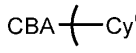
62. The pharmaceutical composition of any one of claims 50-61, wherein the pharmaceutical composition further comprises histidine, trehalose, and polysorbate 20.

63. The pharmaceutical composition of any one of claims 50-57, wherein the pharmaceutical composition further comprises 20 mM histidine, 50 μM sodium bisulfite, 7.2% (w/v) trehalose, 0.02% (w/v) polysorbate 20.

64. The pharmaceutical composition of any one of claims 50-63, wherein the pharmaceutical composition has a pH of 5.5 to 6.5.

65. The pharmaceutical composition of claim 64, wherein the pharmaceutical composition has a pH of 6.1.

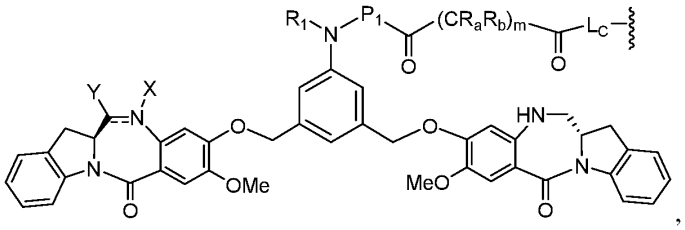
66. A method of reducing the amount of methionine oxidation in an immunoconjugate comprising mixing the immunoconjugate with 0.1 mM to 20 mM methionine to give a pharmaceutical composition comprising the immunoconjugate and methionine, wherein the immunoconjugate is represented by the following formula:



CBA is an antibody or antigen-binding fragment thereof;

W_C is 1 or 2; and

Cy^{Cys} is represented by the following formula:



or a pharmaceutically acceptable salt thereof, wherein:

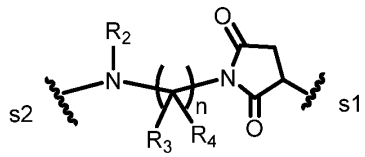
the double line == between N and C represents a single bond or a double bond, provided that when it is a double bond, X is absent and Y is -H or a $(C_1$-$C_4)$alkyl; and when it is a single bond, X is -H or an amine protecting moiety, Y is -OH or $-SO_3H$;

R_1 is -H or a $(C_1$-$C_3)$alkyl;

P_1 is an amino acid residue or a peptide containing 2 to 5 amino acid residues;

R_a and R_b, for each occurrence, are independently -H, $(C_1$-$C_3)$alkyl, or a charged substituent or an ionizable group Q;

m is an integer from 1 to 6;

L_C is represented by , s1 is the site covalently linked to CBA, and s2 is the site covalently linked to the -C(=O)- group on Cy^{C1}; wherein:

R_2 is -H or a $(C_1$-$C_3)$alkyl

R_3 and R_4, for each occurrence, are independently -H or a $(C_1$-$C_3)$alkyl; and

n is an integer between 1 and 10.

67. The method of claim 66, wherein R_a and R_b are both H; and R_1 is H or Me.

68. The method of claim 66 or 67, wherein P is selected from Gly-Gly-Gly, Ala-Val, Val-Ala, Val-Cit, 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:21), β-Ala-Leu-Ala-Leu (SEQ ID NO:22), Gly-Phe-Leu-Gly (SEQ ID NO:23), 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, Ala-Ala, Ala-D-Ala, D-Ala-Ala, D-Ala-D-Ala, Ala-Met, and Met-Ala.

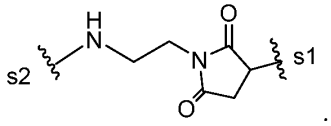
69. The method of claim 68, wherein P is Ala-Ala, Ala-D-Ala, D-Ala-Ala, or D-Ala-D-Ala.

70. The method of any one of claims 66-69, wherein Q is $-SO_3H$.

71. The method of any one of claims 66-69, wherein Q is H.

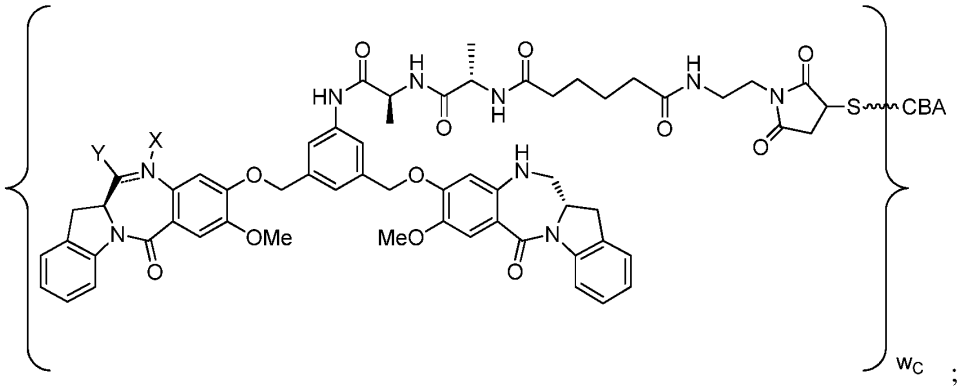
72. The method of any one of claims 66-71, wherein R_3 and R_4 are both H; and n is an integer from 1 to 6.

73. The method of any one of claims 66-72, wherein $-L_C-$ is represented by the following formula:



.

74. The method of claim 66, wherein the immunoconjugate is represented by the following formula:



;

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, and when it is a single bond, X is -H, and Y is -OH or $-SO_3H$.

75. The method of any one of claims 66-74, wherein CBA is an antibody or an antigen-binding fragment thereof that (a) binds an epitope within amino acids 101 to 346 of human CD123 / IL3-Rα antigen, and (b) inhibits IL3-dependent proliferation in antigen-positive TF-1 cells.

76. The method of claim 75, wherein the antibody or antigen-binding fragment thereof comprises:

a) an immunoglobulin heavy chain variable region comprising a CDR1 having an amino acid sequence set forth in SEQ ID NO:4, a CDR2 having an amino acid sequence set forth in SEQ ID NO:5, and a CDR3 having an amino acid sequence set forth in SEQ ID NO:6; and

b) an immunoglobulin light chain variable region comprising a CDR1 having an amino acid sequence set forth in SEQ ID NO:1, a CDR2 having an amino acid sequence set forth in SEQ ID NO:2, and a CDR3 having an amino acid sequence set forth in SEQ ID NO:3.

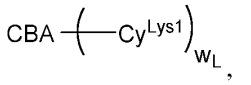
77. The method of claim 75, wherein the antibody or antigen-binding fragment thereof comprises a V_H sequence of SEQ ID NO:7 and a V_L sequence of SEQ ID NO:9.

78. The method of claim 75, wherein the antibody comprises:

a) an immunoglobulin heavy chain having the amino acid sequence set forth in SEQ ID NO:8; and

b) an immunoglobulin light chain having the amino acid sequence set forth in SEQ ID NO:10.

79. A method of reducing the amount of methionine oxidation in an immunoconjugate comprising mixing the immunoconjugate with 0.1 mM to 20 mM methionine to give a pharmaceutical composition comprising the immunoconjugate and methionine, wherein the immunoconjugate is represented by the following formula:

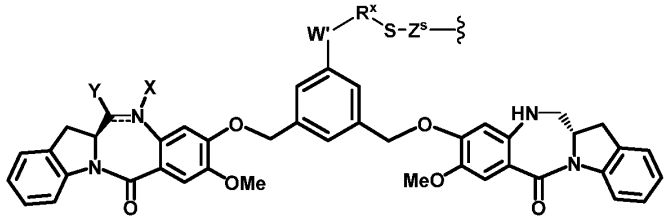


wherein:

CBA is an antibody or antigen-binding fragment thereof;

W_L is an integer from 1 to 20; and

Cy^{Lys1} is represented by the following formula:



;

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, and Y is -OH or -SO_3H;

R^x is independently a (C_1-C_6)alkyl;

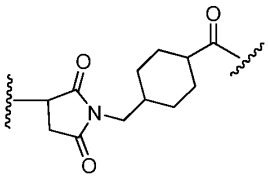
W' is -NR^e,

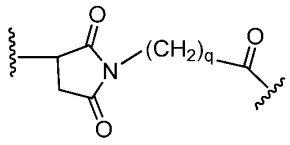
R^e is -$(CH_2$-CH_2-$O)_{n1}$-R^k;

n1 is an integer from 2 to 6;

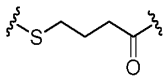
R^k is -H or -Me;

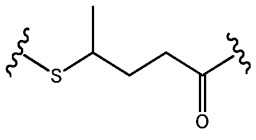
Z^s is selected from any one of the following formulas:

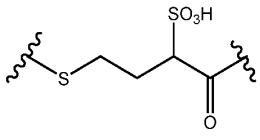

(b1);



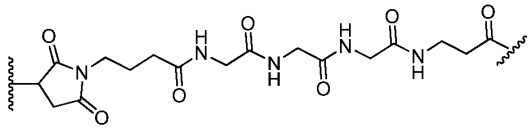
(b2);

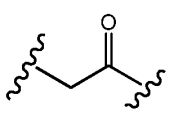

(b3);

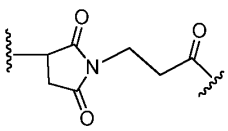

(b4);

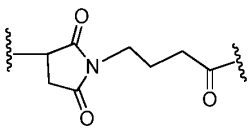


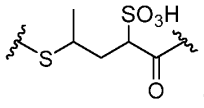
(b5),


(b6),


(b7);


(b8);


(b9); and



(b10),

wherein:

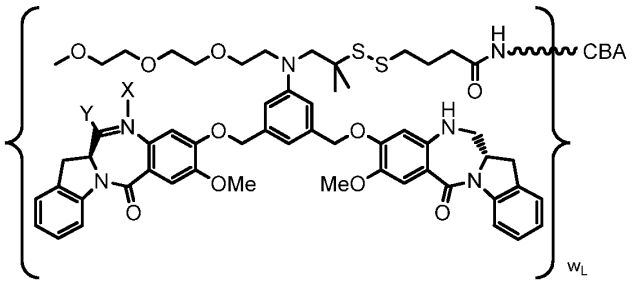
q is an integer from 1 to 5; and

M^+ is $-H^+$ or a cation.

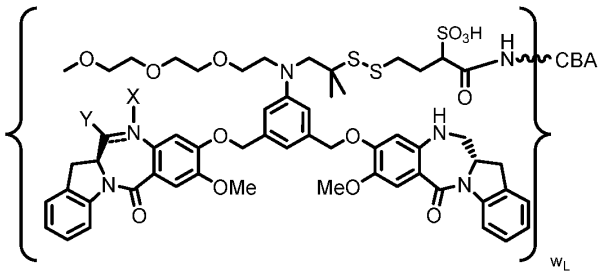
80. The method of claim 79, wherein R^x is independently $-(CH_2)_p-(CR^fR^g)-$, wherein R^f and R^g are each independently -H or a (C_1-C_4)alkyl; and p is 0, 1, 2 or 3.

81. The method of claim 80, wherein R^f and R^g are the same or different, and are selected from -H and -Me.

82. The method of claim 79, wherein the immunoconjugate is represented by:



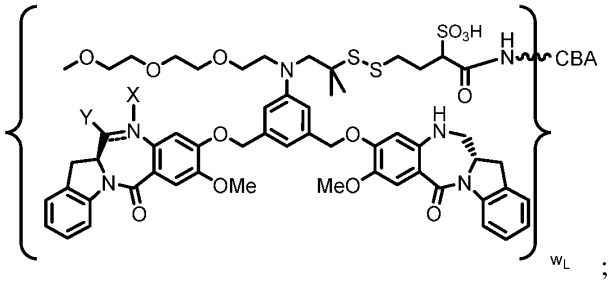
; or



;

or a pharmaceutically acceptable salt thereof, wherein W_L is an integer from 1 to 10; 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 -OH or $-SO_3H$.

83. The method of claim 82, wherein the immunoconjugate is represented by the following formula:



or a pharmaceutically acceptable salt thereof.

84. The method of any one of claims 79-83, wherein the antibody is an anti-CD33 antibody comprising an immunoglobulin heavy chain having the amino acid sequence set forth in SEQ ID NO:18 and an immunoglobulin light chain having the amino acid sequence set forth in SEQ ID NO:20.

85. The method of any one of claims 66-84, wherein the double line == between N and C represents a single bond, X is -H and Y is -SO_3H.

86. The method of any one of claims 66-85, wherein Y is –SO_3H or sodium or potassium salt thereof.

87. The method of any one of claims 66-86, wherein the pharmaceutical composition comprises 0.1 mM to 10 mM methionine.

88. The method of claim 87, wherein the pharmaceutical composition comprises 0.5 to 5 mM methionine.

89. The method of claim 87, wherein the pharmaceutical composition comprises 1.0 to 4.0 mM methionine.

90. The method of claim 89, wherein the pharmaceutical composition comprises 3.0 mM of methionine.

91. The method of any one of claims 66-90, wherein when the pharmaceutical composition is exposed to light at room temperature for 6 hours or more, the immunoconjugate has less than 40%, less than 35%, less than 30%, less than 25%, less than 20% or less than 15% of methionine oxidation

92. The method of any one of claims 66-91, wherein the pharmaceutical composition is in a container that protects the pharmaceutical composition from light exposure.

93. The method of any one of claims 66-92, wherein the pharmaceutical composition comprises 1 mg/mL to 10 mg/mL of the immunoconjugate.

94. The method of any one of claims 66-92, wherein the pharmaceutical composition comprises 1 mg/mL to 5 mg/mL of the immunoconjugate.

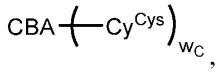
95. The method of any one of claims 66-94, further comprising sodium bisulfite.

96. The method of claim 95, wherein the pharmaceutical composition further comprises 10 μM to 100 μM, 20 μM to 90 μM, or 30 μM to 80 μM of sodium bisulfite.

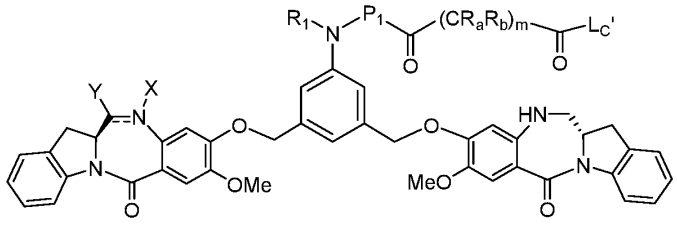
97. The method of claim 95, wherein the pharmaceutical composition further comprises 40 μM to 60 μM of sodium bisulfite.

98. The method of claim 95, wherein the pharmaceutical composition further comprises 50 μM of sodium bisulfite.

99. A method of preparing an immunoconjugate represented by the following formula:



comprising reacting a CBA with a cytotoxic agent represented by the following formula:

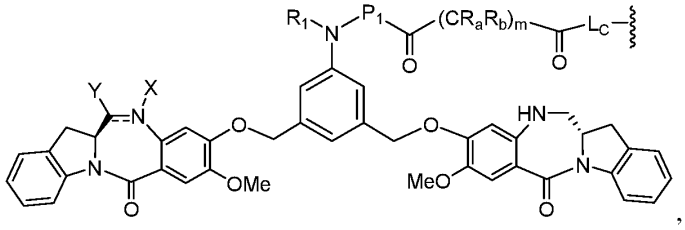


or a pharmaceutically acceptable salt thereof, in the presence of an antioxidant, wherein:

CBA is an antibody or antigen-binding fragment thereof;

W_C is 1 or 2; and

Cy^{Cys} is represented by the following formula:



,

or a pharmaceutically acceptable salt thereof, wherein:

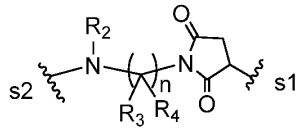
the double line == between N and C represents a single bond or a double bond, provided that when it is a double bond, X is absent and Y is -H or a (C_1-C_4)alkyl; and when it is a single bond, X is -H or an amine protecting moiety, Y is -OH or -SO_3H;

R_1 is -H or a (C_1-C_3)alkyl;

P_1 is an amino acid residue or a peptide containing 2 to 5 amino acid residues;

R_a and R_b, for each occurrence, are independently -H, (C_1-C_3)alkyl, or a charged substituent or an ionizable group Q;

m is an integer from 1 to 6;

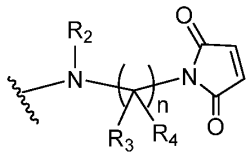
L_C is represented by 

, s1 is the site covalently linked to CBA, and s2 is the site covalently linked to the -C(=O)- group on Cy^{C1};

R_2 is -H or a (C_1-C_3)alkyl

R_3 and R_4, for each occurrence, are independently -H or a (C_1-C_3)alkyl; and

n is an integer between 1 and 10; and

L_C' is represented by 

.

100. The method of claim 99, wherein the antioxidant reduces the amount of methionine oxidation in the immunoconjugate.

101. The method of claim 99, wherein the antioxidant is N-acetylmethionine.

102. The method of any one of claims 99-101, wherein R_a and R_b are both H; and R_1 is H or Me.

103. The method of any one of claims 99-102, wherein P is selected from Gly-Gly-Gly, Ala-Val, Val-Ala, Val-Cit, 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:21), β-Ala-Leu-Ala-Leu (SEQ ID NO:22), Gly-Phe-Leu-Gly (SEQ ID NO:23), 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, Ala-Ala, Ala-D-Ala, D-Ala-Ala, D-Ala-D-Ala, Ala-Met, and Met-Ala.

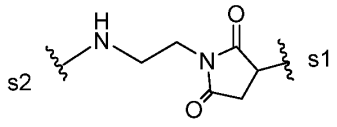
104. The method of claim 103, wherein P is Ala-Ala, Ala-D-Ala, D-Ala-Ala, or D-Ala-D-Ala.

105. The method of any one of claims 99-104, wherein Q is $-SO_3H$.

106. The method of any one of claims 99-104, wherein Q is H.

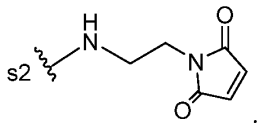
107. The method of any one of claims 99-106, wherein R_3 and R_4 are both H; and n is an integer from 1 to 6.

108. The method of any one of claims 99-107, wherein $-L_C-$ is represented by the following formula:



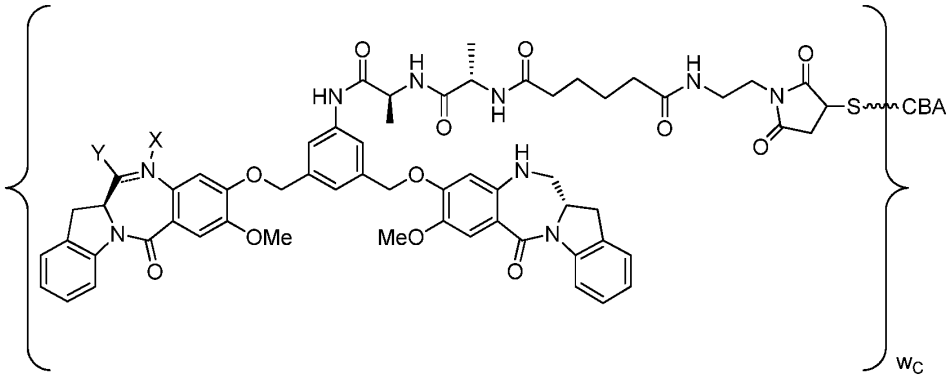
; and

$-L_C'$ is represented by the following formula:



.

109. The method of any one of claims 99-101, wherein the immunoconjugate is represented by the following formula:



;

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, and when it is a single bond, X is -H, and Y is -OH or $-SO_3H$.

110. The method of any one of claims 99-109, wherein CBA is an antibody or an antigen-binding fragment thereof that (a) binds an epitope within amino acids 101 to 346 of human CD123 / IL3-Rα antigen, and (b) inhibits IL3-dependent proliferation in antigen-positive TF-1 cells.

111. The method of claim 110, wherein the antibody or antigen-binding fragment thereof comprises:

a) an immunoglobulin heavy chain variable region comprising a CDR1 having an amino acid sequence set forth in SEQ ID NO:4, a CDR2 having an amino acid sequence set forth in SEQ ID NO:5, and a CDR3 having an amino acid sequence set forth in SEQ ID NO:6; and

b) an immunoglobulin light chain variable region comprising a CDR1 having an amino acid sequence set forth in SEQ ID NO:1, a CDR2 having an amino acid sequence set forth in SEQ ID NO:2, and a CDR3 having an amino acid sequence set forth in SEQ ID NO:3.

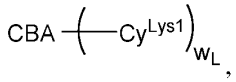
112. The method of claim 110, wherein the antibody or antigen-binding fragment thereof comprises a V_H sequence of SEQ ID NO:7 and a V_L sequence of SEQ ID NO:9.

113. The method of claim 110, wherein the antibody comprises:

a) an immunoglobulin heavy chain having the amino acid sequence set forth in SEQ ID NO:8; and

b) an immunoglobulin light chain having the amino acid sequence set forth in SEQ ID NO:10.

114. A method of preparing an immunoconjugate represented by the following formula:

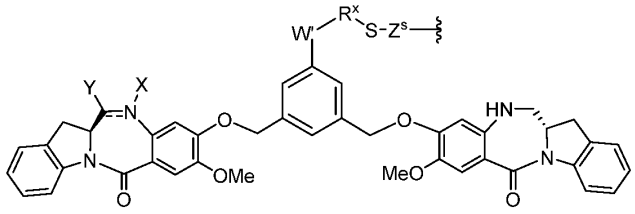


wherein:

CBA is an antibody or antigen-binding fragment thereof;

W_L is an integer from 1 to 20; and

Cy^{Lys1} is represented by the following formula:



;

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, and Y is -OH or -SO_3H;

R^x is independently a (C_1-C_6)alkyl;

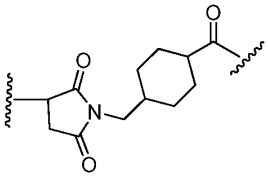
W' is -NR^e,

R^e is -$(CH_2$-CH_2-$O)_{n1}$-R^k;

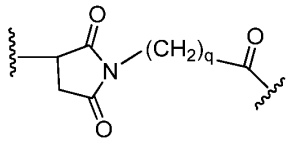
n1 is an integer from 2 to 6;

R^k is -H or -Me;

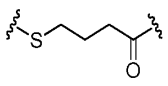
Z^s is selected from any one of the following formulas:



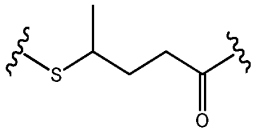
(b1);



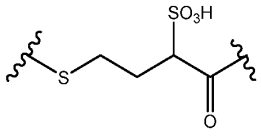
(b2);



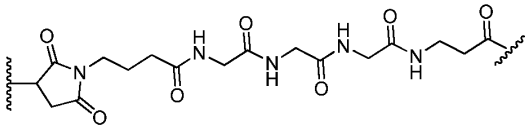
(b3);



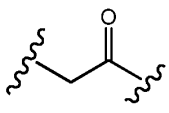
(b4);



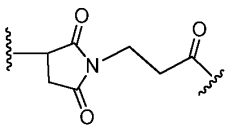
(b5),



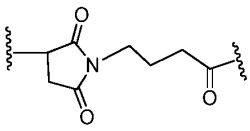
(b6),



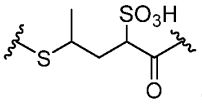
(b7);



(b8);



(b9); and

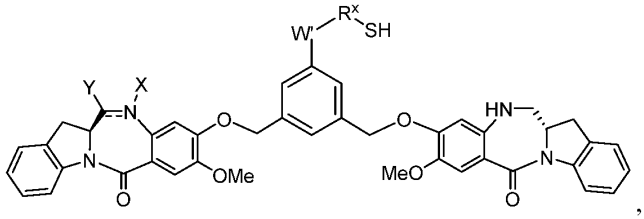


(b10),

or a pharmaceutically acceptable salt thereof, wherein q is an integer from 1 to 5,

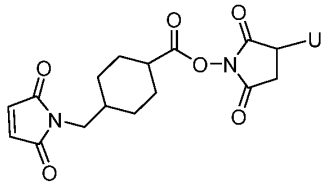
comprising the step of:

(a) reacting a cytotoxic agent represented by the following formula:

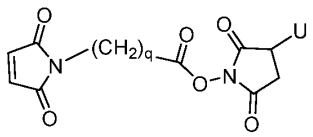


,

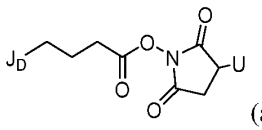
or a pharmaceutically acceptable salt thereof, with a bifunctional crosslinking agent selected from the following:



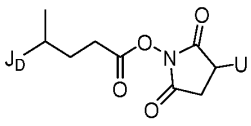
(a1L);



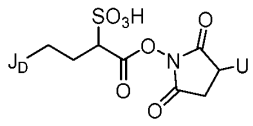
(a2L);



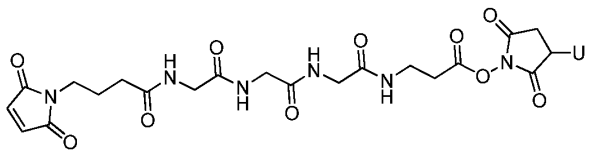
(a3L);



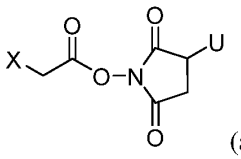
(a4L);



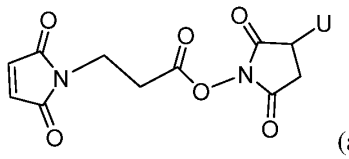
(a5L),



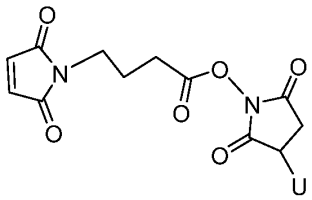
(a6L),



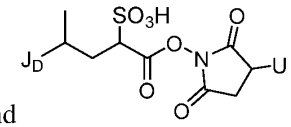
(a7L);



(a8L);



(a9L); and



(a10L),

to form a cytotoxic agent-linker compound, wherein X is halogen; J_D –SH, –SSR^d, or –SC(=O)R^g; R^d is phenyl, nitrophenyl, dinitrophenyl, carboxynitrophenyl, pyridyl or nitropyridyl; R^g is an alkyl; q is an integer from 1 to 5; and U is –H or SO_3H; and

(b) reacting the CBA with the cytotoxic agent-linker compound in the presence of an antioxidant to form the immunoconjugate.

115. The method of claim 114, wherein the cytotoxic agent-linker compound is not purified before reacting with the CBA in step (b).

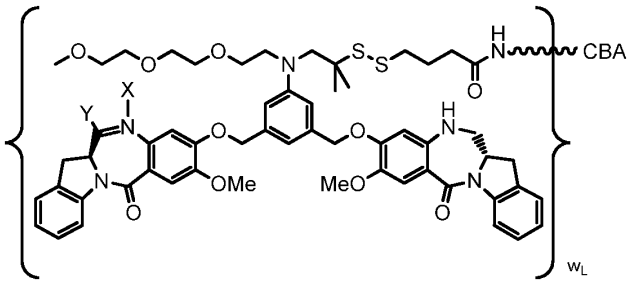
116. The method of claim 114 or 115, wherein the antioxidant reduces the amount of methionine oxidation in the immunoconjugate.

117. The method of claim 116, wherein the antioxidant is N-acetylmethionine methyl ester.

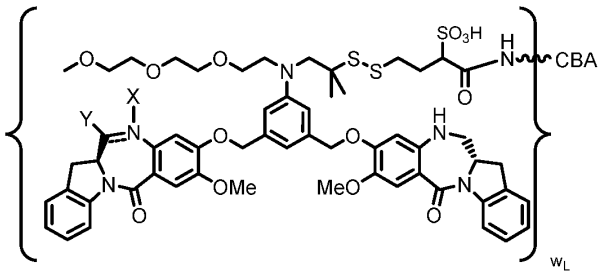
118. The method of any one of claims 114-117, wherein R^x is independently -$(CH_2)_p$-(CR^fR^g)-, wherein R^f and R^g are each independently -H or a (C_1-C_4)alkyl; and p is 0, 1, 2 or 3.

119. The method of claim 118, wherein R^f and R^g are the same or different, and are selected from -H and -Me.

120. The method of any one of claims 114-117, wherein the immunoconjugate is represented by:



; or



;

or a pharmaceutically acceptable salt thereof, wherein W_L is an integer from 1 to 10; 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 -OH or -SO_3H.

121. The method of any one of claims 114-120, wherein the antibody is an anti-CD33 antibody comprising an immunoglobulin heavy chain having the amino acid sequence set forth in SEQ ID NO:18 and an immunoglobulin light chain having the amino acid sequence set forth in SEQ ID NO:20.

122. The method of any one of claims 99-121, wherein the double line == between N and C represents a single bond, X is -H and Y is -OH or $-SO_3H$.

123. The method of any one of claims 99-122, wherein Y is $-SO_3H$ or sodium or potassium salt thereof.

124. The method of any one of claims 99-123, wherein the method further comprises purifying the immunoconjugate into a formulation buffer to give a pharmaceutical composition comprising the immunoconjugate and 0.1 mM to 10 mM methionine.

125. The method of claim 124, wherein the pharmaceutical composition comprises 0.5 mM to 5 mM methionine.

126. The method of claim 124, wherein the pharmaceutical composition comprises 1 mM to 4 mM methionine.

127. The method of claim 124, wherein the pharmaceutical composition comprises 3 mM methionine.

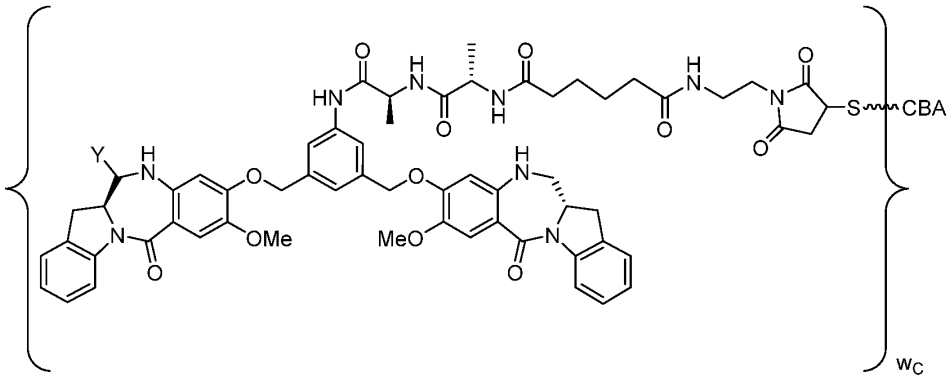
128. The method of any one of claims 124-127, wherein the pharmaceutical composition further comprises sodium bisulfite.

129. The method of claim 128, wherein the pharmaceutical composition further comprises 10 μM to 100 μM, 20 μM to 90 μM, or 30 μM to 80 μM of sodium bisulfite.

130. The method of claim 129, wherein the pharmaceutical composition further comprises 40 μM to 60 μM of sodium bisulfite.

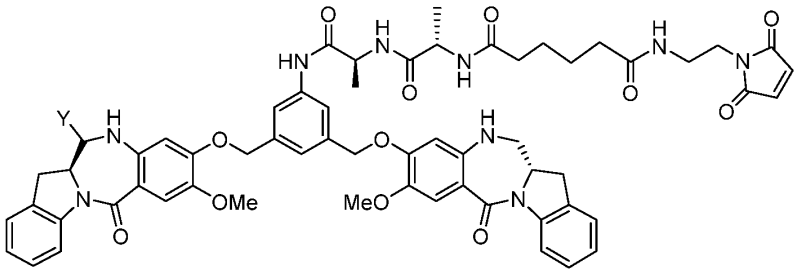
131. The method of claim 129, wherein the pharmaceutical composition further comprises 50 μM of sodium bisulfite.

132. A method of preparing an immunoconjugate represented by the following formula:



;

or a pharmaceutically acceptable salt thereof, comprising reacting the CBA with a cytotoxic agent represented by the following formula:



or a pharmaceutically acceptable salt thereof, in the presence of an antioxidant, wherein:

Y is $-SO_3H$ or sodium salt thereof;

W_C is 2; and

CBA is an anti-CD123 antibody comprising: a) an immunoglobulin heavy chain having the amino acid sequence set forth in SEQ ID NO:8; and b) an immunoglobulin light chain having the amino acid sequence set forth in SEQ ID NO:10.

133. The method of claim 131, wherein the antioxidant reduces the amount of methionine oxidation in the immunoconjugate.

134. The method of claim 131, wherein the antioxidant is N-acetylmethionine.

135. The method of any one of claims 131-134, wherein the method further comprises purifying the immunoconjugate into a formulation buffer to give a pharmaceutical composition comprising the immunoconjugate and 1 mM to 4 mM methionine.

136. The method of claim 135, the pharmaceutical composition comprises 3 mM methionine.

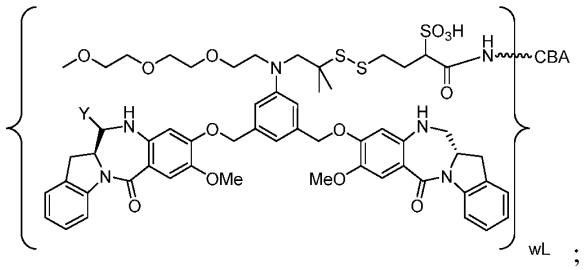
137. The method of claim 135 or 136, wherein the pharmaceutical composition further comprises 10 μM to 100 μM, 20 μM to 90 μM, or 30 μM to 80 μM of sodium bisulfite.

138. The method of claim 137, wherein the pharmaceutical composition further comprises 40 μM to 60 μM of sodium bisulfite.

139. The method of claim 138, wherein the pharmaceutical composition further comprises 50 μM of sodium bisulfite.

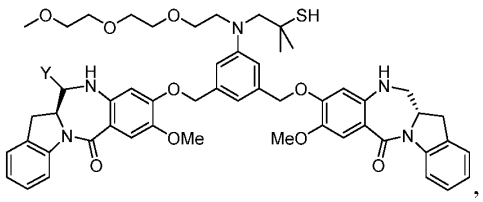
140. The method of claim 135, wherein the pharmaceutical composition comprises further comprises 10 mM succinate, 50 μM sodium bisulfite, 7.2% (w/v) trehalose, and 0.01% (w/v) polysorbate 20.

141. A method of preparing an immunoconjugate represented by the following formula:

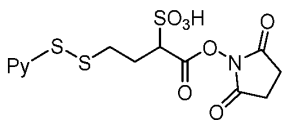


or a pharmaceutically acceptable salt thereof, comprising the steps of:

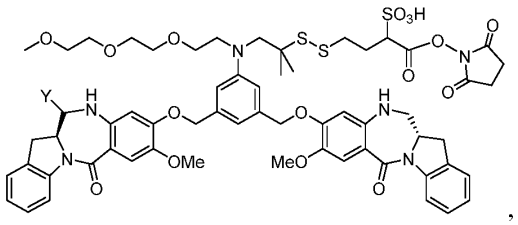
(a) reacting a cytotoxic agent represented by the following formula:



or a pharmaceutically acceptable salt thereof, with a bifunctional crosslinking agent sulfo-SPDB presented by the following formula:



to form a cytotoxic agent-linker compound represented by the following formula:



or a pharmaceutically acceptable salt thereof; and

(b) reacting the CBA with the cytotoxic agent-linker compound in the presence of an antioxidant to form the immunoconjugate, wherein:

Y is $-SO_3H$;

W_L is an integer from 1 to 10; and

CBA is an anti-CD33 antibody comprising an immunoglobulin heavy chain having the amino acid sequence set forth in SEQ ID NO:18 and an immunoglobulin light chain having the amino acid sequence set forth in SEQ ID NO:20.

142. The method of claim 141, wherein the cytotoxic agent-linker compound is not purified before reacting with the CBA in step (b).

143. The method of claim 141 or 142, wherein the antioxidant reduces the amount of methionine oxidation in the immunoconjugate.

144. The method of claim 141 or 142, wherein the antioxidant is N-acetylmethionine methyl ester.

145. The method of any one of claims 141-144, wherein the method further comprises purifying the immunoconjugate into a formulation buffer to give a pharmaceutical composition comprising the immunoconjugate and 1 mM to 4 mM methionine.

146. The method of claim 145, the pharmaceutical composition comprises 3 mM methionine.

147. The method of claim 145 or 146, wherein the pharmaceutical composition further comprises 10 μM to 100 μM, 20 μM to 90 μM, or 30 μM to 80 μM of sodium bisulfite.

148. The method of claim 147, wherein the pharmaceutical composition further comprises 40 μM to 60 μM of sodium bisulfite.

149. The method of claim 148, wherein the pharmaceutical composition further comprises 50 μM of sodium bisulfite.

150. The method of claim 145, wherein the pharmaceutical composition comprises further comprises 20 mM histidine, 50 μM sodium bisulfite, 7.2% (w/v) trehalose, 0.02% (w/v) polysorbate 20.