41. The antibody-drug conjugate compound of claim 40 wherein the anti-CD33 antibody comprises three light chain hypervariable regions (HVR-L1, HVR-L2 and HVR-L3) and three heavy chain hypervariable regions (HVR-H1, HVR-H2 and HVR-H3) wherein:

HVR-L1 comprises the amino acid sequence of SEQ ID NO:51;

HVR-L2 comprises the amino acid sequence of SEQ ID NO:52;

HVR-L3 comprises the amino acid sequence of SEQ ID NO:53;

HVR-H1 comprises the amino acid sequence of SEQ ID NO:54;

HVR-H2 comprises the amino acid sequence of SEQ ID NO:55; and

HVR-H3 comprises the amino acid sequence of SEQ ID NO:56.

42. The antibody-drug conjugate compound of claim 40 wherein the anti-CD33 antibody comprises a light chain hypervariable region of SEQ ID NO:57 or a heavy chain variable region of SEQ ID NO:58.

43. The antibody-drug conjugate compound of claim 40 wherein the anti-CD33 antibody comprises three light chain hypervariable regions (HVR-L1, HVR-L2 and HVR-L3) and three heavy chain hypervariable regions (HVR-H1, HVR-H2 and HVR-H3) wherein:

HVR-L1 comprises the amino acid sequence of SEQ ID NO:59;

HVR-L2 comprises the amino acid sequence of SEQ ID NO:60;

HVR-L3 comprises the amino acid sequence of SEQ ID NO:61;

HVR-H1 comprises the amino acid sequence of SEQ ID NO:62;

HVR-H2 comprises the amino acid sequence of SEQ ID NO:63; and

HVR-H3 comprises the amino acid sequence of SEQ ID NO:64.

44. The antibody-drug conjugate compound of claim 40 wherein the anti-CD33 antibody comprises a light chain hypervariable region selected from SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, and SEQ ID NO:71; or a heavy chain variable region selected from of SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, and SEQ ID NO:72.

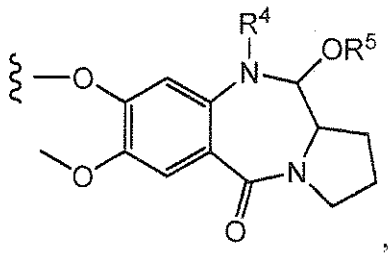
45. A pharmaceutical composition comprising the antibody-drug conjugate compound of any one of claims 1 to 44, and a pharmaceutically acceptable carrier, glidant, diluent, or excipient.

46. A method of treating cancer comprising administering to a patient a therapeutically-effective amount of the pharmaceutical composition of claim 45.

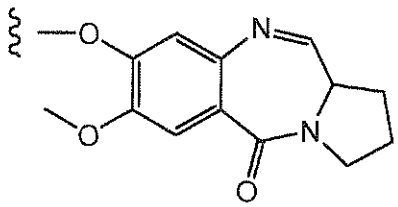
47. A kit for treating cancer, comprising:

a) the pharmaceutical composition of claim 45; and

b) instructions for use.



, and



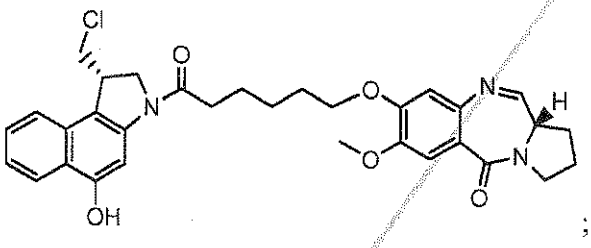
where the wavy line indicates the site of attachment to T;

X^1 and X^2 are independently selected from O and NR^3, where R^3 is selected from H and C_1–C_6 alkyl optionally substituted with one or more F;

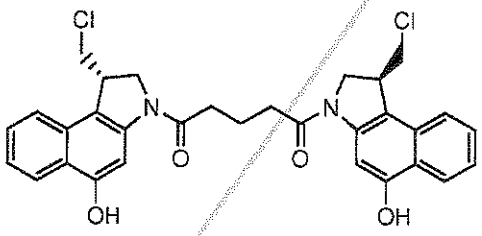
R^4 is H, CO_2R, where R is C_1–C_6 alkyl or benzyl; and

R^5 is H or C_1–C_6 alkyl.

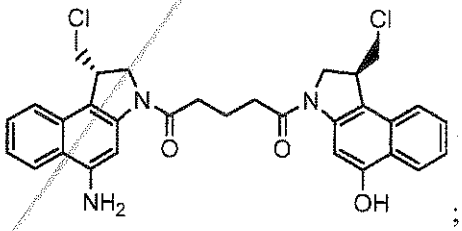
54. The CBI dimer drug moiety compound of claim 41 selected from:



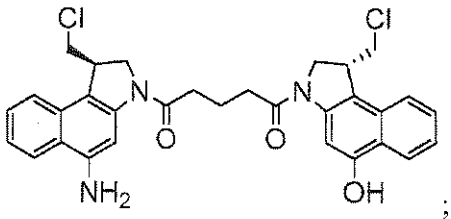
;



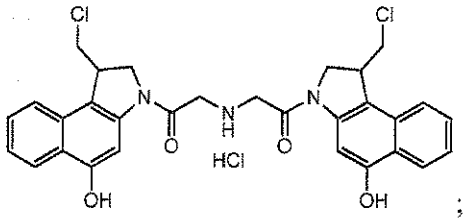
;



;

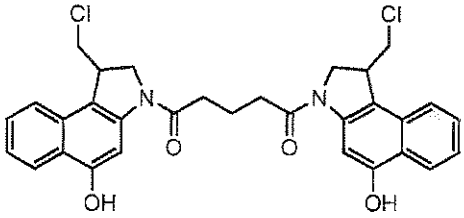


;



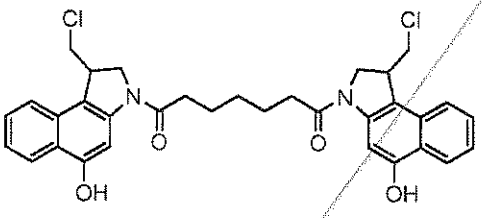
;

(racemic)



; and

(racemic)

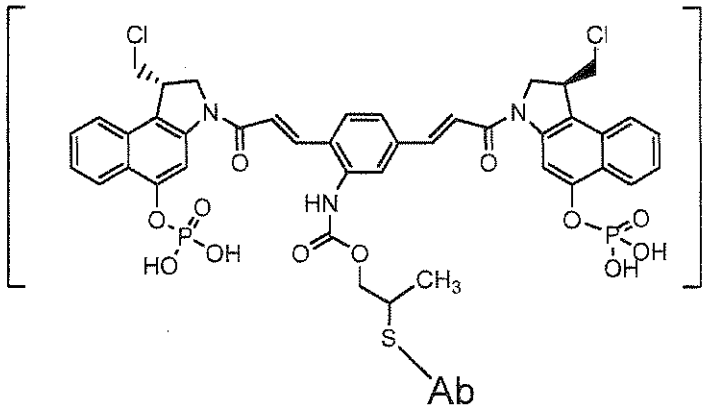


(racemic)

55. A conjugate according to any one of claims 1 to 22, wherein p is 1, 2, 3 or 4.

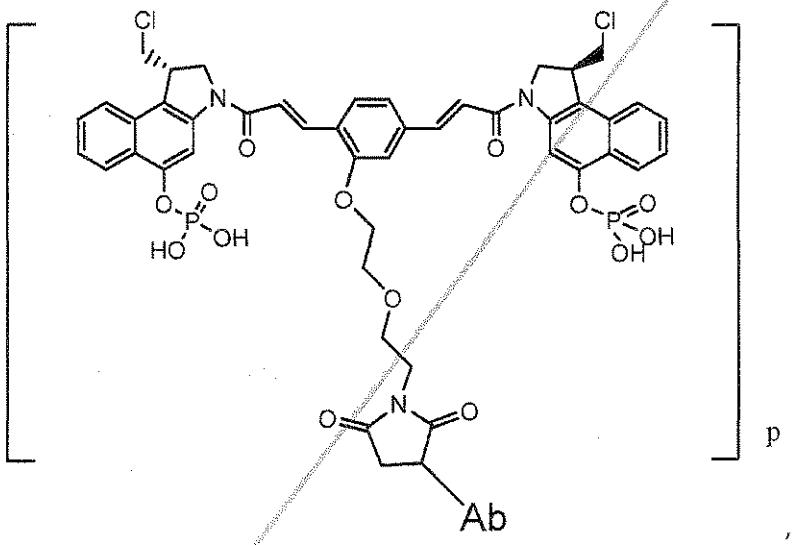
56. A conjugate according to any one of claims 1 to 22, wherein p is 1 or 2.

57. A conjugate of claim 1, which is



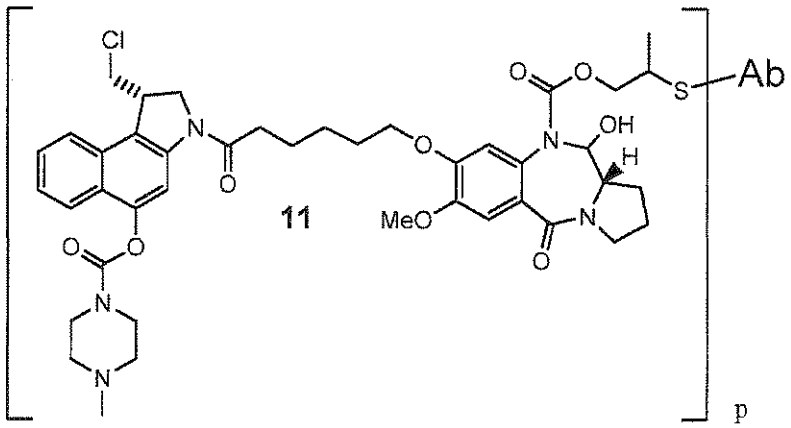
wherein p is 1-4, and Ab is an anti-HER2, anti-CD33, anti-CLL-1, anti-CD22, anti-CD79b or anti-NaPi3b antibody .

58. A conjugate of claim 1, which is



wherein p is 1-4, and Ab is an anti-HER2, anti-CD33, or anti-NaPi3b antibody .

59. A conjugate of claim 1, which is



wherein p is 1-4, and Ab is an anti-HER2, anti-CD33, anti-CLL-1, anti-CD22, anti-CD79b or anti-NaPi3b antibody .

60. A conjugate of any one of claims 57-59, wherein Ab is an anti-HER2 antibody.

61. A conjugate of any one of claims 57-59, wherein Ab is an anti-CLL-1 antibody.

62. A conjugate of any one of claims 57-59, wherein Ab is an anti-CD22 antibody.

63. A conjugate of any one of claims 57-59, wherein Ab is an anti-CD79b antibody.

41. The antibody-drug conjugate compound of claim 40 wherein the anti-CD33 antibody comprises three light chain hypervariable regions (HVR-L1, HVR-L2 and HVR-L3) and three heavy chain hypervariable regions (HVR-H1, HVR-H2 and HVR-H3) wherein:

HVR-L1 comprises the amino acid sequence of SEQ ID NO:51;
HVR-L2 comprises the amino acid sequence of SEQ ID NO:52;
HVR-L3 comprises the amino acid sequence of SEQ ID NO:53;
HVR-H1 comprises the amino acid sequence of SEQ ID NO:54;
HVR-H2 comprises the amino acid sequence of SEQ ID NO:55; and
HVR-H3 comprises the amino acid sequence of SEQ ID NO:56.

42. The antibody-drug conjugate compound of claim 40 wherein the anti-CD33 antibody comprises a light chain hypervariable region of SEQ ID NO:57 or a heavy chain variable region of SEQ ID NO:58.

43. The antibody-drug conjugate compound of claim 40 wherein the anti-CD33 antibody comprises three light chain hypervariable regions (HVR-L1, HVR-L2 and HVR-L3) and three heavy chain hypervariable regions (HVR-H1, HVR-H2 and HVR-H3) wherein:

HVR-L1 comprises the amino acid sequence of SEQ ID NO:59;
HVR-L2 comprises the amino acid sequence of SEQ ID NO:60;
HVR-L3 comprises the amino acid sequence of SEQ ID NO:61;
HVR-H1 comprises the amino acid sequence of SEQ ID NO:62;
HVR-H2 comprises the amino acid sequence of SEQ ID NO:63; and
HVR-H3 comprises the amino acid sequence of SEQ ID NO:64.

44. The antibody-drug conjugate compound of claim 40 wherein the anti-CD33 antibody comprises a light chain hypervariable region selected from SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, and SEQ ID NO:71; or a heavy chain variable region selected from of SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, and SEQ ID NO:72.

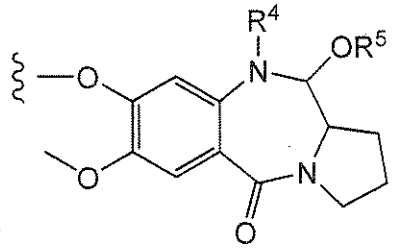
45. A pharmaceutical composition comprising the antibody-drug conjugate compound of any one of claims 1 to 44, and a pharmaceutically acceptable excipient.

46. Use of the pharmaceutical composition of claim 45 in the manufacture of a medicament for treating cancer in a patient.

47. A kit for treating cancer, comprising:

a) the pharmaceutical composition of claim 45; and

b) instructions for use.

and 

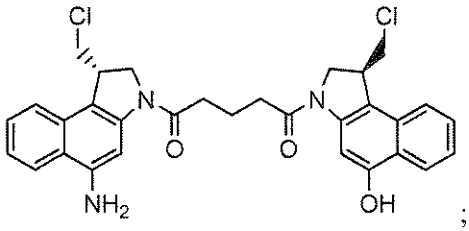
where the wavy line indicates the site of attachment to T;

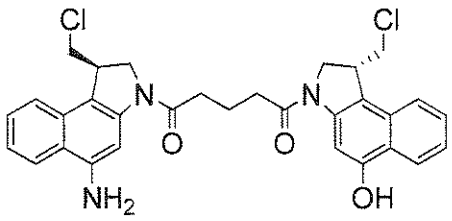
X^1 and X^2 are independently selected from O and NR^3, where R^3 is selected from H and C_1–C_6 alkyl optionally substituted with one or more F;

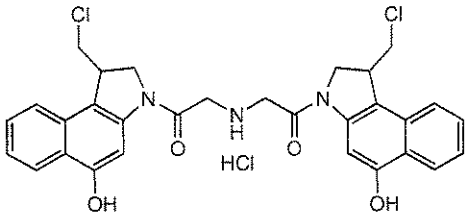
R^4 is H, CO_2R, where R is C_1–C_6 alkyl or benzyl; and

R^5 is H or C_1–C_6 alkyl, provided that when one of X^1-R^1 or X^2-R^1 is OH, T is C_3-C_{12} alkylene, and R^2 is H, then the other of X^1-R^1 or X^2-R^1 is not OH.

54. The CBI dimer drug moiety compound of claim 41 selected from:


;

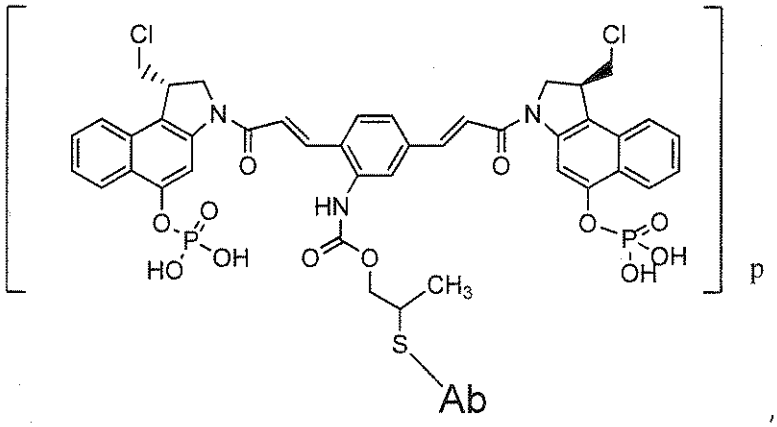

; and


.

55. A conjugate according to any one of claims 1 to 22, wherein p is 1, 2, 3 or 4.

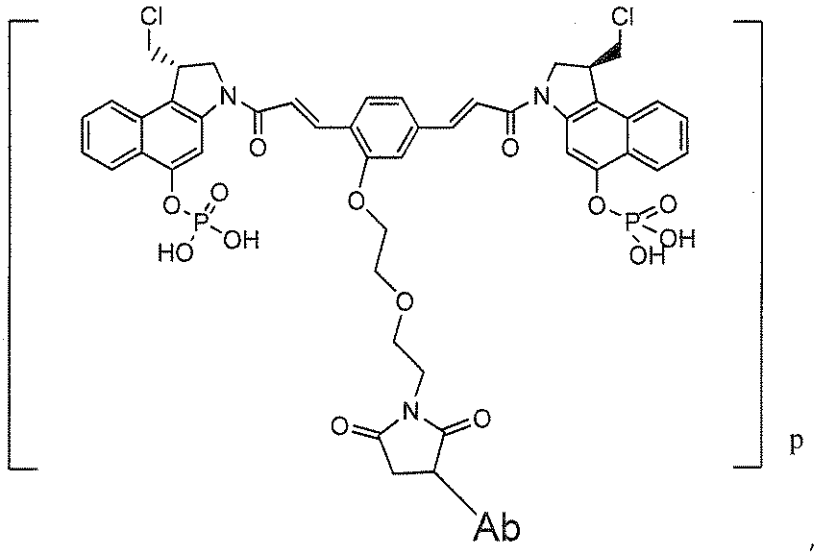
56. A conjugate according to any one of claims 1 to 22, wherein p is 1 or 2.

57. A conjugate of claim 1, which is



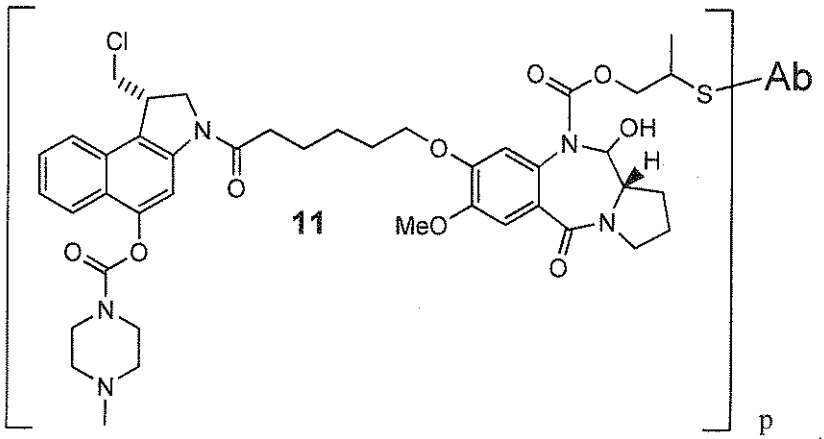
wherein p is 1-4, and Ab is an anti-HER2, anti-CD33, anti-CLL-1, anti-CD22, anti-CD79b or anti-NaPi3b antibody .

58. A conjugate of claim 1, which is



wherein p is 1-4, and Ab is an anti-HER2, anti-CD33, anti-CLL-1, anti-CD22, anti-CD79b or anti-NaPi3b antibody .

59. A conjugate of claim 1, which is



wherein p is 1-4, and Ab is an anti-HER2, anti-CD33, anti-CLL-1, anti-CD22, anti-CD79b or anti-NaPi3b antibody .

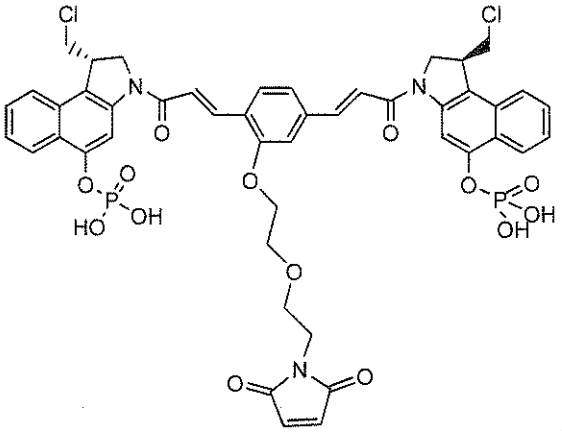
60. A conjugate of any one of claims 57-59, wherein Ab is an anti-HER2 antibody.

61. A conjugate of any one of claims 57-59, wherein Ab is an anti-CLL-1 antibody.

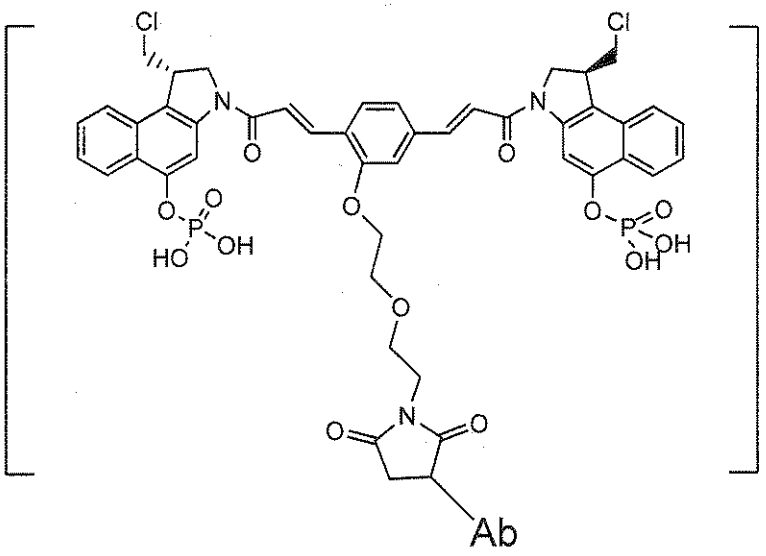
62. A conjugate of any one of claims 57-59, wherein Ab is an anti-CD22 antibody.

63. A conjugate of any one of claims 57-59, wherein Ab is an anti-CD79b antibody.

64. A linker-drug intermediate having the formula

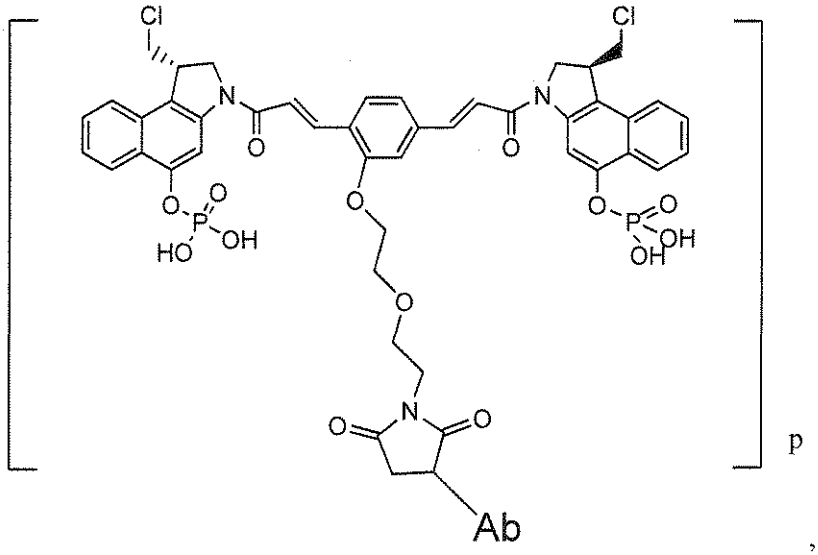


65. An antibody-drug conjugate having the formula



wherein p is 1-4 and Ab is an anti-CD22 antibody.

66. An antibody-drug conjugate having the formula



,

wherein p is 1-4, and Ab is an anti-CD22 antibody that comprises three light chain hypervariable regions (HVR-L1, HVR-L2 and HVR-L3) and three heavy chain hypervariable regions (HVR-H1, HVR-H2 and HVR-H3) wherein:

HVR-L1 comprises the amino acid sequence of SEQ ID NO:1;

HVR-L2 comprises the amino acid sequence of SEQ ID NO:2;

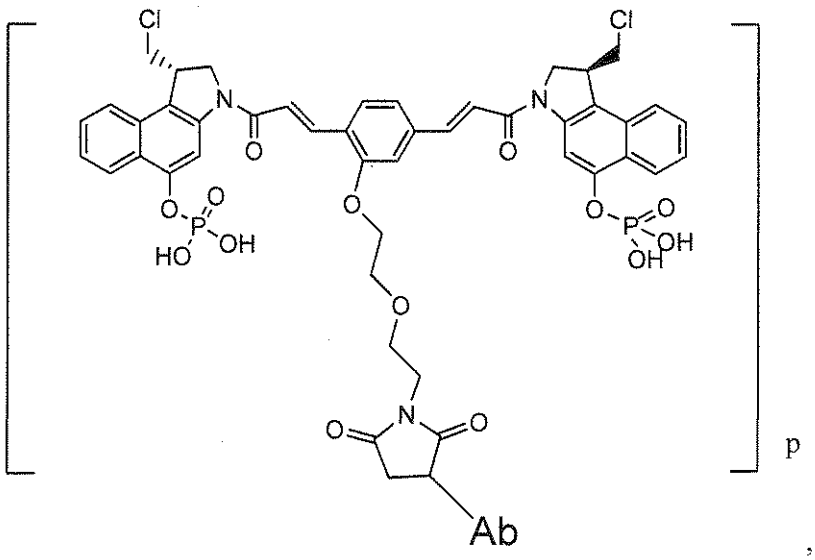
HVR-L3 comprises the amino acid sequence of SEQ ID NO:3;

HVR-H1 comprises the amino acid sequence of SEQ ID NO:4;

HVR-H2 comprises the amino acid sequence of SEQ ID NO:5; and

HVR-H3 comprises the amino acid sequence of SEQ ID NO:6.

67. An antibody-drug conjugate having the formula



,

wherein p is 1-4, and Ab is an anti-CD22 antibody that comprises three light chain hypervariable regions (HVR-L1, HVR-L2 and HVR-L3) and three heavy chain hypervariable regions (HVR-H1, HVR-H2 and HVR-H3) wherein:

HVR-L1 comprises the amino acid sequence of SEQ ID NO:1;

HVR-L2 comprises the amino acid sequence of SEQ ID NO:2;

HVR-L3 comprises the amino acid sequence of SEQ ID NO:3;

HVR-H1 comprises the amino acid sequence of SEQ ID NO:4;

HVR-H2 comprises the amino acid sequence of SEQ ID NO:5; and

HVR-H3 comprises the amino acid sequence of SEQ ID NO:6; and the anti-CD22 antibody is a cysteine engineered antibody.

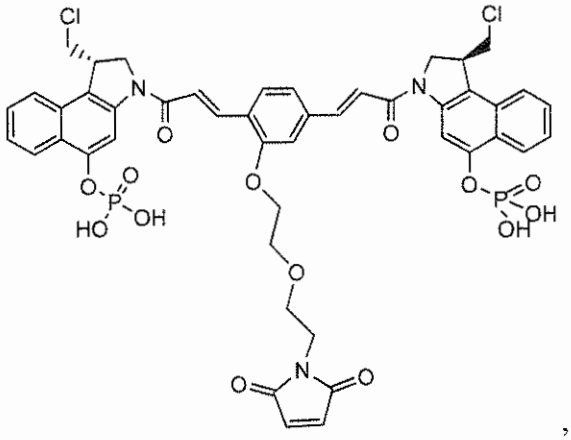
68. A pharmaceutical composition comprising an antibody drug conjugate of any one of claims 65 to 67 and a pharmaceutically acceptable excipient.

69. Use of an antibody drug conjugate of any one of claims 65 to 67 in the manufacture of a medicament for treating a hematological disorder in a patient.

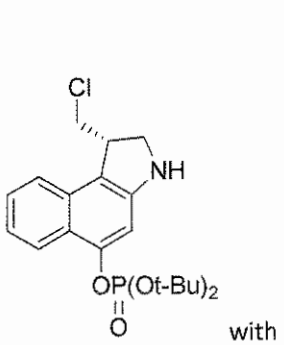
70. Use of an antibody drug conjugate of any one of claims 65 to 67 in the manufacture of a medicament for treating a leukemia in a patient.

71. Use of an antibody drug conjugate of any one of claims 65 to 67 in the manufacture of a medicament for treating a lymphoma in a patient.

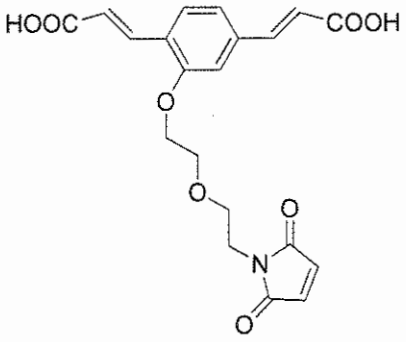
72. A method of making a linker-drug intermediate having the formula



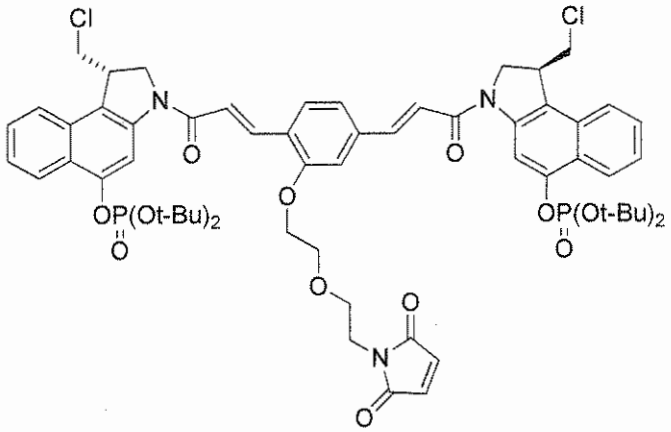
the method comprising reacting



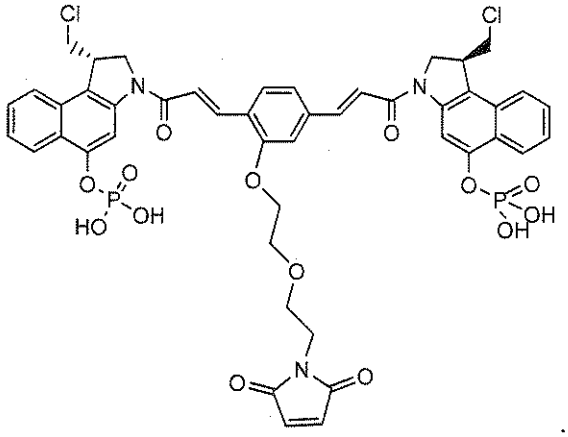
with



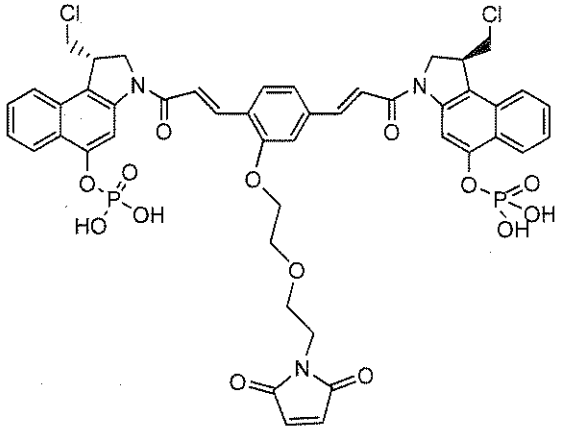
to form



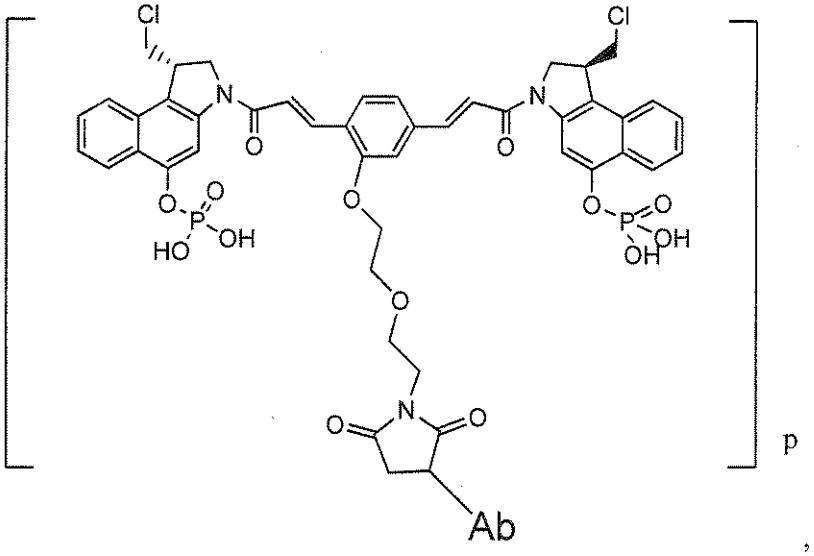
, which is then hydrolyzed to form



73. The method of claim 72 wherein the

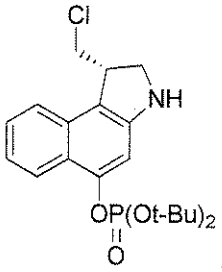


is further reacted with an anti CD22antibody, Ab, to form

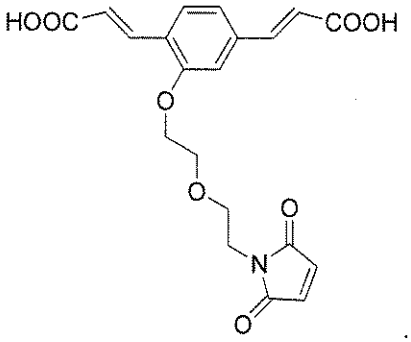


wherein p is 1-4.

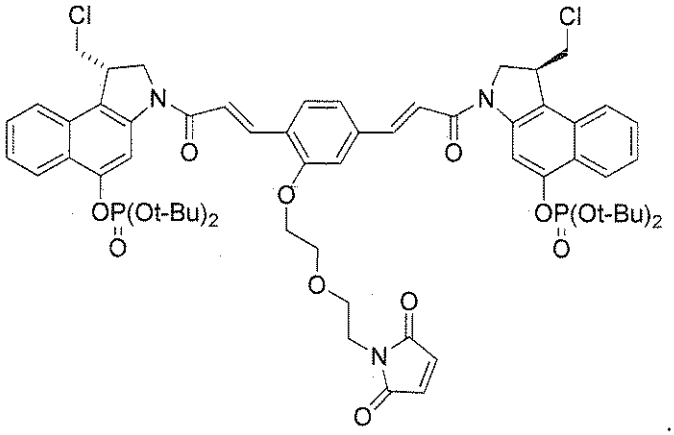
74. The compound having the formula



75. The compound having the formula



76. The compound having the formula



for cancer therapy. These have included antibody conjugates, (Jeffrey, et al. (2005) J. Med. Chem., 48:1344), prodrugs for gene therapy based on nitrobenzyl carbamates (Hay, et al (2003) J. Med. Chem. 46:2456) and the corresponding nitro-CBI derivatives as hypoxia-activated prodrugs (Tercel, et al (2011) Angew. Chem., Int. Ed., 50:2606-2609). The CBI and pyrrolo[2,1-c][1,4]benzodiazepine (PBD) pharmacophores have been linked together by an alkyl chain (Tercel et al (2003) J. Med. Chem 46:2132-2151). PBD dimers, where two pyrrolo[2,1-c][1,4]benzodiazepine units are tethered by an alkylene or alkylene-arylene chain are highly efficient interstrand crosslinking agents that react with guanine in the DNA minor groove (Rahman et al (2009) Jour. Amer. Chem. Soc. 131(38):13756-13766; Thurston et al (1994) Chem. Rev., 94:433-465; Bose et al (1992) J. Am. Chem. Soc. 114:4939-4941; Gregson et al (2004) Jour. Med. Chem. 47(5):1161-1174; US 7511032; US 7528126; US 7557099; US 7049311; US 7067511; US 7265105) and have activity against gram-positive bacteria (Doyle et al (2009) Jour. Antimicrob. Chemo. 65(5):949-959; Hadjivassileva et al (2005) Jour. Antimicrob. Chemo. 56(3):513-518), human B-cell chronic lymphocytic leukemia (CLL) cells (Pepper et al (2004) Cancer Res. 64(18):6750-6755), and solid tumors (Hochhauser et al (2009) Clin. Cancer Res. 15(6):2140-2147; Alley et al (2004) 64(18):6700-6706; Hartley et al (2004) Cancer Res. 64(18):6693-6699). Dimeric forms of PBD have been linked to antibodies to form ADC (US 2009/304710; US 2010/047257; US 2009/036431; WO 2011/130598, WO 2011/130616; US 2013/0028919).

SUMMARY

The ~~invention~~ dislosure includes 1-(chloromethyl)-2,3-dihydro-1H-benzo[e]indole (CBI) dimer drug moieties covalently attached by a linker to form antibody-drug conjugate (ADC) compounds with therapeutic or diagnostic applications.

In one~~An~~ aspect, there is provided ~~of the invention is~~ an antibody-drug conjugate compound having the formula:

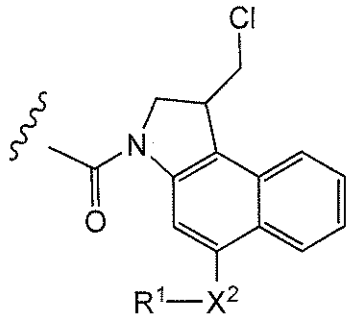
$$Ab–(L–D)_p$$

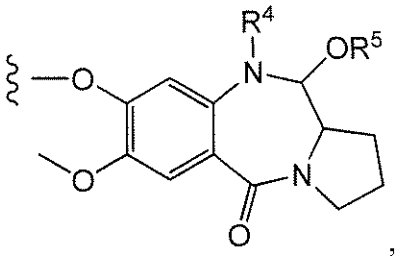
wherein:

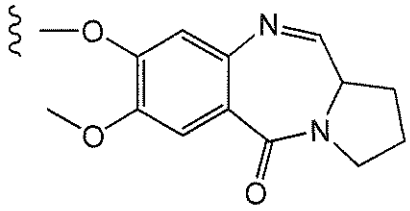
Ab is an antibody;

L is a linker having the formula:

$$–Str–(Pep)_m–(Sp)_n–$$



,


, and


where the wavy line indicates the site of attachment to T;

X^1 and X^2 are independently selected from O and NR^3, where R^3 is selected from H and C_1–C_6 alkyl optionally substituted with one or more F;

R^4 is H, CO_2R, or a bond to L, where R is C_1–C_6 alkyl or benzyl; and

R^5 is H or C_1–C_6 alkyl.

In another aspect, there is provided a pharmaceutical composition comprising any one of the antibody-drug conjugate compounds described herein, and a pharmaceutically acceptable excipient.

~~An aspect of the invention~~Disclosed is a pharmaceutical composition of the antibody-drug conjugate compound, and a pharmaceutically acceptable carrier, glidant, diluent, or excipient.

In another aspect, there is provided use of the pharmaceutical composition described herein in the manufacture of a medicament for treating cancer in a patient.

~~An aspect of the invention~~Disclosed is a method of treating cancer comprising administering to a patient a therapeutically-effective amount of the antibody-drug conjugate compound.

In another~~An~~ aspect, there is provided ~~of the invention is~~ a kit for treating cancer, comprising:

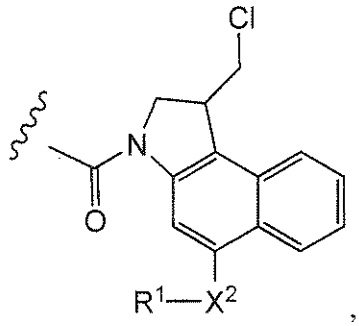
a) the pharmaceutical composition described herein; and

b) instructions for use.

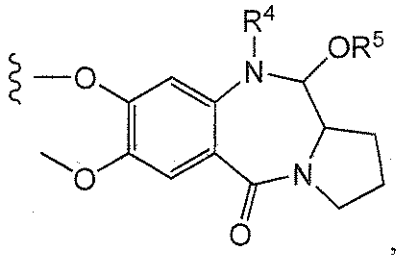
In another~~An~~ aspect, there is provided ~~of the invention is~~ a linker-drug intermediate selected from:

X–L–D

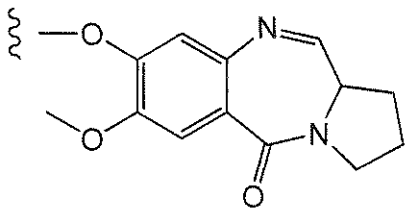
wherein:



,



, and



where the wavy line indicates the site of attachment to T;

X^1 and X^2 are independently selected from O and NR^3, where R^3 is selected from H and C_1–C_6 alkyl optionally substituted with one or more F;

R^4 is H, CO_2R, or a bond to L, where R is C_1–C_6 alkyl or benzyl; and

R^5 is H or C_1–C_6 alkyl.

~~An aspect of the invention~~Disclosed is a process for making an antibody-drug conjugate by conjugating the antibody to a linker-drug intermediate.

In another aspect, there is provided a process for making the antibody-drug conjugate compound described herein comprising conjugating the antibody to a linker-drug intermediate having the formula:

X–L–D

wherein:

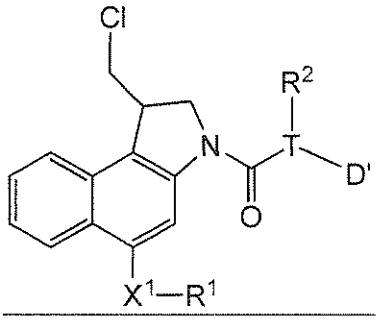
X is a reactive functional group selected from maleimide, thiol, amino, bromide, bromoacetamido, iodoacetamido, p-toluenesulfonate, iodide, hydroxyl, carboxyl, pyridyl disulfide, and N-hydroxysuccinimide;

L is a linker having the formula:

$$-Str-(Pep)_m-(Sp)_n-$$

where Str is a stretcher unit covalently attached to X; Pep is an optional peptide unit of two to twelve amino acid residues, Sp is an optional spacer unit covalently attached to a dimer drug moiety, and m and n are independently selected from 0 and 1;

D is the dimer drug moiety having the formula:



where

R^1 is selected from H, $P(O)_3H_2$, $C(O)NR^aR^b$, or a bond to L;

R^2 is selected from H, $P(O)_3H_2$, $C(O)NR^aR^b$, or a bond to L;

R^a and R^b are independently selected from H and C_1–C_6 alkyl optionally substituted with one or more F, or R^a and R^b form a five or six membered heterocyclyl group;

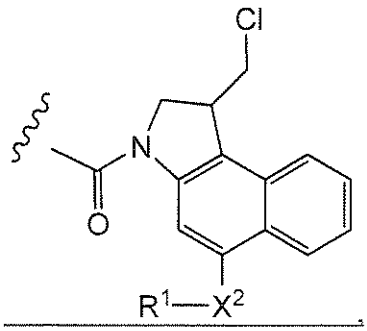
T is a tether group selected from C_3–C_{12} alkylene, Y, (C_1–C_6 alkylene)–Y–(C_1–C_6 alkylene), (C_1–C_6 alkylene)–Y–(C_1–C_6 alkylene)–Y–(C_1–C_6 alkylene), (C_2–C_6 alkenylene)–Y–(C_2–C_6 alkenylene), and (C_2–C_6 alkynylene)–Y–(C_2–C_6 alkynylene);

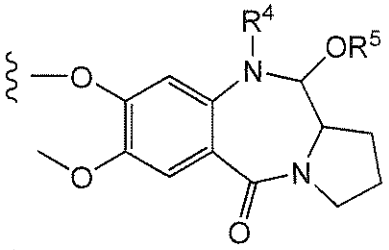
where Y is independently selected from O, S, NR^1, aryl, and heteroaryl;

where alkylene, alkenylene, aryl, and heteroaryl are independently and optionally substituted with F, OH, O(C_1–C_6 alkyl), NH_2, $NHCH_3$, $N(CH_3)_2$, $OP(O)_3H_2$, and C_1–C_6 alkyl, where alkyl is optionally substituted with one or more F;

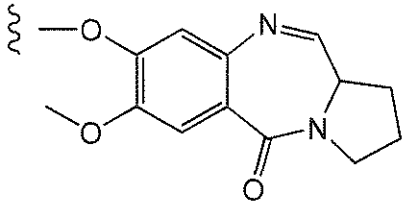
or alkylene, alkenylene, aryl, and heteroaryl are independently and optionally substituted with a bond to L;

D' is a drug moiety selected from:





, and



where the wavy line indicates the site of attachment to T;

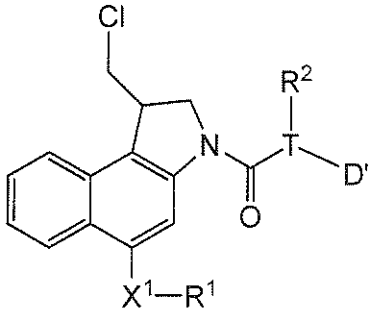
X^1 and X^2 are independently selected from O and NR^3, where R^3 is selected from H and C_1–C_6 alkyl optionally substituted with one or more F;

R^4 is H, CO_2R, or a bond to L, where R is C_1–C_6 alkyl or benzyl; and

R^5 is H or C_1–C_6 alkyl,

whereby the antibody-drug conjugate compound is formed.

~~An aspect of the invention~~Disclosed is a CBI dimer drug moiety compound having the formula:



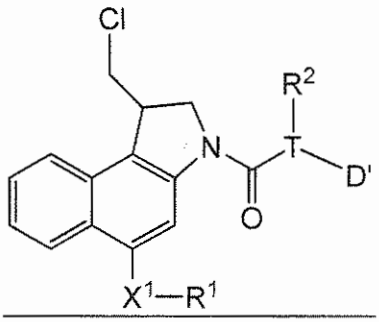
where

R^1 is selected from H, $P(O)_3H_2$, $C(O)NR^aR^b$;

R^2 is selected from H, $P(O)_3H_2$, $C(O)NR^aR^b$;

R^5 is H or C_1–C_6 alkyl.

In another aspect, there is provided a CBI dimer drug moiety compound having the formula:



where

R^1 is selected from H, $P(O)_3H_2$, $C(O)NR^aR^b$;

R^2 is selected from H, $P(O)_3H_2$, $C(O)NR^aR^b$;

R^a and R^b are independently selected from H and C_1–C_6 alkyl optionally substituted with one or more F,

or R^a and R^b form a five or six membered heterocyclyl group;

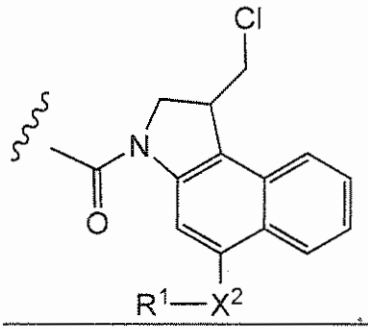
T is a tether group selected from C_3–C_{12} alkylene, Y, (C_1–C_6 alkylene)–Y–(C_1–C_6 alkylene), (C_1–C_6 alkylene)–Y–(C_1–C_6 alkylene)–Y–(C_1–C_6 alkylene), (C_2–C_6 alkenylene)–Y–(C_2–C_6 alkenylene), and (C_2–C_6 alkynylene)–Y–(C_2–C_6 alkynylene);

where Y is independently selected from O, S, NR^1, aryl, and heteroaryl;

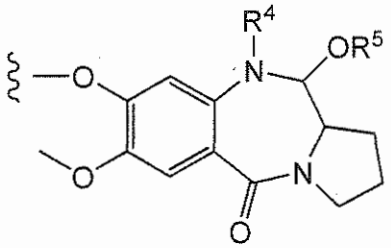
where alkylene, alkenylene, aryl, and heteroaryl are independently and optionally substituted with F, OH, O(C_1–C_6 alkyl), NH_2, $NHCH_3$, $N(CH_3)_2$, $OP(O)_3H_2$, and C_1–C_6 alkyl, where alkyl is optionally substituted with one or more F;

or alkylene, alkenylene, aryl, and heteroaryl are independently and optionally substituted with a bond to L;

D' is a drug moiety selected from:



, and



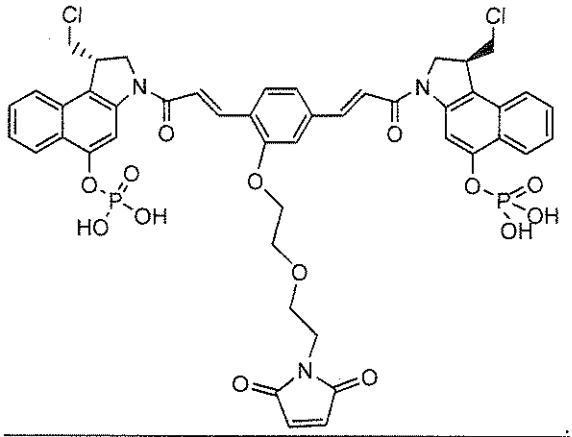
where the wavy line indicates the site of attachment to T;

X^1 and X^2 are independently selected from O and NR^3, where R^3 is selected from H and C_1–C_6 alkyl optionally substituted with one or more F;

R^4 is H, CO_2R, where R is C_1–C_6 alkyl or benzyl; and

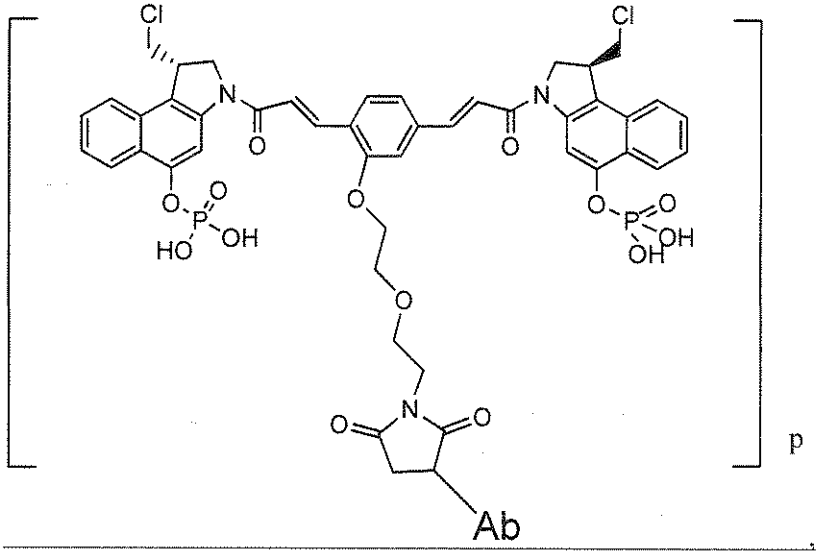
R^5 is H or C_1–C_6 alkyl, provided that when one of X^1-R^1 or X^2-R^1 is OH, T is C_3-C_{12} alkylene, and R^2 is H, then the other of X^1-R^1 or X^2-R^1 is not OH.

In another aspect, there is provided a linker-drug intermediate having the formula



.

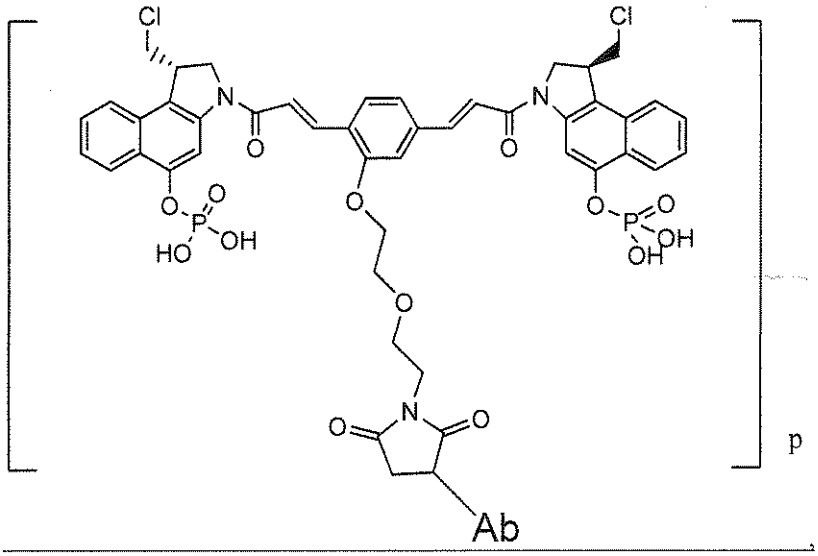
In another aspect, there is provided an antibody-drug conjugate having the formula



,

wherein p is 1-4 and Ab is an anti-CD22 antibody.

In another aspect,t here is provided an antibody-drug conjugate having the formula



wherein p is 1-4, and Ab is an anti-CD22 antibody that comprises three light chain hypervariable regions (HVR-L1, HVR-L2 and HVR-L3) and three heavy chain hypervariable regions (HVR-H1, HVR-H2 and HVR-H3) wherein:

HVR-L1 comprises the amino acid sequence of SEQ ID NO:1;

HVR-L2 comprises the amino acid sequence of SEQ ID NO:2;

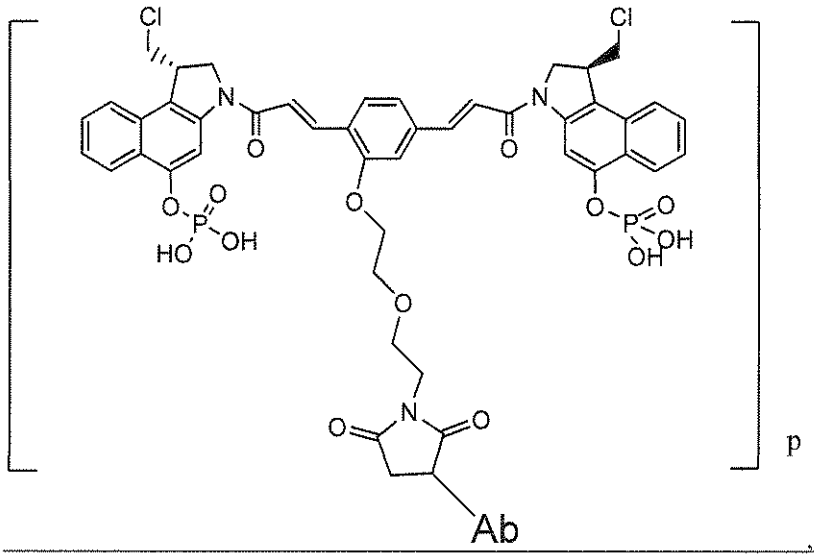
HVR-L3 comprises the amino acid sequence of SEQ ID NO:3;

HVR-H1 comprises the amino acid sequence of SEQ ID NO:4;

HVR-H2 comprises the amino acid sequence of SEQ ID NO:5; and

HVR-H3 comprises the amino acid sequence of SEQ ID NO:6.

In another aspect, there is provided an antibody-drug conjugate having the formula



wherein p is 1-4, and Ab is an anti-CD22 antibody that comprises three light chain hypervariable regions (HVR-L1, HVR-L2 and HVR-L3) and three heavy chain hypervariable regions (HVR-H1, HVR-H2 and HVR-H3) wherein:

HVR-L1 comprises the amino acid sequence of SEQ ID NO:1;

HVR-L2 comprises the amino acid sequence of SEQ ID NO:2;

HVR-L3 comprises the amino acid sequence of SEQ ID NO:3;

HVR-H1 comprises the amino acid sequence of SEQ ID NO:4;

HVR-H2 comprises the amino acid sequence of SEQ ID NO:5; and

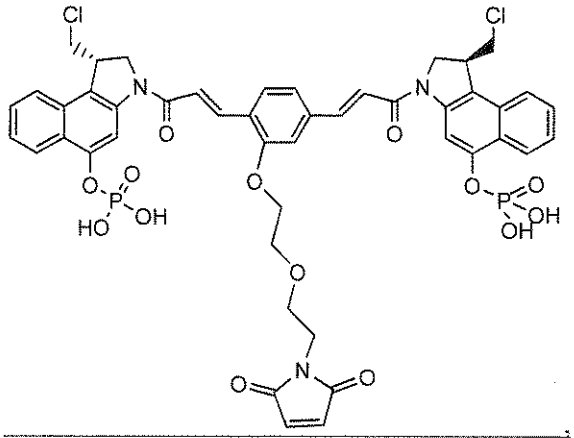
HVR-H3 comprises the amino acid sequence of SEQ ID NO:6; and the anti-CD22 antibody is a cysteine engineered antibody.

In another aspect, there is provided use of any one of the antibody drug conjugates described herein in the manufacture of a medicament for treating a hematological disorder in a patient.

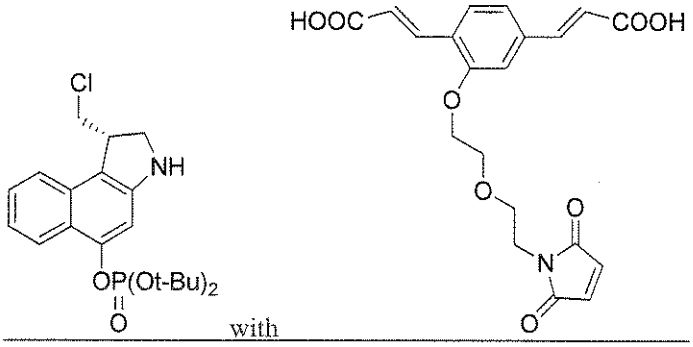
In another aspect, there is provided use of any one of the antibody drug conjugates described herein in the manufacture of a medicament for treating a leukemia in a patient.

In another aspect, there is provided use of any one of the antibody drug conjugates described in the manufacture of a medicament for treating a lymphoma in a patient.

In another aspect, there is provided a method of making a linker-drug intermediate having the formula

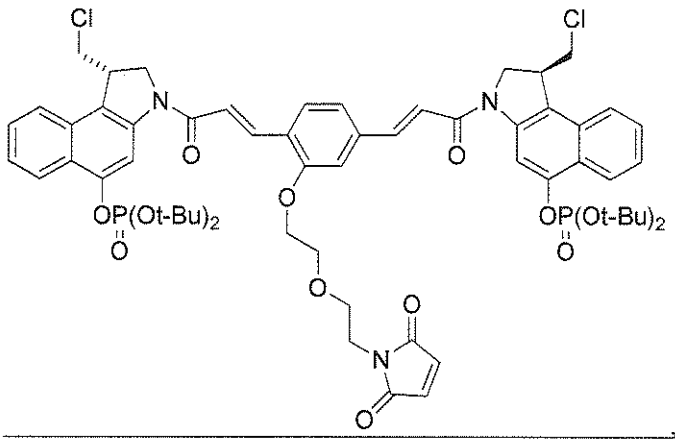


the method comprising reacting

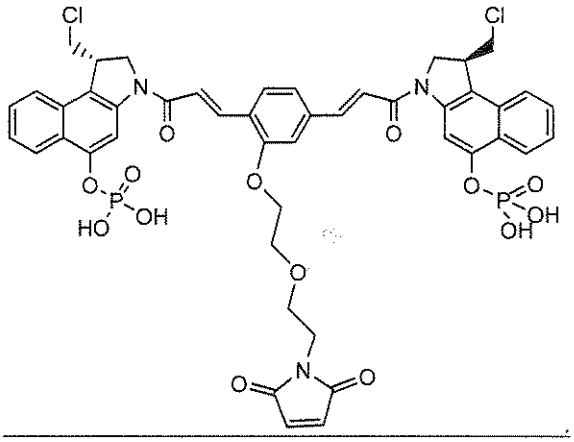


with

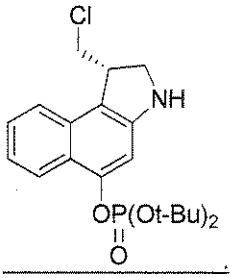
to form



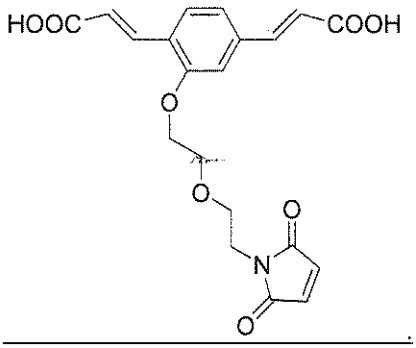
, which is then hydrolyzed to form



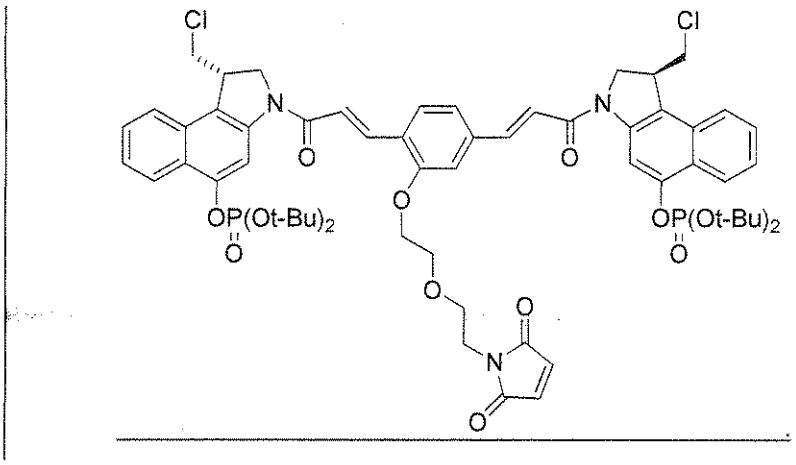
In another aspect, there is provided the compound having the formula



In another aspect, there is provided compound having the formula



In another aspect, there is provided the compound having the formula



BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the synthesis of (S)-1-(chloromethyl)-3-(5-((S)-1-(chloromethyl)-5-hydroxy-1H-benzo[e]indol-3(2H)-yl)-5-oxopentanoyl)-2,3-dihydro-1H-benzo[e]indol-5-yl 2-(2-bromo-N-methylacetamido)ethyl(methyl)carbamate **51** from (S)-tert-Butyl 1-(chloromethyl)-5-hydroxy-1H-benzo[e]indole-3(2H)-carboxylate **51a**.

Figure 2 shows the synthesis of N-((R)-1-(chloromethyl)-3-(5-((R)-1-(chloromethyl)-5-hydroxy-1H-benzo[e]indol-3(2H)-yl)-5-oxopentanoyl)-2,3-dihydro-1H-benzo[e]indol-5-yl)-6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamide **53** from (R)-tert-butyl 1-(chloromethyl)-5-(diphenylmethyleneamino)-1H-benzo[e]indole-3(2H)-carboxylate **53a**.

Figure 3 shows the synthesis of 1-((S)-5-amino-1-(chloromethyl)-1H-benzo[e]indol-3(2H)-yl)-5-((S)-1-(chloromethyl)-5-hydroxy-1H-benzo[e]indol-3(2H)-yl)pentane-1,5-dione **53j** from (S)-5-(5-(benzyloxy)-1-(chloromethyl)-1H-benzo[e]indol-3(2H)-yl)-5-oxopentanoic acid **57c**.

Figure 4 shows the synthesis of unnatural enantiomer, 1-((R)-5-amino-1-(chloromethyl)-1H-benzo[e]indol-3(2H)-yl)-5-((R)-1-(chloromethyl)-5-hydroxy-1H-benzo[e]indol-3(2H)-yl)pentane-1,5-dione **53p** from (R)-tert-butyl 1-(chloromethyl)-5-hydroxy-1H-benzo[e]indole-3(2H)-carboxylate **53k**.

Figure 5 shows the synthesis of N-(4-(((S)-1-(chloromethyl)-3-(6-((S)-7-methoxy-5-oxo-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yloxy)hexanoyl)-2,3-dihydro-1H-benzo[e]indol-5-yloxy)methyl)phenyl)-6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamide **54** from (S)-(2-amino-4-hydroxy-5-methoxyphenyl)(2-(hydroxymethyl)pyrrolidin-1-yl)methanone **54a**.

Figure 6 shows the synthesis of N-((S)-1-((S)-1-(4-(((S)-1-(chloromethyl)-3-(5-((S)-1-(chloromethyl)-5-hydroxy-1H-benzo[e]indol-3(2H)-yl)-5-oxopentanoyl)-2,3-dihydro-1H-benzo[e]indol-5-yloxy)methyl)phenylamino)-1-oxo-5-ureidopentan-2-ylamino)-3-methyl-1-oxobutan-2-yl)-6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamide **55** from (S)-tert-butyl 1-(chloromethyl)-5-hydroxy-1H-benzo[e]indole-3(2H)-carboxylate **51a**.

Figure 7 shows the synthesis of (S)-tert-butyl 8-(6-((S)-5-(4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)benzyloxy)-1-(chloromethyl)-1H-benzo[e]indol-3(2H)-yl)-6-oxohexyloxy)-11-hydroxy-7-methoxy-5-oxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate **56d** from (S)-tert-Butyl 8-(6-((S)-1-(chloromethyl)-5-(4-nitrobenzyloxy)-1H-benzo[e]indol-3(2H)-yl)-6-oxohexyloxy)-11-