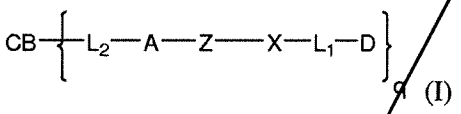
What is claimed is:

1. A cell-binding agent-cytotoxic agent conjugate represented by the following structural formula:



(I)

or a pharmaceutically acceptable salt thereof, wherein:

CB is a cell-binding agent;

L_2 is absent or a spacer;

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

Z is -NH- or -C(=O)-;

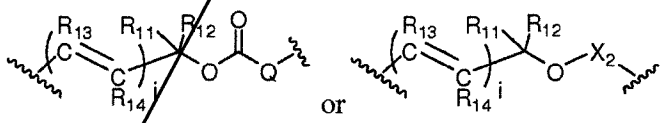
D is a cytotoxic agent;

L_1 is absent or a spacer;

X is an aryl or a heteroaryl, each of which is optionally substituted; and

q is an integer from 1 to 20;

provided that when Z is -NH-, L_1 does not comprise one of the following moieties directly connected to X:



wherein i is 0 or an integer from 1 to 5; Q is -O-, $-NR_{15}$, S or $-CR_{11}R_{12}$-; X_2 is an aryl or a heteroaryl; and R_{11}, R_{12}, R_{13}, R_{14} and R_{15} are each independently H or an alkyl group.

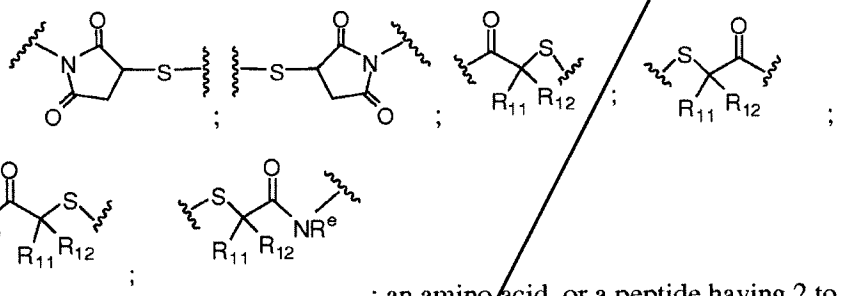
2. The conjugate of claim 1, wherein L_1 is represented by:

$$-J_X-R^{A1}-V_1-W_1-R^{B1}-V_1'-R^{C1}-J_D-,$$

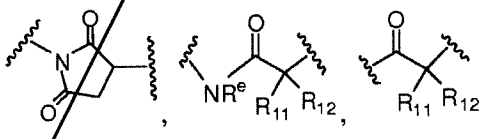
wherein:

R^{A1}, R^{B1} and R^{C1} are each absent, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, an aryl, a heteroaryl or a heterocyclyl, wherein the alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, and heterocyclyl are optionally substituted;

J_X and W_1 are each independently absent, -O-, -O-C(=O)-, -C(=O)-O-, -S-, -SO-, $-SO_2$-, $-CR_{11}R_{12}$-S-, $-CR_{11}R_{12}$-O-, $-CR_{11}R_{12}NR^e$-, -O-(C=O)O-, -O-(C=O)N(R^e)-, -N(R^e)-, -N(R^e)-C(=O)-, -C(=O)-N(R^e)-, -N(R^e)-C(=O)O-, -N(C(=O)R^e)C(=O)- , -N(C(=O)R^e)-, -SS-, -C(=O)-,



; an amino acid, or a peptide having 2 to 8 amino acids, provided that when Z is -NH-, J_x and V_1 are both absent, and R^{A1} is $-CR_{11}R_{12}-$, then W_1 is not -O-C(=O)-;

J_D is absent, -C(=O)-, -S-, , -O-, -O-C(=O)-, -C(=O)-O-, -SO-, $-SO_2-$, -O-(C=O)-, $-N(R^e)-$, $-N(R^e)-C(=O)-$, or $-N(C(=O)R^e)C(=O)-$, or $-N(C(=O)R^e)-$;

V_1 and V_1' are each independently $-(O-CH_2-CH_2)_{p1}-$, $-(CH_2-CH_2-O)_{p1}-$, $-(NR^m-CH_2-CH_2)_{p1}-$ or $-(CH_2-CH_2-NR^m)_{p1}-$;

R^e is H, an alkyl, an alkenyl, an alkynyl, or $-(CH_2-CH_2-O)_{n'}-R^k$;

R^k and R^m are each independently H or an alkyl;

R_{11} and R_{12} are each independently H or an alkyl;

n' is an integer from 1 to 24;

p1 is 0 or an integer from 1 to 1000; and

p1' is 0 or an integer from 1 to 10.

3. The conjugate of claim 2, wherein L_2 is represented by the following structural formula:

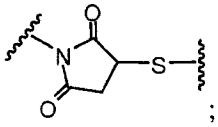
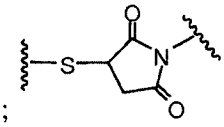
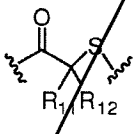
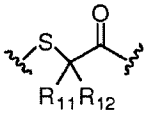
$$—J_{CB}—R^{A2}—V_2—W_2—R^{B2}—V_2'—R^{C2}—J_A—;$$

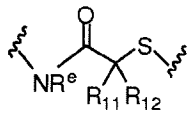
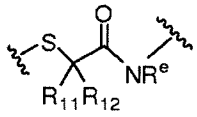
wherein:

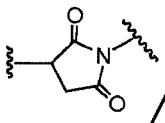
R^{A2}, R^{B2} and R^{C2} are each independently absent, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, an aryl, a heteroaryl, a heterocyclyl, a cycloalkylalkyl, an arylalkyl, a heteroarylalkyl, or a heterocyclylalkyl;

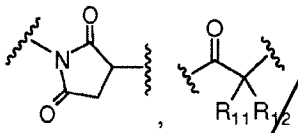
V_2 and V_2' are each independently $-(O-CH_2-CH_2)_{p2}-$, $-(CH_2-CH_2-O)_{p2}-$, $-(NR^m-CH_2-CH_2)_{p2'}-$ or $-(CH_2-CH_2-NR^m)_{p2'}-$;

W_2 is absent, -O-, -O-C(=O)-, -C(=O)-O-, -S-, -SO-, $-SO_2-$, $-CR_{11}R_{12}-S-$, $-CR_{11}R_{12}-O-$, $-CR_{11}R_{12}-NR^e-$, -O-(C=O)O-, $-O-(C=O)N(R^e)-$, $-N(R^e)-$, $-N(R^e)-$

C(=O)-, -C(=O)-N(R^e)-, -N(R^e)-C(=O)O-, -N(C(=O)R^e)C(=O)- , -N(C(=O)R^e)-, -$(O\text{-}CH_2\text{-}CH_2)_{n'}$-, -SS-, -C(=O)-, an amino acid, a peptide having 2 to 8 amino acids,  ;  ;  ;  ;

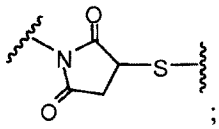
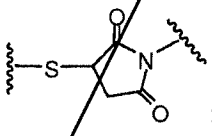
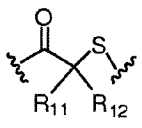
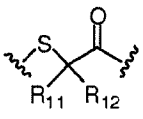
 ;  ;

J_{CB} is -C(=O)-, -C(=O)-NR^e-, -C(=O)-O- -C(=NH)-, -C(=NH)-NR^e-, -S-, -NR^e-, -NH-NR^e-,  , -C(=NR^e)-, =NNR^e-, -CH_2-C(=O)-, or -CH_2-C(=O)-NR^e-;

J_A is -NR^e-, -C(=O)-, =N-, -NR^e-C(=O)-, -O-C(=O)- , -SO_2-, -S-,  ;

p2 is 0 or an integer from 1 to 1000; and

p2' is 0 or an integer from 1 to 10.

4. The conjugate of claim 3, wherein W_2 is absent,  ;  ;  ;  ; -S-S-, -NH-C(=O)- or -C(=O)-NH-.

5. The conjugate of claim 4, wherein p2 and p2' are both 0.

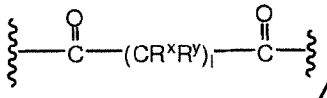
6. The conjugate of any one of claims 3-5, wherein R^{A2} is an alkyl.

7. The conjugate of any one of claims 3-6, wherein one of R^{B2} and R^{C2} is absent, and the other is an alkyl.

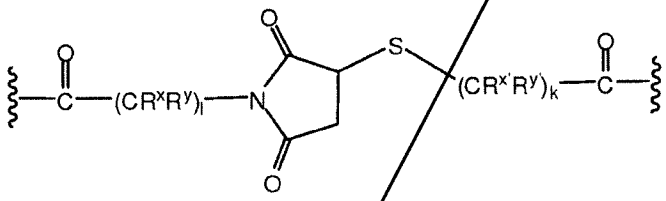
8. The conjugate of any one of claims 3-5, wherein R^{B2} and R^{C2} are both absent.

9. The conjugate of any one of claims 3-7, wherein CB comprises a -NH- group that covalently linked to J_{CB}.

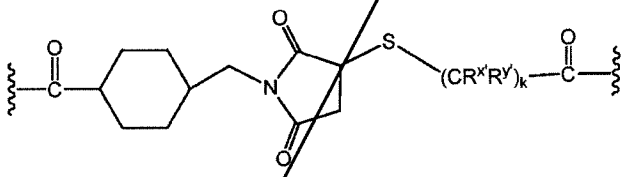
10. The conjugate of claim 9, wherein L_2 is represented by the following structural formulas:



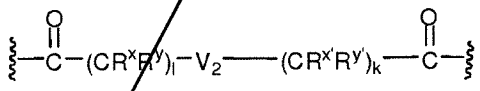
(L2a);



(L2b);



(L2b'); or



(L2c);

wherein:

R^x, R^y, $R^{x'}$ and $R^{y'}$, for each occurrence, are independently H, -OH, halogen, -O-(C_{1-4} alkyl), $-SO_3^-$, $-NR_{40}R_{41}R_{42}^+$, or a C_{1-4} alkyl optionally substituted with -OH, halogen, SO_3^- or $NR_{40}R_{41}R_{42}^+$, wherein R_{40}, R_{41} and R_{42} are each independently H or a C_{1-4} alkyl;

V_2 is $-(CH_2-CH_2-O)_{p2}-$ or $-(O-CH_2-CH_2)_{p2}-$;

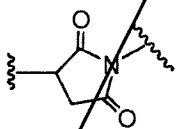
l and k are each independently 0 or an integer from 1 to 10; and

p2 is an integer from 1 to 24.

11. The conjugate of claim 10, wherein R^x, R^y, $R^{x'}$ and $R^{y'}$ are all H.

12. The conjugate of claim 11, wherein p2 is an integer from 2 to 24; l and k are each independently an integer from 1 to 6.

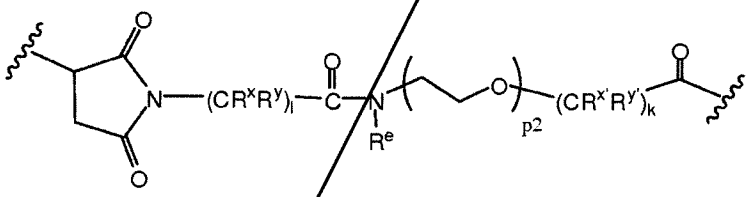
13. The conjugate of any one of claims 3-7, wherein CB comprises a -S- group that covalently linked with J_{CB}.

14. The conjugate of claim 13, wherein J_{CB} is  , $-CH_2-C(=O)-$ or $-CH_2-C(=O)-NH-$.

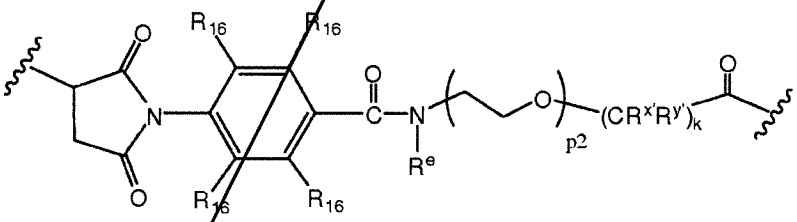
15. The conjugate of claim 14, wherein V_2 and V_2' are both $-(O-CH_2-CH_2)_{p2}-$ or $-(CH_2-CH_2-O)_{p2}-$.

16. The conjugate of claim 15, wherein p2 for one of V_2 and V_2' is 0 and the other is an integer from 2 to 24.

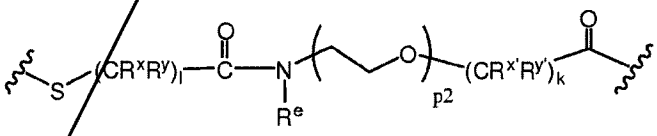
17. The conjugate of claim 13, wherein L_2 is represented by:



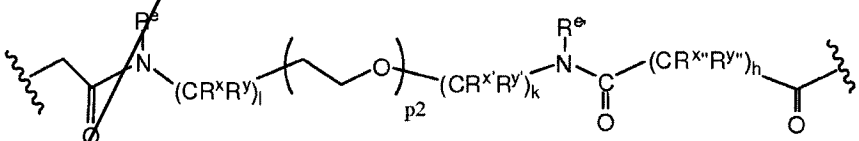
(L2d);



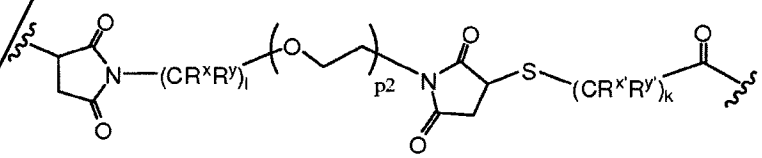
(L2e);



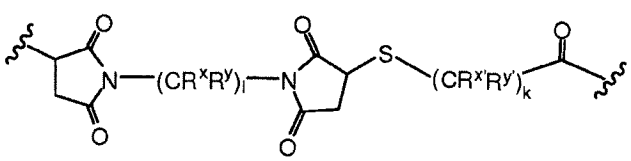
(L2f);



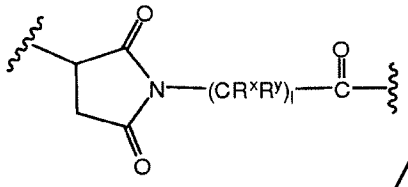
(L2g);



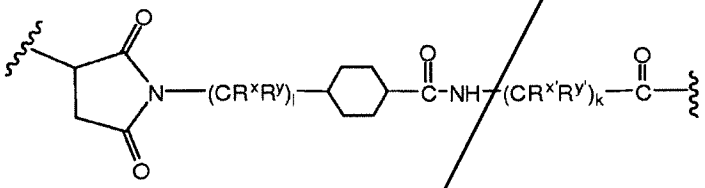
(L2h);



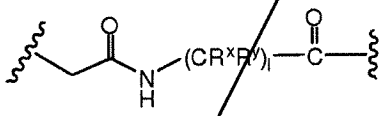
(L2i);



(L2j);



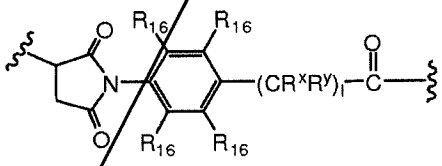
(L2k);



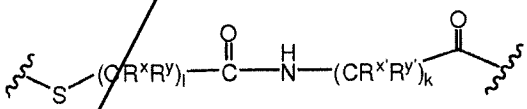
(L2l);



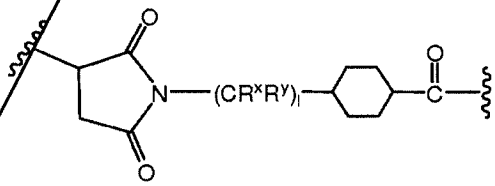
(L2m);



(L2n);



(L2o); or



(L2p);

wherein:

R^x, R^y, $R^{x'}$, $R^{y'}$, $R^{x''}$ and $R^{y''}$, for each occurrence, are each independently H, C_{1-4} alkyl, -OH, halogen or -O-(C_{1-4} alkyl);

R_{16}, for each occurrence, is independently H, an alkyl, halogen, -OH, -O-alkyl, $-NO_2$, or -CN; and

l, k and h are each independently an integer from 1 to 10.

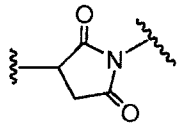
18. The conjugate of claim 17, wherein R_{16} is H.

19. The conjugate of claim 17 or 18, wherein R^x, R^y, $R^{x'}$, $R^{y'}$, $R^{x''}$ and $R^{y''}$ are all H.

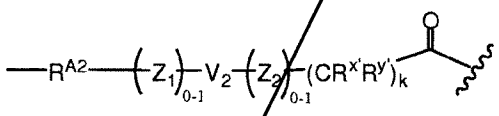
20. The conjugate of claim 19, wherein p2 or each occurrence is independently an integer from 2 to 24.

21. The conjugate of claim 20, wherein l, k and h are each independently an integer from 1 to 6.

22. The conjugate of claim 3, wherein:

J_{CB} is , -C(=O)-, -S-, $-CH_2-C(=O)-$, or $-CH_2-C(=O)-N(R^e)-$;

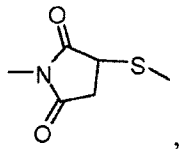
$-R^{A2}-V_2-W_2-R^{B2}-V_{2'}-R^{C2}-J_A-$ is:

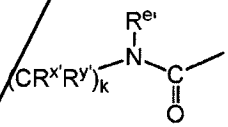
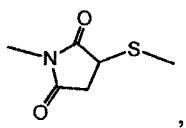
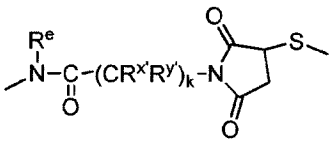
; wherein

R^{A2} is $-(CR^xR^y)_l-$ or $-Cy-(CR^xR^y)_l-$;

Cy is a cycloalkyl (*e.g.*, cyclohexyl);

V_2 is $-(CH_2-CH_2-O)_{p2}-$ or $-(O-CH_2-CH_2)_{p2}-$;

Z_1 is $-C(=O)-N(R^e)-$, $-N(R^e)-C(=O)-$, or , provided that Z_1-V_2 does not contain an N-O bond;

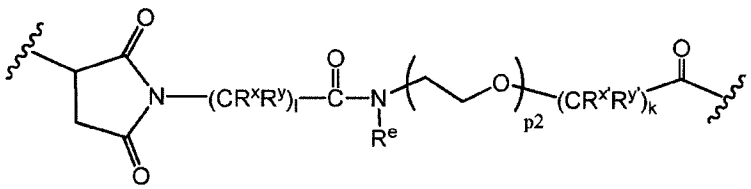
Z_2 is , , or , provided that V_2-Z_2 does not contain an O-N bond;

l is an integer from 0 to 10;

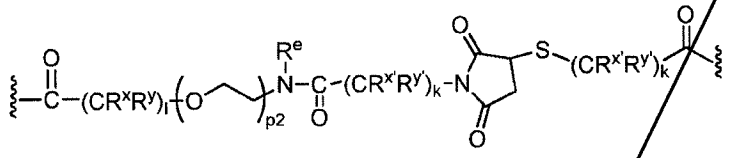
p2 is an integer from 0 to 200; and,

Z is -NH-.

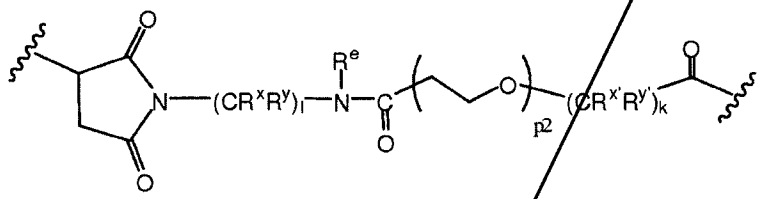
23. The conjugate of claim 22, wherein L_2 is represented by:



(L2d);



(L2r); or



(L2s);

p2 is an integer from 0 to 200.

24. The conjugate of claim 22 or 23, wherein A is Val-Cit or Gly-Gly-Gly.

25. The conjugate of any one of claims 22-24, wherein L_1 is $-CH_2-S-R^{B1}-C(=O)-$, wherein R^{B1} is a C_{1-10} alkyl.

26. The conjugate of claim 25, wherein R^{B1} is $-CH_2-CH_2-$.

27. The conjugate of any one of claims 22-26, wherein X is phenyl.

28. The conjugate of any one of claims 1-27, wherein A is a peptide cleavable by a protease.

29. The conjugate of claim 28, wherein A is a peptide cleavable by a protease expressed in tumor tissue.

30. The conjugate of claim 28, wherein A is a peptide cleavable by a lysosomal protease.

31. The conjugate of any one of claims 1-27, wherein A is a peptide having an amino acid that is covalent linked with -Z-X-L_1-D selected from the group consisting of Ala, Arg, Asn, Asp, Cit, Cys, selino-Cys, Gln, Glu, Gly, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr and Val, each independently as L or D isomers.

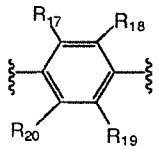
32. The conjugate of any one of claims 1-27, wherein A is selected from the group consisting of Val-Cit, Val-Lys, Phe-Lys, Lys-Lys, Ala-Lys, Phe-Cit, Leu-Cit, Ile-Cit, Trp, Cit, Phe-Ala, Phe-N^9-tosyl-Arg, Phe-N^9-nitro-Arg, Phe-Phe-Lys, D-Phe-Phe-Lys, Gly-Phe-Lys, Leu-Ala-Leu, Ile-Ala-Leu, Val-Ala-Val, Ala-Leu-Ala-Leu (SEQ ID NO: 1), β-Ala-Leu-Ala-Leu (SEQ ID NO: 2) and Gly-Phe-Leu-Gly (SEQ ID NO: 3), Val-Arg, Arg-Val, Arg-Arg, Val-D-Cit, Val-D-Lys,

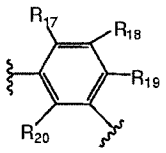
Val-D-Arg, D-Val-Cit, D-Val-Lys, D-Val-Arg, D-Val-D-Cit, D-Val-D-Lys, D-Val-D-Arg, D-Arg-D-Arg, Ala-Ala, Ala-D-Ala, D-Ala-Ala, and D-Ala-D-Ala.

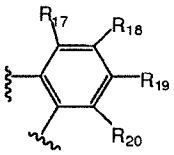
33. The conjugate of claim 32, wherein A is Val-Cit, Val-Lys, Val-D-Cit or Val-D-Lys.

34. The conjugate of any one of claims 1-27, wherein A is Cys or a peptide comprising a Cys that is covalently linked to L_2, wherein the Cys is linked to L_2 through the -SH group of Cys side chain.

35. The conjugate of any one of claims 1-34, wherein X is an optionally substituted phenyl.

36. The conjugate of claim 35, wherein X is , wherein R_{17}, R_{18}, R_{19} and R_{20} each are independently H, an alkyl, halogen, -OH, -O-alkyl, $-C(=O)OR_{30}$, $-C(=O)R_{30}$, $-NO_2$, or –CN, wherein R_{30} is H or an alkyl.

37. The conjugate of claim 35, wherein X is , wherein R_{17}, R_{18}, R_{19} and R_{20} each are independently H, an alkyl, halogen, -OH, -O-alkyl, $-C(=O)OR_{30}$, $-C(=O)R_{30}$, $-NO_2$, or -CN, wherein R_{30} is H or an alkyl.

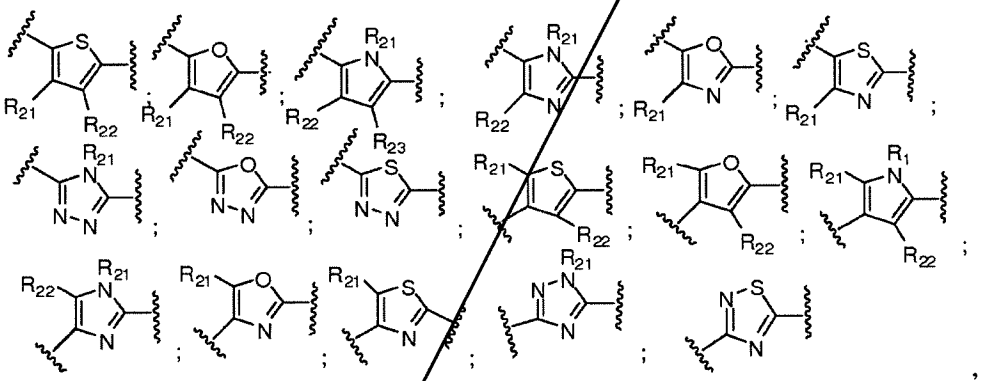
38. The conjugate of claim 35, wherein X is , wherein R_{17}, R_{18}, R_{19} and R_{20} each are independently H, an alkyl, halogen, -OH, -O-alkyl, $-C(=O)OR_{30}$, $-C(=O)R_{30}$, $-NO_2$, or -CN, wherein R_{30} is H or an alkyl.

39. The conjugate of any one of claims 36-38, wherein R_{17}, R_{18}, R_{19} and R_{20} are all H.

40. The conjugate of claim 1-34, wherein X is a six-membered heteroaryl selected from the group consisting of pyridine, pyridazine, pyrimidine, pyrazine, a triazine, and tetrazine.

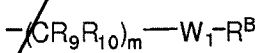
41. The conjugate of claim 1-34, wherein X is a six-membered heteroaryl selected from the group consisting of pyridine, pyridazine, pyrimidine, pyrazine, a triazine, and tetrazine, wherein the heteroaryl is connected to the -NH group and -L_1 group at carbon atoms of the heteroaryl.

42. The conjugate of claims 1-34, wherein X is selected from the group consisting of:



wherein R_{21}, R_{22} and R_{23} are each independently H or an alkyl.

43. The conjugate of any one of claims 1-42, wherein L_1 is represented by:



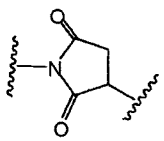
wherein:

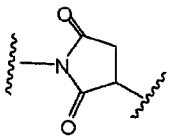
R_9 and R_{10} are each independently H or a C_{1-4} alkyl;

m is an integer from 1 to 10;

W_1 is absent, -NH-C(=O)-, -C(=O)-NH-, -C(=O)-O- or –O-C(=O)-, provided when Z is -NH- and m is 1, then W_1 is not -O-C(=O)-;

R^{B1} is absent or a C_{1-10} alkyl; and

J_D is absent, -C(=O)-, , -NH-C(=O)-CH_2-, or -S-.

44. The conjugate of claim 43, wherein J_D is absent, -C(=O)- or .

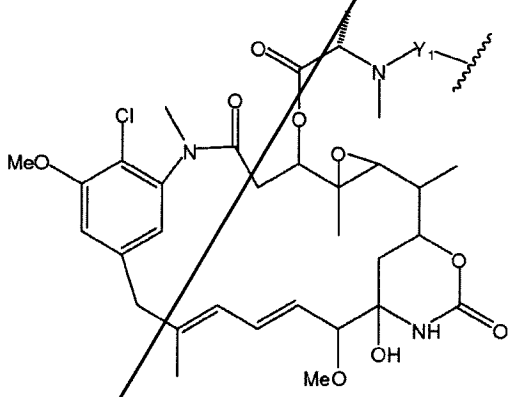
45. The conjugate of claim 43 or 44, wherein R_9 and R_{10} are both H; and m is 1, 2 or 3.

46. The conjugate of any one of claims 43-45, wherein W_1 is absent or -NH-C(=O)- and R^{B1} is absent or $-(CH_2)_{m'}-$, wherein m' is 1, 2, or 3.

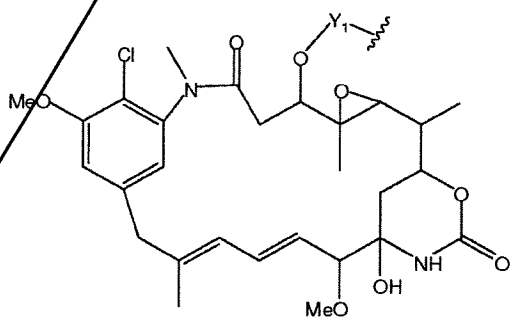
47. The conjugate of any one of claims 1-42, wherein L_1 is $-CH_2-S-R^{B1}-C(=O)-$, wherein R^{B1} is a C_{1-10} alkyl.

48. The conjugate of any one of claims 1-47, wherein D is a maytansinoid.

49. The conjugate of claim 48, wherein D is represented by the following structural formula:



(A), or



(B),

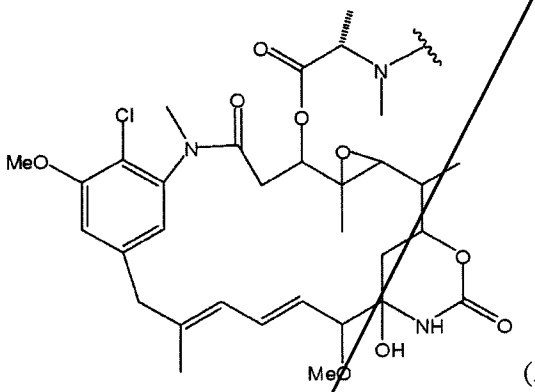
wherein:

Y_1 is absent or $-C(=O)(CR_7R_8)_{l1}(CR_5R_6)_{m1}(CR_3R_4)_{n1}CR_1R_2S-$;

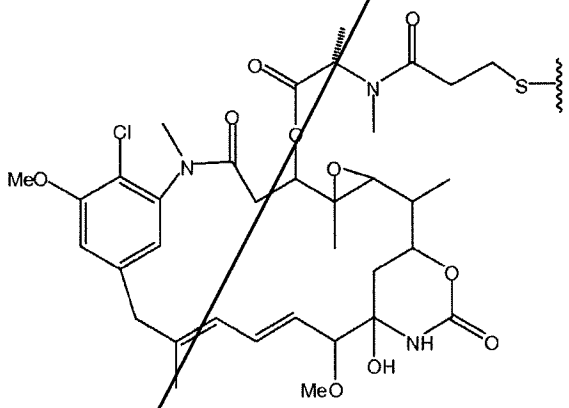
R_1, R_2, R_3, R_4, R_5, R_6, R_7 and R_8 are each independently H, an alkyl, an alkenyl, a cycloalkyl, a heteroaryl, a heterocyclyl, or an aryl; and,

l1, m1 and n1 are each independently 0 or an integer from 1 to 5.

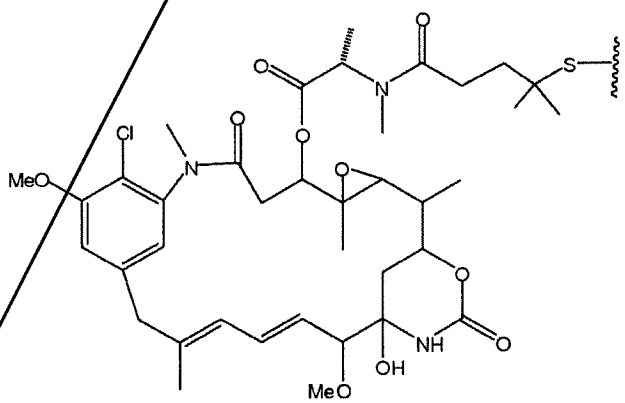
50. The conjugate of claim 49, wherein D is represented by any one of the following structural formulas:



(A1);

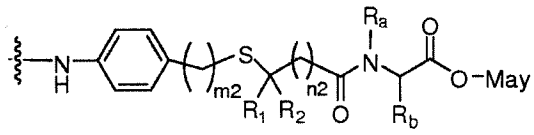


(A2); or

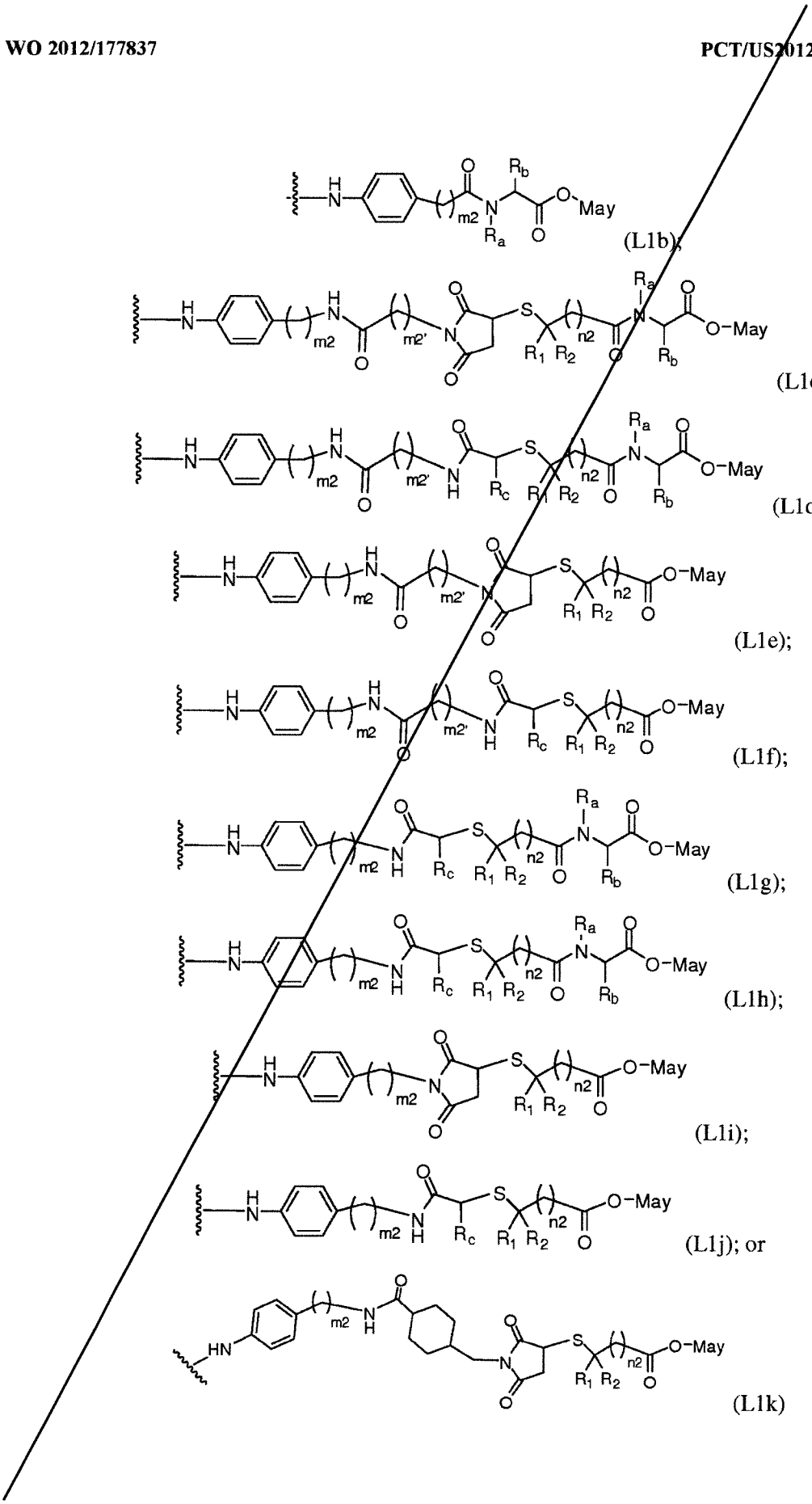


(A3).

51. The conjugate of any one of claims 1-34, wherein -Z-X-L_1-D is represented by one of the following structural formulas:



(L1a);



(L1b);

(L1c);

(L1d);

(L1e);

(L1f);

(L1g);

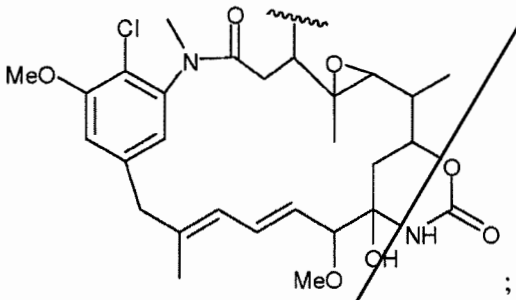
(L1h);

(L1i);

(L1j); or

(L1k)

wherein May is represented by the following structural formula:



;

R_1, R_2, R_a, R_b and R_c are each independently H or a C_{1-4} alkyl; and

m2, m2' and n2 are each independently an integer from 1 to 10.

52. The conjugate of claim 51, wherein R_a and R_b are both methyl; and R_c is H.

53. The conjugate of claim 52, wherein R_1 and R_2 are both H and n is 1.

54. The conjugate of claim 4552 wherein R_1 and R_2 are both methyl and n is 2.

55. The conjugate of any one of claims 51-54, wherein m2 and m2' are each independently an integer from 1 to 3.

56. A compound represented by the following structural formula:

$$Z'—X—L_1—D \quad (II),$$

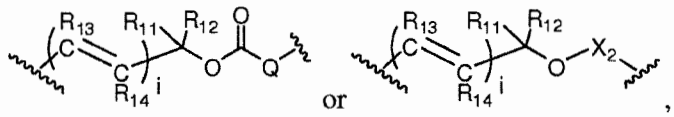
or a salt thereof, wherein:

Z' is $-NH_2$ or $-C(=O)-OH$;

D is a cytotoxic agent;

L_1 is absent or a spacer;

X is an optionally substituted aryl or an optionally substituted heteroaryl, provided when Z' is $-NH_2$, L_1 does not comprise one of the following moieties directly connected to X:



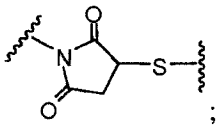
wherein i is 0 or an integer from 1 to 5; Q is -O-, $-NR_{15}$, S or $-CR_{11}R_{12}$-; X_2 is an aryl or a heteroaryl; and R_{11}, R_{12}, R_{13}, R_{14} and R_{15} are each independently H or an alkyl group.

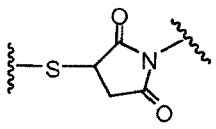
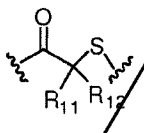
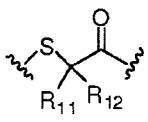
57. The compound of claim 56, wherein L_1 is represented by:

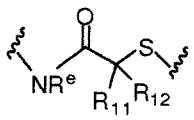
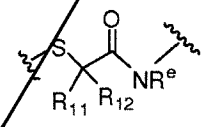
$$—J_x—R^{A1}—V_1—W_1—R^{B1}—V_1'—R^{C1}—J_D—,$$

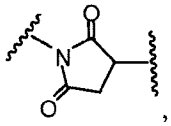
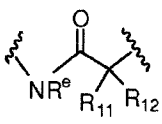
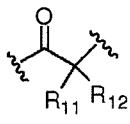
wherein:

R^{A1}, R^{B1} and R^{C1} are each absent, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, an aryl, a heteroaryl or a heterocyclyl, wherein the alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, and heterocyclyl are optionally substituted;

J_X and W_1 are each independently absent, -O-, -O-C(=O)-, -C(=O)-O-, -S-, -SO-, $-SO_2-$, $-CR_{11}R_{12}-S-$, $-CR_{11}R_{12}-O-$, $-CR_{11}R_{12}NR^e-$, -O-(C=O)O-, -O-(C=O)N(R^e)-, -N(R^e)-, -N(R^e)-C(=O)-, -C(=O)-N(R^e)-, -N(R^e)-C(=O)O-, -N(C(=O)R^e)C(=O)- , -N(C(=O)R^e)-, -SS-, -C(=O)-,  ;

 ;  ;  ;

 ;  ; an amino acid, or a peptide having 2 to 8 amino acids, provided when Z' is $-NH_2$, J_x and V_1 are both absent, R^{A1} is -$CR_{11}R_{12}$-, then W_1 is not -O-C(=O)-;

J_D is absent, –C(=O)-, -S-,  ,  ,  , -O-, -O-C(=O)-, -C(=O)-O-, -SO-, $-SO_2-$, -O-(C=O)-, -N(R^e)-, -N(R^e)-C(=O)-, or -N(C(=O)R^e)C(=O)- , or -N(C(=O)R^e)-;

V_1 and V_1' are each independently $–(O-CH_2-CH_2)_{p1}-$, $-(CH_2-CH_2-O)_{p1}-$, $-(NR^m-CH_2-CH_2)_{p1'}-$ or $–(CH_2-CH_2-NR^m)_{p1'}-$;

R^e is H, an alkyl, an alkenyl, an alkynyl, or $-(CH_2-CH_2-O)_{n'}-R^k$;

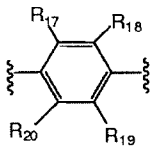
R^k and R^m are each independently H or an alkyl;

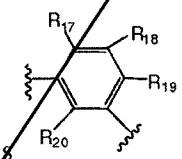
n' is an integer from 1 to 24;

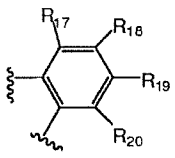
p1 is 0 or an integer from 1 to 1000; and

p1' is 0 or an integer from 1 to 10.

58. The compound of claim 56 or 57, wherein X is an optionally substituted phenyl.

59. The compound of claim 58, wherein X is  , wherein R_{17}, R_{18}, R_{19} and R_{20} each are independently H, an alkyl, halogen, -OH, -O-alkyl, -C(=O)OR_{30}, -C(=O)R_{30}, -NO_2, or –CN, wherein R_{30} is H or an alkyl.

60. The compound of claim 58, wherein X is  , wherein R_{17}, R_{18}, R_{19} and R_{20} each are independently H, an alkyl, halogen, -OH, -O-alkyl, -C(=O)OR_{30}, -C(=O)R_{30}, -NO_2, or –CN, wherein R_{30} is H or an alkyl.

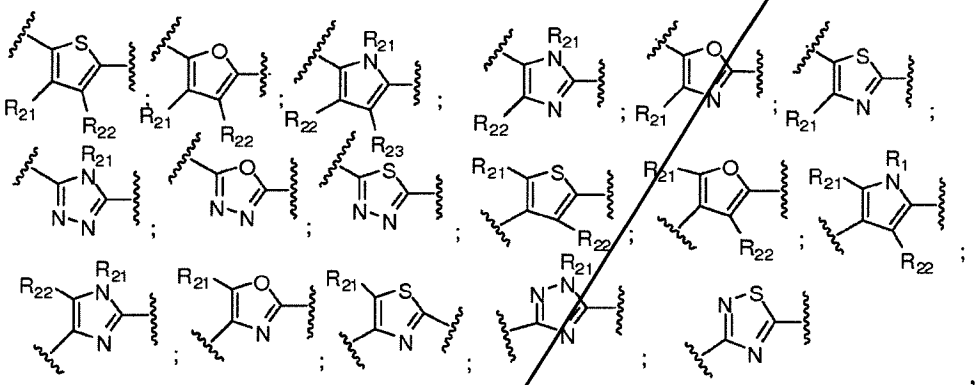
61. The compound of claim 60, wherein X is  , wherein R_{17}, R_{18}, R_{19} and R_{20} each are independently H, an alkyl, halogen, -OH, -O-alkyl, -C(=O)OR_{30}, -C(=O)R_{30}, -NO_2, or –CN, wherein R_{30} is H or an alkyl.

62. The compound of any one of claims 59-61, wherein R_{17}, R_{18}, R_{19} and R_{20} are all H.

63. The compound of any one of claims 56-62, wherein X is a six-membered heteroaryl selected from the group consisting of pyridine, pyridazine, pyrimidine, pyrazine, a triazine, and tetrazine.

64. The compound of any one of claim 56-62, wherein X is a six-membered heteroaryl selected from the group consisting of pyridine, pyridazine, pyrimidine, pyrazine, a triazine, and tetrazine, wherein the heteroaryl is connected to the -NH group and -L_1 group at carbon atoms of the heteroaryl.

65. The compound of any one of claims 56-62, wherein X is selected from the group consisting of:



wherein R_{21}, R_{22} and R_{23} are each independently H or an alkyl.

66. The compound of any one of claims 56-65, wherein L_1 is represented by:

$$-(CR_9R_{10})_m—W_1-R^{B1}—J_D-$$

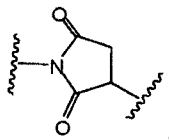
wherein:

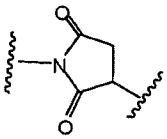
R_9 and R_{10} are each independently H or a C_{1-4} alkyl;

m is an integer from 1 to 10;

W_1 is absent, -NH-C(=O)-, -C(=O)-NH-, -C(=O)-O- or –O-C(=O)-, provided when Z' is $-NH_2$ and m is 1, then W_1 is not -O-C(=O)-;

R^{B1} is absent or a C_{1-10} alkyl; and

J_D is absent, -C(=O)-, , -NH-C(=O)-CH_2-, or -S-.

67. The compound of claim 66, wherein J_D is absent, -C(=O)- or .

68. The compound of claim 66 or 67, wherein R_9 and R_{10} are both H; and m is 1, 2 or 3.

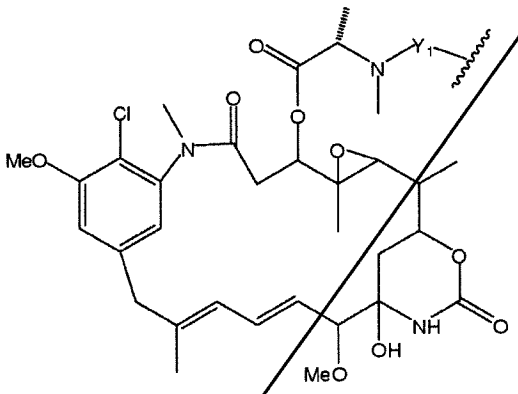
69. The compound of any one of claims 57-65, wherein W_1 is absent or -NH-C(=O)- and R^{B1} is absent or $-(CH_2)_{m'}-$, wherein m' is 1, 2, or 3.

70. The compound of any one of claims 56-65, wherein L_1 is $-CH_2-S-R^{B1}-C(=O)-$, wherein R^{B1} is a C_{1-10} alkyl.

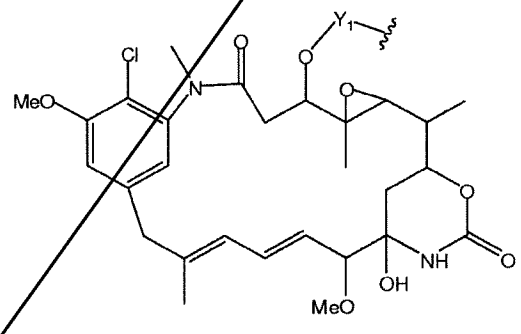
71. The compound of claim 70, wherein R^{B1} is $-CH_2-CH_2-$.

72. The compound of any one of claims 66-69, wherein D is a maytansinoid.

73. The compound of claim 72, wherein D is represented by the following structural formula:



(A), or



(B),

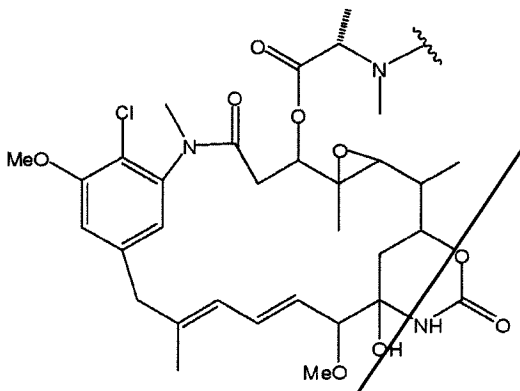
wherein:

Y_1 is absent or $-C(=O)(CR_7R_8)_{l1}(CR_5R_6)_{m1}(CR_3R_4)_{n1}CR_1R_2S-$,

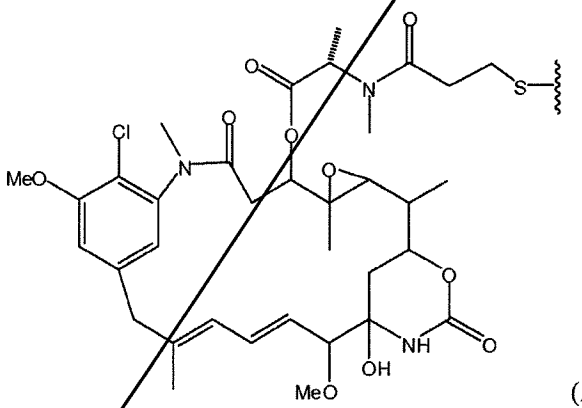
R_1, R_2, R_3, R_4, R_5, R_6, R_7 and R_8 are each independently H, an alkyl, an alkenyl, a cycloalkyl, a heteroaryl, a heterocyclyl, or an aryl; and

l1, m1 and n1 are each independently 0 or an integer from 1 to 5.

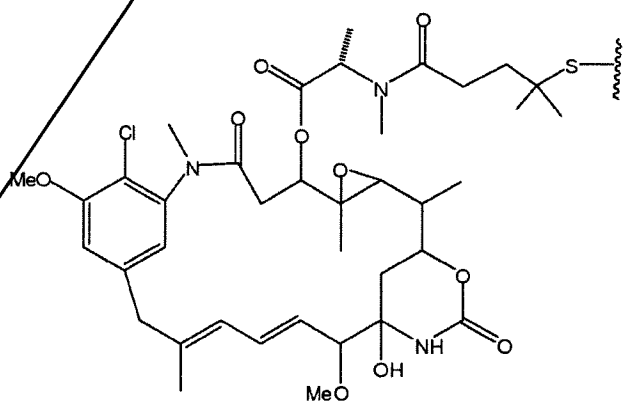
74. The conjugate of claim 73, wherein D is represented by any one of the following structural formulas:



(A1);

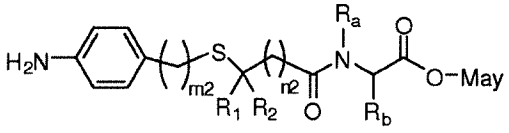


(A2); or

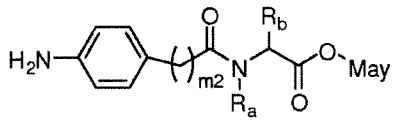


(A3).

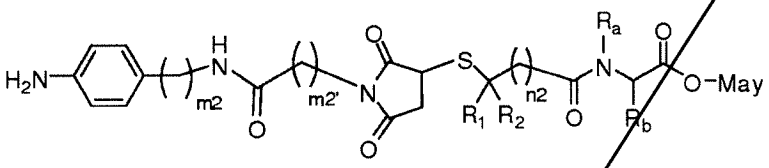
75. The compound of claim 56, wherein the compound is represented by one of the following structural formulas:



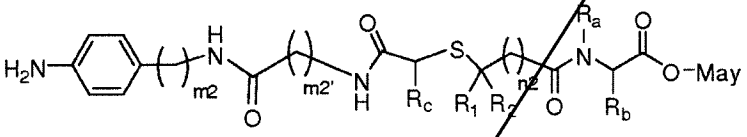
(IIa);



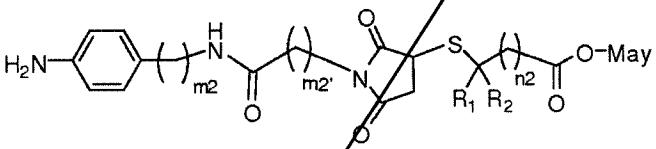
(IIb);



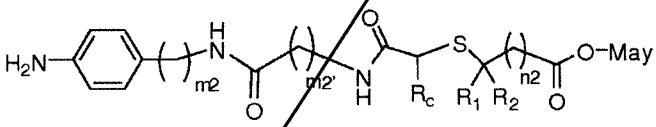
(IIc);



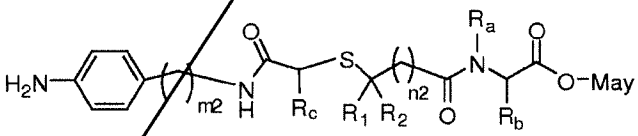
(IId);



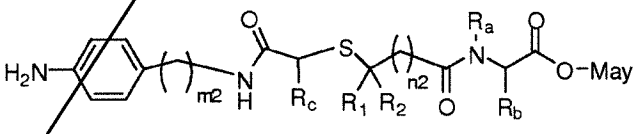
(IIe);



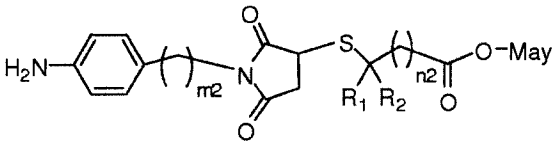
(IIf);



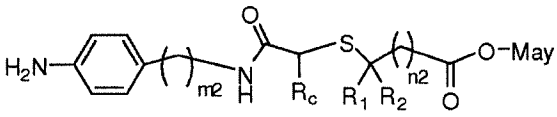
(IIg);



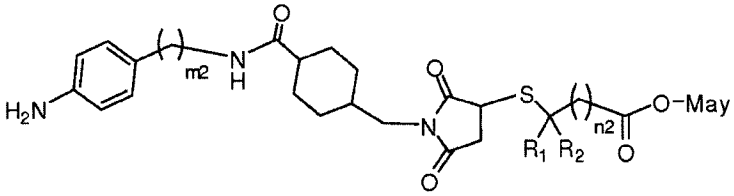
(IIh);



(IIi);

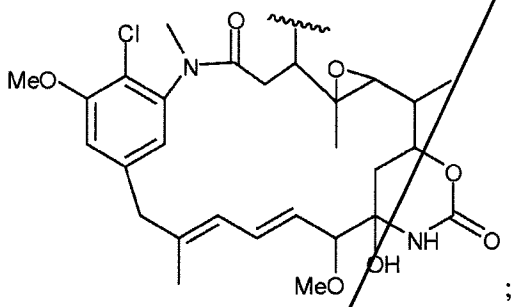


(IIj); or



(IIk)

or a pharmaceutically acceptable salt thereof, wherein May is represented by the following structural formula:



;

R_1, R_2, R_a, R_b and R_c are each independently H or a C_{1-4} alkyl; and

m2, m2' and n2 are each independently an integer from 1 to 10.

76. The compound of claim 75, wherein R_a and R_b are both methyl; and R_c is H.

77. The compound of claim 75 or 76, wherein R_1 and R_2 are both H and n is 1.

78. The compound of claim 75 or 76, wherein R_1 and R_2 are both methyl and n is 2.

79. The compound of any one of claims 75-78, wherein m2 and m2' are each independently an integer from 1 to 3.

80. A compound represented by the following structural formula:

$$L_2'—A—Z—X—L_1' \quad (III),$$

or a salt thereof, wherein:

L_2' is absent or a spacer comprising a reactive moiety that can form a covalent bond with a cell-binding agent (CB);

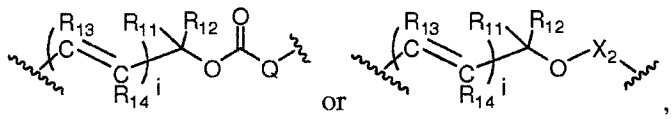
A is an amino acid or a peptide comprising 2 to 20 amino acids;

Z is -NH- or -C(=O)-;

L_1' is a spacer comprising a reactive moiety that can form a covalent bond with a cytotoxic agent; and

X is an optionally substituted aryl or an optionally substituted heteroaryl;

provided that when Z is -NH-, L_1' does not comprise one of the following moieties directly connected to X:



wherein i is 0 or an integer from 1 to 5; Q is -O-, -NR_{15}, S or -$CR_{11}R_{12}$-;

X_2 is an aryl or a heteroaryl; and R_{11}, R_{12}, R_{13}, R_{14} and R_{15} are each independently H or an alkyl group.

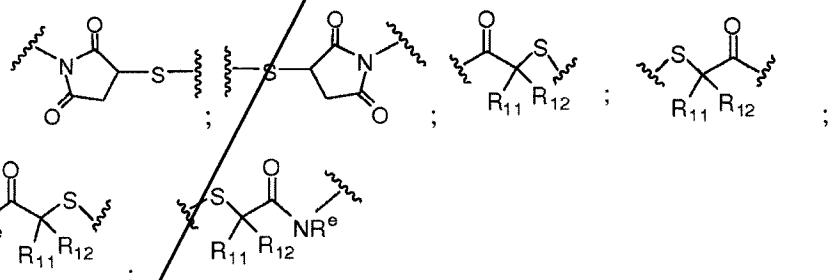
81. The compound of claim 80, wherein L_1' is represented by:

$$—J_x—R^{A1}—V_1—W_1—R^{B1}—V_1'—R^{C1}—J_D',$$

wherein:

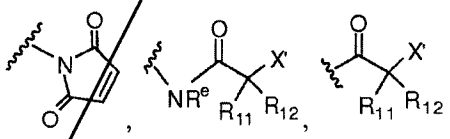
R^{A1}, R^{B1} and R^{C1} are each absent, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, an aryl, a heteroaryl or a heterocyclyl, wherein the alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, and heterocyclyl are optionally substituted;

J_X and W_1 are each independently absent, -O-, -O-C(=O)-, -C(=O)-O-, -S-, -SO-, -SO_2-, -$CR_{11}R_{12}$-S-, -$CR_{11}R_{12}$-O-, -$CR_{11}R_{12}NR^e$-, -O-(C=O)O-, -O-(C=O)N(R^e)-, -N(R^e)-, -N(R^e)-C(=O)-, -C(=O)-N(R^e)-, -N(R^e)-C(=O)O-, -N(C(=O)R^e)C(=O)- , -N(C(=O)R^e)-, -SS-, -C(=O)-,



; an amino acid, or a peptide having 2 to 8 amino acids, provided when Z is -NH-, J_X and V_1 are both absent, R^{A1} is -$CR_{11}R_{12}$-, then W_1 is not -O-C(=O)-;

J_D' is -C(=O)-OH, -COE, -SH, -S-S(=O)-R^d, S-$S(=O)_2R^d$, -SSR^d,



, -OH, -O-C(=O)X', -S(=O)X'-, -SO_2X', -N(R^e)H, -N(R^e)-C(=O)-X', or -N(C(=O)R^e)C(=O)X', -C(=O)NH(C(=O)R^e), or -NH(C(=O)R^e), wherein COE represents a reactive ester; X' is a leaving group (*e.g.*, halogen, mesylate or tosylate); and R^d is selected from phenyl, nitrophenyl (*e.g.*, 2 or 4-nitrophenyl), dinitrophenyl (*e.g.*, 2 or 4-nitrophenyl), carboxynitrophenyl (*e.g.*, 3-carboxy-4-nitrophenyl), pyridyl or nitropyridyl (*e.g.*, 4-nitropyridyl);

V_1 and V_1' are each independently -$(O-CH_2-CH_2)_{p1}$-, -$(CH_2-CH_2-O)_{p1}$-, -$(NR^m-CH_2-CH_2)_{p1}$'- or -$(CH_2-CH_2-NR^m)_{p1}$'-;

R^e is H, an alkyl, an alkenyl, an alkynyl, or -$(CH_2$-CH_2-$O)_{n'}$-R^k;

R^k and R^m are each independently H or an alkyl;

R_{11} and R_{12} are each independently H or an alkyl;

n' is an integer from 1 to 24;

p1 is 0 or an integer from 1 to 1000; and

p1' is 0 or an integer from 1 to 10.

82. The compound of claim 81, -COE is a reactive ester selected from N-hydroxysuccinimde ester, N-hydroxy sulfosuccinimide ester, nitrophenyl (*e.g.*, 2 or 4-nitrophenyl) ester, dinitrophenyl (*e.g.*, 2,4-dinitrophenyl) ester, sulfo-tetraflurophenyl (*e.g.*, 4-sulfo-2,3,5,6-tetrafluorophenyl) ester, and pentafluorophenyl ester.

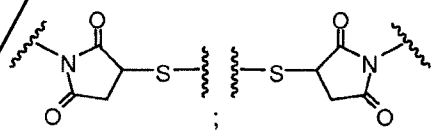
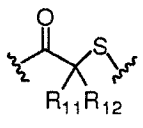
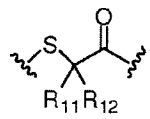
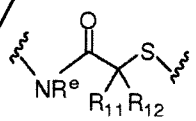
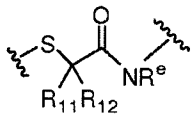
83. The compound of claim 81 or 82, wherein L_2' is represented by the following structural formula:

$$J_{CB}'—R^{A2}—V_2—W_2—R^{B2}—V_2'—R^{C2}-J_A—;$$

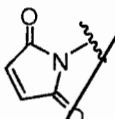
wherein:

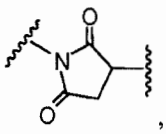
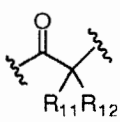
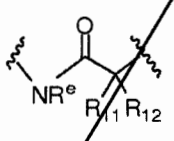
R^{A2}, R^{B2} and R^{C2} are each independently absent, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, an aryl, a heteroaryl, a heterocyclyl, a cycloalkylalkyl, an arylalkyl, a heteroarylalkyl or a heterocyclylalkyl;

V_2 and V_2' are each independently -$(O$-CH_2-$CH_2)_{p2}$-, -$(CH_2$-CH_2-$O)_{p2}$-, -$(NR^m$-CH_2-$CH_2)_{p2}$- or -$(CH_2$-CH_2-$NR^m)_{p2'}$-;

W_2 is absent, -O-, -O-C(=O)-, -C(=O)-O-, -S-, -SO-, -SO_2-, -$CR_{11}R_{12}$-S-, -$CR_{11}R_{12}$-O-, -$CR_{11}R_{12}$-NR^e-, -O-(C=O)O-, -O-(C=O)N(R^e)-, -N(R^e)-, -N(R^e)-C(=O)-, -C(=O)-N(R^e)-, -N(R^e)-C(=O)O-, -N(C(=O)R^e)C(=O)- , -N(C(=O)R^e)-, -$(O$-CH_2-$CH_2)_{n'}$-, -SS-, -C(=O)-, an amino acid, a peptide having 2 to 8 amino acids,  ;  ;  ;  ;  ;

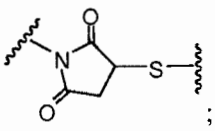
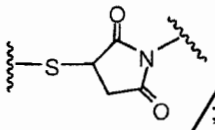
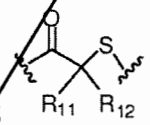
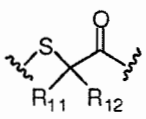
J_{CB}' is -C(=O)OH, -COE, -N(R^e)-C(=O)-X', -O-C(=O)X', -C(=NH)X', -

NR^e-C(=NH)X', -SH, -SSR^d, -NR^eH, -NH-NR^eH, , -C(=NR^e)X', -NR^eNH, X'-CR_1R_2-C(=O)-, or X'-CR_1R_2-C(=O)-NR^e-, wherein COE is a reactive ester, X' is a halogen; and R^d is selected from phenyl, nitrophenyl (*e.g.*, 2 or 4-nitrophenyl), dinitrophenyl (*e.g.*, 2 or 4-nitrophenyl), carboxynitrophenyl (*e.g.*, 3-carboxy-4-nitrophenyl), pyridyl or nitropyridyl (*e.g.*, 4-nitropyridyl);

J_A is -NR^e-, -C(=O)-, =N-, -NR^e-C(=O)-, -O-C(=O)- , -SO_2-, -S-, , , ;

p2 is 0 or an integer from 1 to 1000; and

p2' is 0 or an integer from 1 to 10.

84. The compound of claim 83, wherein W_2 is absent, ; ; ; ; -S-S-, -NH-C(=O)- or -C(=O)-NH-.

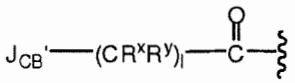
85. The compound of claim 84, wherein p2 for V_2 and V_2' are both 0.

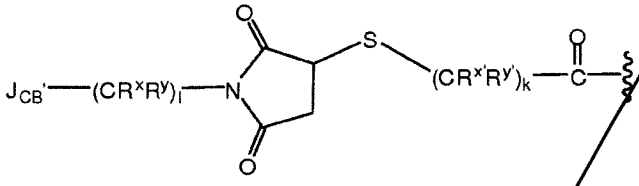
86. The compound of any one of claims 83-85, wherein R^{A2} is an alkyl.

87. The compound of any one of claims 83-86, wherein one of R^{B2} and R^{C2} is absent, and the other is an alkyl.

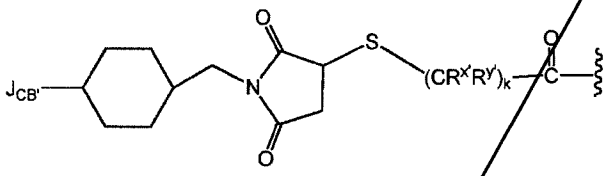
88. The compound of any one of claims 83-85, wherein R^{B2} and R^{C2} are both absent.

89. The compound of any one of claims 83, wherein L_2' is represented by the following structural formulas:

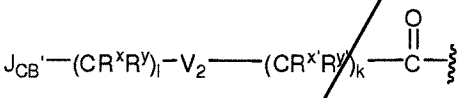
 (L2'a);



(L2'b);



(L2'b'); or



(L2'c);

wherein:

J_{CB}' is -COOH or -COE, wherein -COE is a reactive ester selected from N-hydroxysuccinimde ester, N-hydroxy sulfosuccinimide ester, nitrophenyl (*e.g.*, 2 or 4-nitrophenyl) ester, dinitrophenyl (*e.g.*, 2,4-dinitrophenyl) ester, sulfo-tetraflurophenyl (*e.g.*, 4-sulfo-2,3,5,6-tetrafluorophenyl) ester, and pentafluorophenyl ester;

R^x, R^y, $R^{x'}$ and $R^{y'}$, for each occurrence, are independently H, -OH, halogen, -O-(C_{1-4} alkyl), $-SO_3^-$, $-NR_{40}R_{41}R_{42}^+$, or a C_{1-4} alkyl optionally substituted with -OH, halogen, SO_3^- or $NR_{40}R_{41}R_{42}^+$, wherein R_{40}, R_{41} and R_{42} are each independently H or a C_{1-4} alkyl;

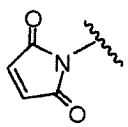
V_2 is $-(CH_2\text{-}CH_2\text{-}O)_{p2}$- or $-(O\text{-}CH_2\text{-}CH_2)_{p2}$-;

l and k are each independently 0 or an integer from 1 to 10; and,

p2 is an integer from 1 to 24.

90. The compound of claim 89, wherein R^x, R^y, $R^{x'}$ and $R^{y'}$ are all H.

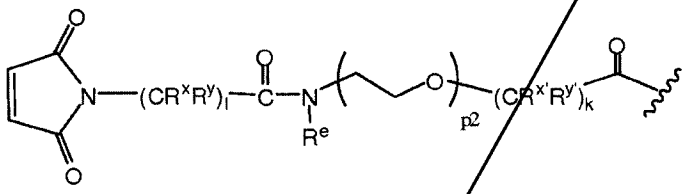
91. The compound of claim 90, wherein p2 is an integer from 2 to 24; l and k are each independently an integer from 1 to 6.

92. The compound of claim 83, wherein J_{CB}' is , X'-CH_2-C(=O)- or X'-CH_2-C(=O)-NH-.

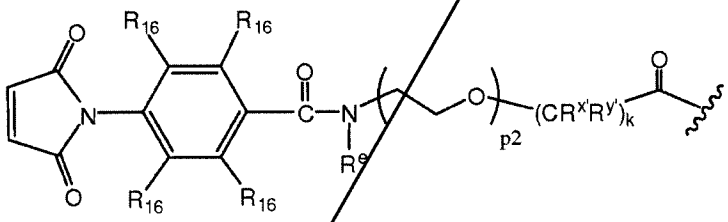
93. The compound of claim 92, wherein V_2 and V_2' are both $-(O\text{-}CH_2\text{-}CH_2)_{p2}$- or $-(CH_2\text{-}CH_2\text{-}O)_{p2}$-.

94. The compound of claim 93, wherein one of p2 for one of V_2 and V_2' is 0 and the other is an integer from 2 to 24.

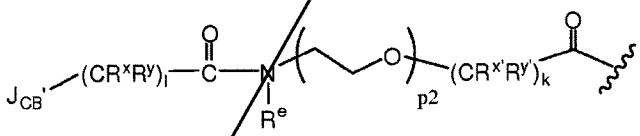
95. The compound of claim 83, wherein L_2' is represented by:



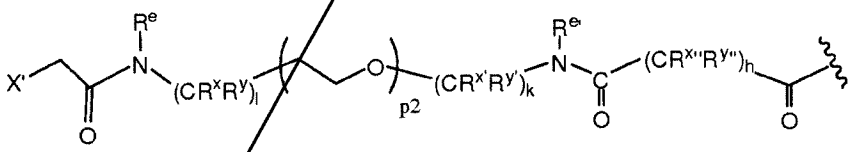
(L2'd);



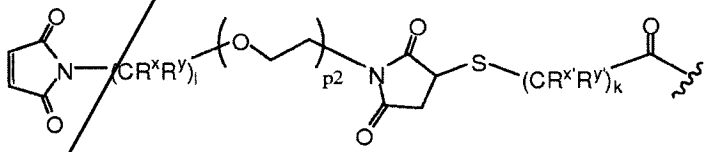
(L2'e);



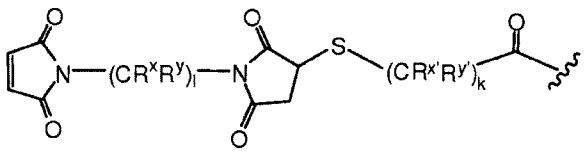
(L2'f);



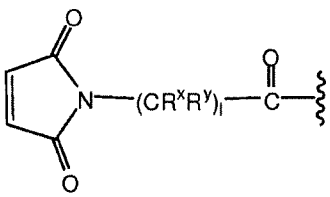
(L2'g);



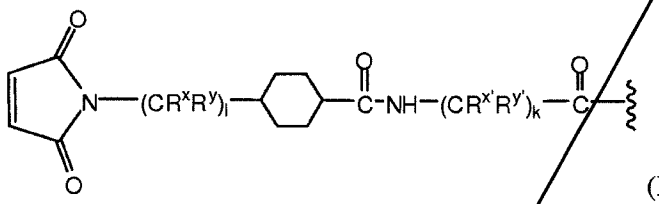
(L2'h);



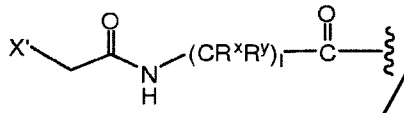
(L2'i);



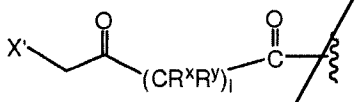
(L2'j);



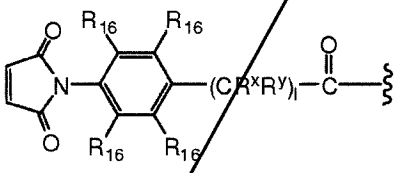
(L2'k);



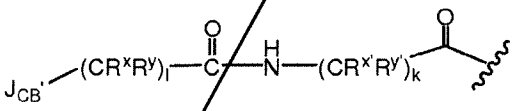
(L2'l);



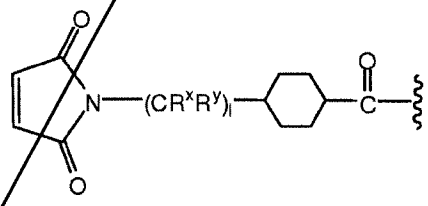
(L2'm);



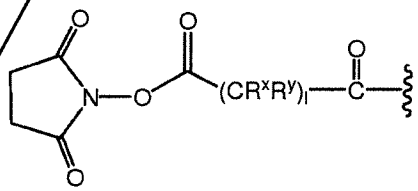
(L2'n);



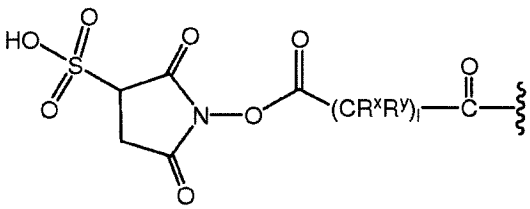
(L2'o);



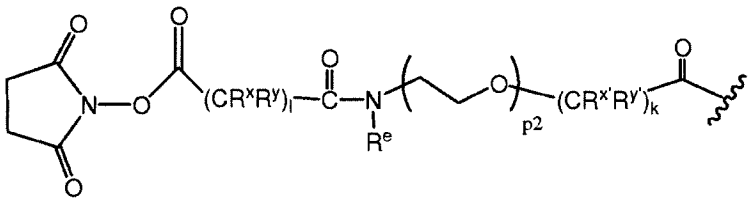
(L2'p);



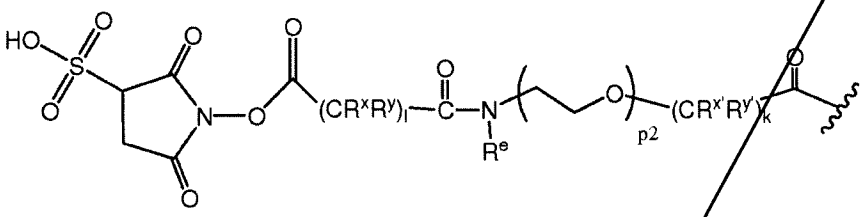
(L2'q);



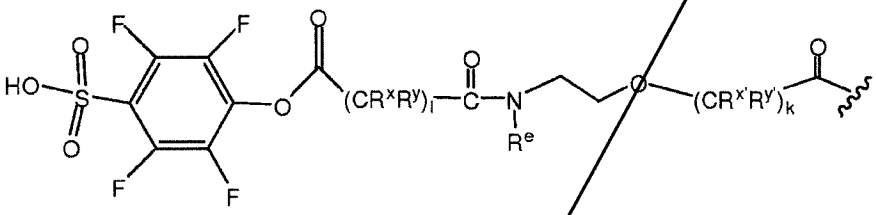
(L2'r);



(L2's);



(L2't); or



(L2'u);

wherein:

J_{CB}' is -SH or -SSR^d,

R^x, R^y, $R^{x'}$, $R^{y'}$, $R^{x''}$ and $R^{y''}$, for each occurrence, are each independently H, -OH, halogen, -O-(C_{1-4} alkyl), -SO_3^-, -$NR_{40}R_{41}R_{42}^+$, or a C_{1-4} alkyl optionally substituted with -OH, halogen, SO_3^- or $NR_{40}R_{41}R_{42}^+$, wherein R_{40}, R_{41} and R_{42} are each independently H or a C_{1-4} alkyl;

R_{16}, for each occurrence, is independently H, an alkyl, halogen, -OH, -O-alkyl, -NO_2, or -CN; and,

l, k and h are each independently an integer from 1 to 10.

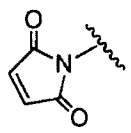
96. The compound of claim 95, wherein R_{16} is H.

97. The compound of claim 95 or 96, wherein R^x, R^y, $R^{x'}$, $R^{y'}$, $R^{x''}$ and $R^{y''}$ are all H.

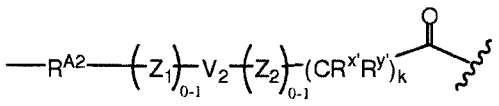
98. The compound of claim 97, wherein p2 for each occurrence is independently an integer from 2 to 24.

99. The compound of claim 98, wherein l, k and h are each independently an integer from 1 to 6.

100. The compound of claim 83, wherein:

$J_{CB'}$ is  , -C(=O)OH, -COE, -SH, -SSR^d, X'-CR_1R_2-C(=O)-, or X'-CR_1R_2-C(=O)-NR^e-, wherein COE is a reactive ester, X' is a halogen; and R^d is selected from phenyl, nitrophenyl (*e.g.*, 2 or 4-nitrophenyl), dinitrophenyl (*e.g.*, 2 or 4-nitrophenyl), carboxynitrophenyl (*e.g.*, 3-carboxy-4-nitrophenyl), pyridyl or nitropyridyl (*e.g.*, 4-nitropyridyl);

$-R^{A2}-V_2-W_2-R^{B2}-V_{2'}-R^{C2}-J_A-$ is:

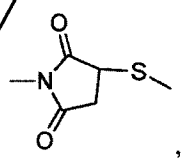


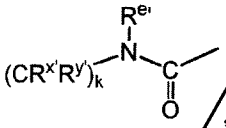
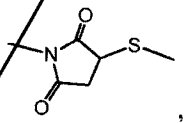
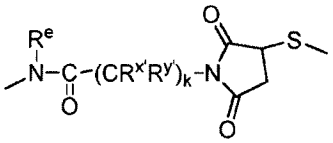
; wherein

R^{A2} is $-(CR^xR^y)_l-$ or $-Cy-(CR^xR^y)_l-$;

Cy is a cycloalkyl (*e.g.*, cyclohexyl);

V_2 is $-(CH_2-CH_2-O)_{p2}-$ or $-(O-CH_2-CH_2)_{p2}-$;

Z_1 is $-C(=O)-N(R^e)-$, $-N(R^e)-C(=O)-$, or , provided that Z_1-V_2 does not contain an N-O bond;

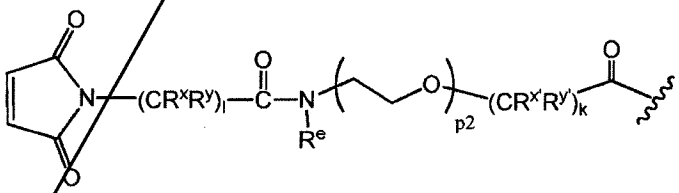
Z_2 is , , or , provided that V_2-Z_2 does not contain an O-N bond;

l is an integer from 0 to 10;

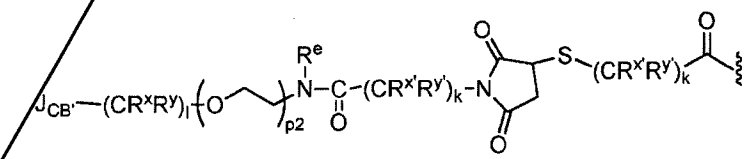
p2 is an integer from 0 to 200;

Z is -NH-.

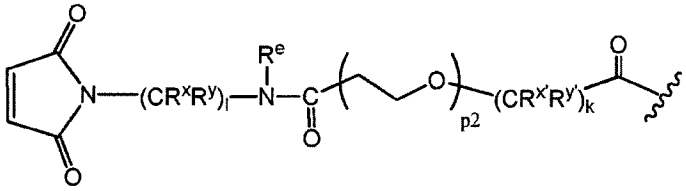
101. The compound of claim 100, wherein L_2' is represented by:



(L2'd);



(L2'r); or



(L2's);

wherein:

p2 is an integer from 0 to 200;

J_{CB}' is -COOH or -COE, wherein -COE is a reactive ester selected from N-hydroxysuccinimde ester, N-hydroxy sulfosuccinimide ester, nitrophenyl (*e.g.*, 2 or 4-nitrophenyl) ester, dinitrophenyl (*e.g.*, 2,4-dinitrophenyl) ester, sulfo-tetraflurophenyl (*e.g.*, 4-sulfo-2,3,5,6-tetrafluorophenyl) ester, and pentafluorophenyl ester;

R^x, R^y, $R^{x'}$, and $R^{y'}$, for each occurrence, are each independently H, -OH, halogen, -O-(C_{1-4} alkyl), $-SO_3^-$, $-NR_{40}R_{41}R_{42}^+$, or a C_{1-4} alkyl optionally substituted with -OH, halogen, SO_3^- or $NR_{40}R_{41}R_{42}^+$, wherein R_{40}, R_{41} and R_{42} are each independently H or a C_{1-4} alkyl;

l and k, for each occurrence, are independently an integer from 1 to 10.

102. The compound of claim 100 or 101, wherein A is Val-Cit or Gly-Gly-Gly.

103. The compound of any one of claims 100-102, wherein X is phenyl.

104. The compound of any one of claims 80-103, wherein A is a peptide cleavable by a protease.

105. The compound of claim 104, wherein A is a peptide cleavable by a protease expressed in tumor tissue.

106. The compound of claim 104, wherein A is a peptide cleavable by a lysosomal protease.

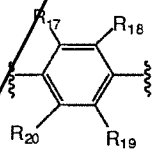
107. The compound of any one of claims 80-103, wherein A is a peptide having an amino acid that is covalent linked with -NH-X-L_1-D selected from the group consisting of Ala, Arg, Asn, Asp, Cit, Cys, selino-Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr and Val, each independently as L or D isomers.

108. The compound of any one of claims 80-103, wherein A is selected from the group consisting of 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: 1), β-Ala-Leu-Ala-Leu (SEQ ID NO: 2) and Gly-Phe-Leu-Gly (SEQ ID NO: 3), 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, and D-Ala-D-Ala.

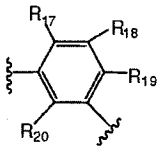
109. The compound of claim 108, wherein A is Val-Cit, Val-Lys, Val-D-Cit or Val-D-Lys.

110. The compound of any one of claims 80-103, wherein A is Cys or a peptide comprising a Cys that is covalently linked to L_2, wherein the Cys is linked to L_2 through the –SH group of Cys side chain.

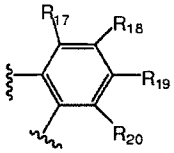
111. The compound of any one of claims 80-110, wherein X is an optionally substituted phenyl.



112. The compound of claim 111, wherein X is , wherein R_{17}, R_{18}, R_{19} and R_{20} each are independently H, an alkyl, halogen, -OH, -O-alkyl, -C(=O)OR_{30}, -C(=O)R_{30}, -NO_2, or –CN, wherein R_{30} is H or an alkyl.



113. The compound of claim 111, wherein X is , wherein R_{17}, R_{18}, R_{19} and R_{20} each are independently H, an alkyl, halogen, -OH, -O-alkyl, -C(=O)OR_{30}, -C(=O)R_{30}, -NO_2, or –CN, wherein R_{30} is H or an alkyl.



114. The compound of claim 111, wherein X is , wherein R_{17}, R_{18}, R_{19} and R_{20} each are independently H, an alkyl, halogen, -OH, -O-alkyl, -C(=O)OR_{30}, -C(=O)R_{30}, -NO_2, or –CN, wherein R_{30} is H or an alkyl.

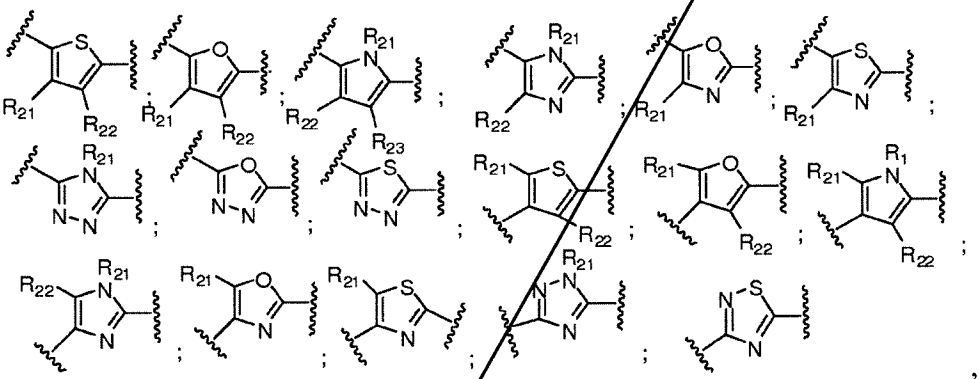
115. The compound of any one of claims 112-114, wherein R_{17}, R_{18}, R_{19} and R_{20} are all H.

116. The compound of any one of claims 80-110, wherein X is a six-membered heteroaryl selected from the group consisting of pyridine, pyridazine, pyrimidine, pyrazine, a triazine, and tetrazine.

117. The compound of any one of claims 80-110, wherein X is a six-membered heteroaryl selected from the group consisting of pyridine, pyridazine, pyrimidine,

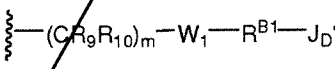
pyrazine, a triazine, and tetrazine, wherein the heteroaryl is connected to the –NH group and $-L_1$ group at carbon atoms of the heteroaryl.

118. The compound of any one of claims 80-110, wherein X is selected from the group consisting of:



wherein R_{21}, R_{22} and R_{23} are each independently H or an alkyl.

119. The compound of any one of claims 80-110, wherein L_1' is represented by:



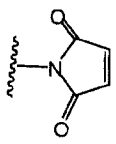
wherein:

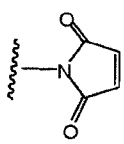
R_9 and R_{10} are each independently H or a C_{1-4} alkyl;

m is an integer from 1 to 10;

W_1 is absent, -NH-C(=O)-, -C(=O)-NH-, -C(=O)-O- or –O-C(=O)-, provided when Z is –NH- and m is 1, W_1 is not -O-C(=O)-;

R^{B1} is absent or a C_{1-10} alkyl; and

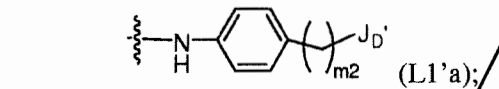
J_D' is absent, -C(=O)OH, COE, , -NH-C(=O)-CH_2-X', -SH, -S-S(=O)-R^d, S-S(=O)$_2R^d$ or -SSR^d.

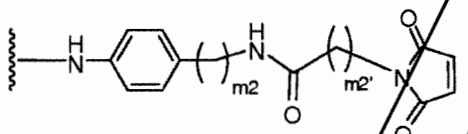
120. The compound of claim 119, wherein J_D' is -C(=O)OH, -COE or .

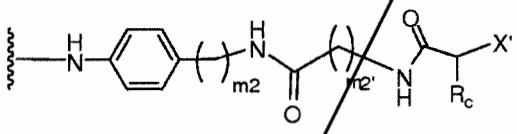
121. The compound of claim 119 or 120, wherein R_9 and R_{10} are both H; and m is 1, 2 or 3.

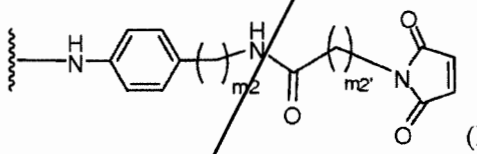
122. The compound of any one of claims 119-121, wherein W_1 is absent or -NH-C(=O)- and R^{B1} is absent or $-(CH_2)_{m'}-$, wherein m' is 1, 2, or 3.

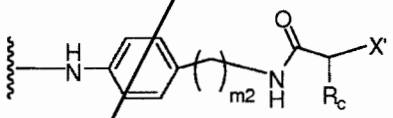
123. The compound of any one of claims 80-99, wherein -Z-X-L_1' is represented by one of the following structural formulas:

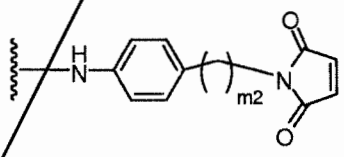

(L1'a);

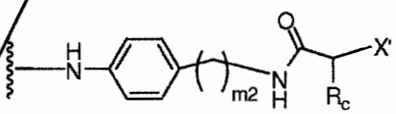

(L1'b);

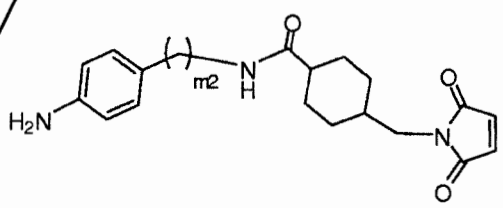

(L1'c);

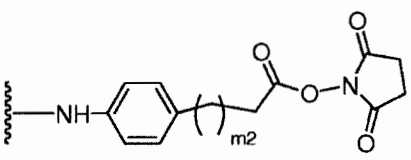

(L1'd);

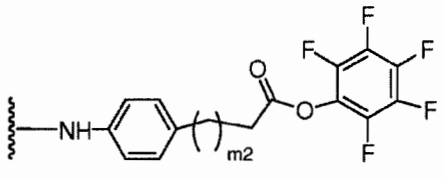

(L1'e);


(L1'f);


(L1'g);


(L1'h);


(L1'i); or



(I.1'j);

wherein R_c is H or a C_{1-4} alkyl; and m2 and m2' are each independently an integer from 1 to 10.

124. The compound of claim 123, wherein R_c is H.

125. The compound of claim 123 or 124, wherein m2 and m2' are each independently an integer from 1 to 3.

126. A compound represented by the following structural formula:

$$L_2'—A—Z—X—L_1—D \quad (IV),$$

or a salt thereof, wherein:

L_2' is absent or a spacer comprising a reactive moiety that can form a covalent bond with a cell-binding agent (CB);

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

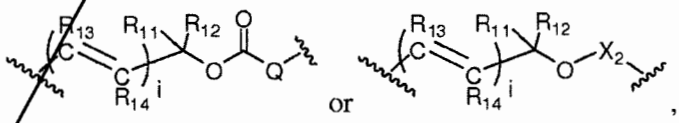
Z is -NH- or –C(=O)-;

D is a cytotoxic agent;

L_1 is absent or a spacer; and

X is an optionally substituted aryl or an optionally substituted heteroaryl;

provided that when Z is -NH-, L_1 does not comprise one of the following moieties directly connected to X:



wherein i is 0 or an integer from 1 to 5; Q is -O-, -NR_{15}, S or -$CR_{11}R_{12}$-; X_2 is an aryl or a heteroaryl; and R_{11}, R_{12}, R_{13}, R_{14} and R_{15} are each independently H or an alkyl group.

127. The compound of claim 126, wherein L_1 is represented by:

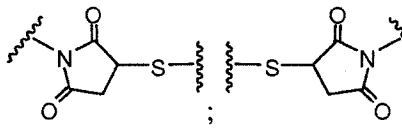
$$—J_x—R^{A1}—V_1—W_1—R^{B1}—V_1'—R^{C1}—J_D—,$$

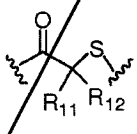
wherein:

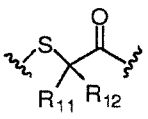
R^{A1}, R^{B1} and R^{C1} are each absent, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, an aryl, a heteroaryl or a heterocyclyl, wherein the alkyl, alkenyl,

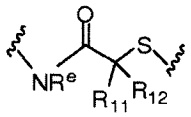
alkynyl, cycloalkyl, aryl, heteroaryl, and heterocyclyl are optionally substituted;

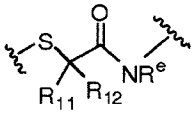
J_X and W_1 are each independently absent, -O-, -O-C(=O)-, -C(=O)-O-, -S-, -SO-, -SO_2-, -$CR_{11}R_{12}$-S-, -$CR_{11}R_{12}$-O-, -$CR_{11}R_{12}NR^e$-, -O-(C=O)O-, -O-(C=O)N(R^e)-, -N(R^e)-, -N(R^e)-C(=O)-, -C(=O)-N(R^e)-, -N(R^e)-C(=O)O-, -N(C(=O)R^e)C(=O)- , -N(C(=O)R^e)-, -SS-, -C(=O)-,

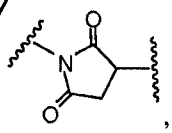
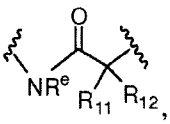
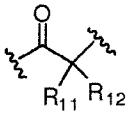

;


;


;


;


; an amino acid, or a peptide having 2 to 8 amino acids, provided when Z is -NH-, J_X and V_1 are both absent, R^{A1} is -$CR_{11}R_{12}$-, then W_1 is not –O-C(=O)-;

J_D is absent, -C(=O)-, -S-, , , , -O-, -O-C(=O)-, -C(=O)-O-, -SO-, -SO_2-, -O-(C=O)-, -N(R^e)-, -N(R^e)-C(=O)-, or -N(C(=O)R^e)C(=O)- , or -N(C(=O)R^e)-;

V_1 and V_1' are each independently -$(O\text{-}CH_2\text{-}CH_2)_{p1}$-, -$(CH_2\text{-}CH_2\text{-}O_{p1}$-, -$(NR^m\text{-}CH_2\text{-}CH_2)_{p1'}$- or -$(CH_2\text{-}CH_2\text{-}NR^m)_{p1'}$-;

R^e is H, an alkyl, an alkenyl, an alkynyl, or -$(CH_2\text{-}CH_2\text{-}O)_{n'}$-$R^k$;

R^k and R^m are each independently H or an alkyl;

R_{11} and R_{12} are each independently H or an alkyl;

n' is an integer from 1 to 24;

p1 is 0 or an integer from 1 to 1000; and

p1' is 0 or an integer from 1 to 10.

128. The compound of claim 126 or 127, wherein L_2' is represented by the following structural formula:

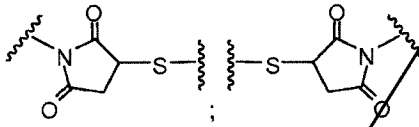
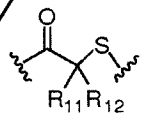
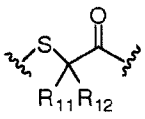
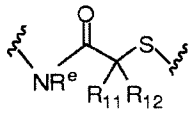
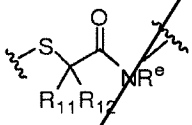
$$J_{CB'}—R^{A2}—V_2—W_2—R^{B2}—V_2'—R^{C2}—J_A—;$$

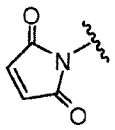
wherein:

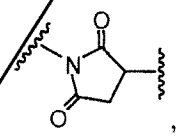
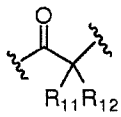
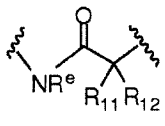
R^{A2}, R^{B2} and R^{C2} are each independently absent, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, an aryl, a heteroaryl, a heterocyclyl, a cycloalkylalkyl, an

arylalkyl, a heteroarylalkyl or a heterocyclylalkyl;

V_2 and V_2' are each independently $-(O\text{-}CH_2\text{-}CH_2)_{p2}$-, $-(CH_2\text{-}CH_2\text{-}O)_{p2}$-, $-(NR^m\text{-}CH_2\text{-}CH_2)_{p2'}$- or $-(CH_2\text{-}CH_2\text{-}NR^m)_{p2'}$-;

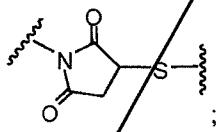
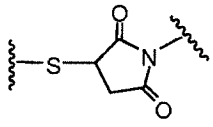
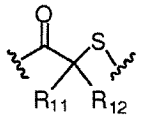
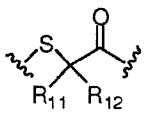
W_2 is absent, -O-, -O-C(=O)-, -C(=O)-O-, -S-, -SO-, $-SO_2$-, $-CR_{11}R_{12}$-S-, $-CR_{11}R_{12}$-O-, - $CR_{11}R_{12}$-NR^e-, -O-(C=O)O-, -O-(C=O)$N(R^e)$-, $-N(R^e)$-, $-N(R^e)$-C(=O)-, -C(=O)-$N(R^e)$-, $-N(R^e)$-C(=O)O-, $-N(C(=O)R^e)C(=O)$- , $-N(C(=O)R^e)$-, $-(O\text{-}CH_2\text{-}CH_2)_{n'}$-, -SS-, -C(=O)-, an amino acid, a peptide having 2 to 8 amino acids,  ;  ;  ;  ;  ;

J_{CB}' is –C(=O)OH, -COE, $-N(R^e)$-C(=O)-X', -O-C(=O)X', -C(=NH)X', $-NR^e$-C(=NH)X', -SH, $-SSR^d$, $-NR^eH$, $-NH\text{-}NR^eH$,  , -C(=NR^e)X', $-NR^eNH$, X'-CR_1R_2-C(=O)-, or X'-CR_1R_2-C(=O)-NR^e-, wherein COE is a reactive ester, X' is a halogen; and R^d is selected from phenyl, nitrophenyl (*e.g.*, 2 or 4-nitrophenyl), dinitrophenyl (*e.g.*, 2 or 4-nitrophenyl), carboxynitrophenyl (*e.g.*, 3-carboxy-4-nitrophenyl), pyridyl or nitropyridyl (*e.g.*, 4-nitropyridyl);

J_A is $-NR^e$-, -C(=O)-, =N-, $-NR^e$-C(=O)-, -O-C(=O)- , $-SO_2$-, -S-,  ,  ,  ;

p2 is 0 or an integer from 1 to 1000; and

p2' is 0 or an integer from 1 to 10.

129. The compound of claim 128, wherein W_2 is absent,  ;  ;  ;  ; -S-S-, -NH-C(=O)- or -C(=O)-NH-.

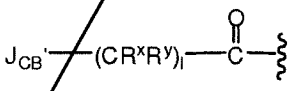
130. The compound of claim 129, wherein p2 for both V_2 and V_2' are 0.

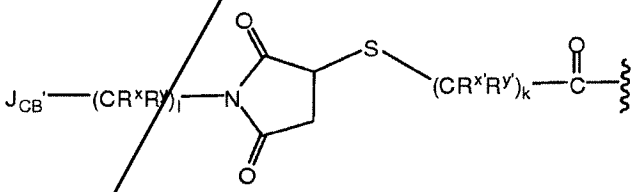
131. The compound of any one of claims 128-130, wherein R^{A2} is an alkyl.

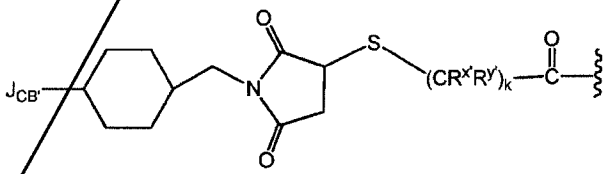
132. The compound of any one of claims 128-131, wherein one of R^{B2} and R^{C2} is absent, and the other is an alkyl.

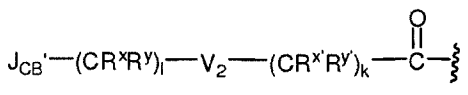
133. The compound of any one of claims 128-130, wherein R^{B2} and R^{C2} are both absent.

134. The compound of claim 128, wherein L_2' is represented by the following structural formulas:

 (L2'a);

 (L2'b);

 (L2'b'); or

 (L2'c);

wherein:

J_{CB}' is -COOH or -COE, wherein -COE is a reactive ester selected from N-hydroxysuccinimde ester, N-hydroxy sulfosuccinimide ester, nitrophenyl (*e.g.*, 2 or 4-nitrophenyl) ester, dinitrophenyl (*e.g.*, 2,4-dinitrophenyl) ester, sulfo-

tetraflurophenyl (*e.g.*, 4-sulfo-2,3,5,6-tetrafluorophenyl) ester, and pentafluorophenyl ester;

R^x, R^y, $R^{x'}$ and $R^{y'}$, for each occurrence, are independently H, -OH, halogen, -O-(C_{1-4} alkyl), $-SO_3^-$, $-NR_{40}R_{41}R_{42}^+$, or a C_{1-4} alkyl optionally substituted with -OH, halogen, SO_3^- or $NR_{40}R_{41}R_{42}^+$, wherein R_{40}, R_{41} and R_{42} are each independently H or a C_{1-4} alkyl;

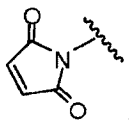
V_2 is $-(CH_2-CH_2-O)_{p2}-$ or $-(O-CH_2-CH_2)_{p2}-$;

l and k are each independently 0 or an integer from 1 to 10; and,

p2 is an integer from 1 to 24.

135. The compound of claim 134, wherein R^x, R^y, $R^{x'}$ and $R^{y'}$ are all H.

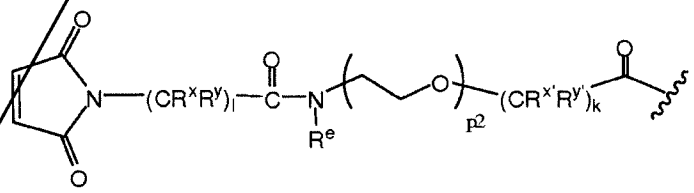
136. The compound of claim 135, wherein p2 is an integer from 2 to 24; l and k are each independently an integer from 1 to 6.

137. The compound of claim 128, wherein J_{CB}' is , X'-CH_2-C(=O)- or X'-CH_2-C(=O)-NH-.

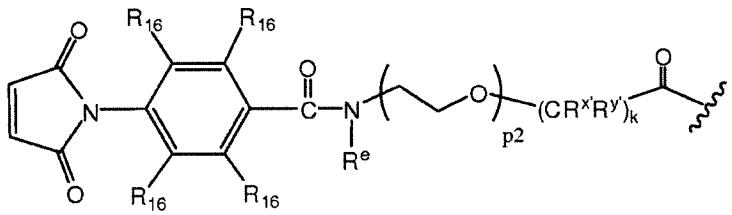
138. The compound of claim 137, wherein V_2 and V_2' are both $-(O-CH_2-CH_2)_{p2}-$ or $-(CH_2-CH_2-O)_{p2}-$.

139. The compound of claim 138, wherein p2 for one of V_2 and V_2' is 0 and the other is an integer from 2 to 24.

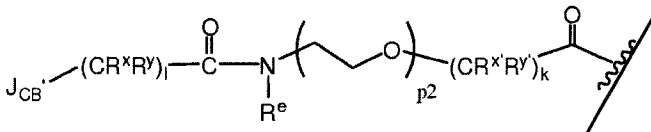
140. The compound of claim 128, wherein L_2' is represented by:



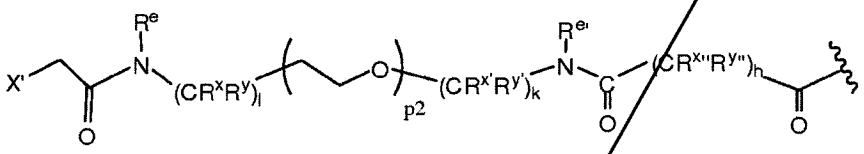
(L2'd);



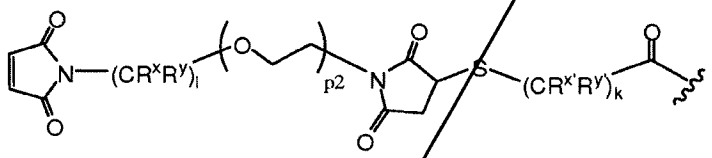
(L2'e);



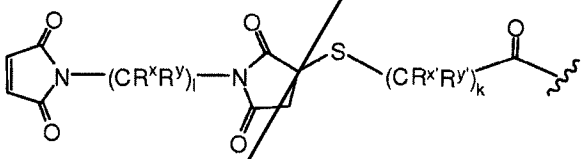
(L2'f);



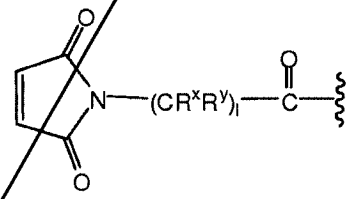
(L2'g);



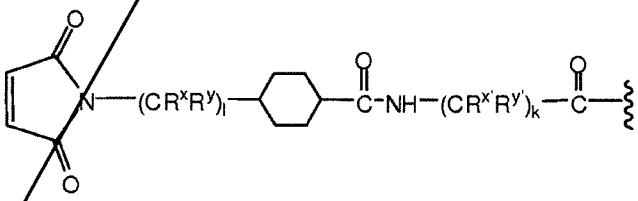
(L2'h);



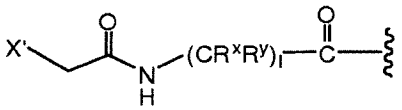
(L2'i);



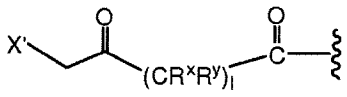
(L2'j);



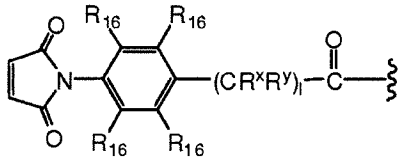
(L2'k);



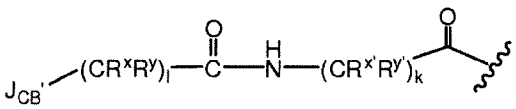
(L2'l);



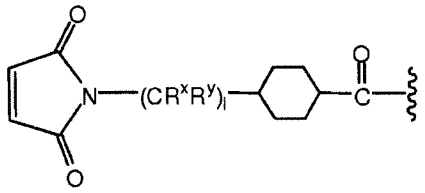
(L2'm);



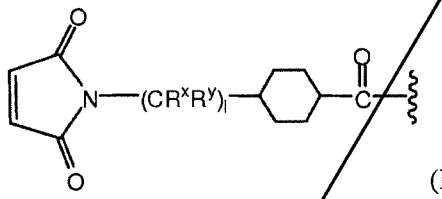
(L2'n);



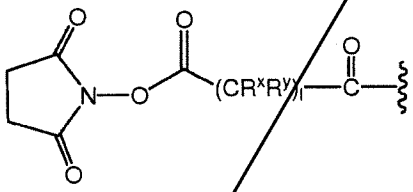
(L2'o);



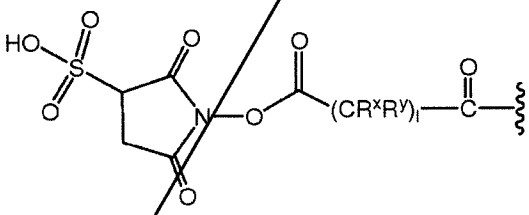
(L2'p);



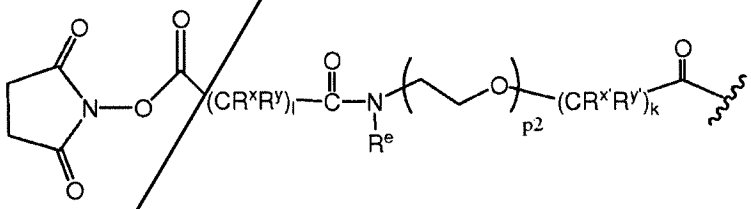
(L2'p);



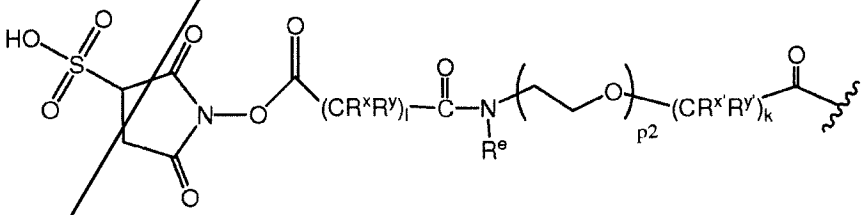
(L2'q);



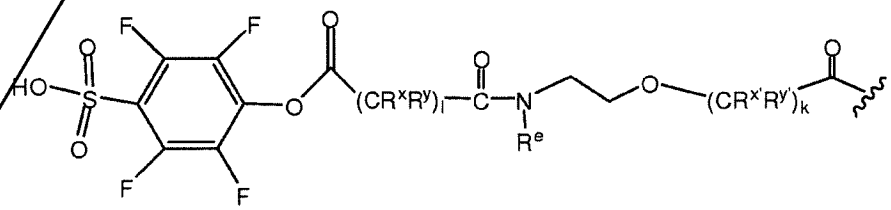
(L2'r);



(L2's);



(L2't); or



(L2'u);

wherein:

J_{CB}' is -SH or -SSR^d,

R^x, R^y, $R^{x'}$, $R^{y'}$, $R^{x''}$ and $R^{y''}$, for each occurrence, are each independently H, -OH, halogen, -O-(C_{1-4} alkyl), $-SO_3^-$, $-NR_{40}R_{41}R_{42}^+$, or a C_{1-4} alkyl optionally substituted with -OH, halogen, SO_3^- or $NR_{40}R_{41}R_{42}^+$, wherein R_{40}, R_{41} and R_{42} are each independently H or a C_{1-4} alkyl;

R_{16}, for each occurrence, is independently H, an alkyl, halogen, -OH, -O-alkyl, $-NO_2$, or -CN; and,

l, k and h are each independently an integer from 1 to 10.

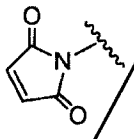
141. The compound of claim 140, wherein R_{16} is H.

142. The compound of claim 140 or 141, wherein R^x, R^y, $R^{x'}$, $R^{y'}$, $R^{x''}$ and $R^{y''}$ are all H.

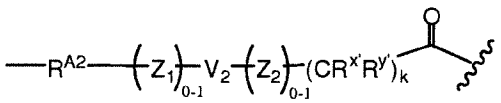
143. The compound of claim 142, wherein p2 for each occurrence is independently an integer from 2 to 24.

144. The compound of claim 143, wherein l, k and h are each independently an integer from 1 to 6.

145. The compound of claim 128, wherein:

$J_{CB'}$ is , -C(=O)OH, -COE, -SH, $-SSR^d$, X'-CR_1R_2-C(=O)-, or X'-CR_1R_2-C(=O)-NR^e-, wherein COE is a reactive ester, X' is a halogen; and R^d is selected from phenyl, nitrophenyl (*e.g.*, 2 or 4-nitrophenyl), dinitrophenyl (*e.g.*, 2 or 4-nitrophenyl), carboxynitrophenyl (*e.g.*, 3-carboxy-4-nitrophenyl), pyridyl or nitropyridyl (*e.g.*, 4-nitropyridyl);

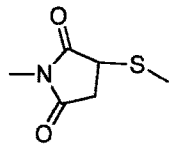
$-R^{A2}-V_2-W_2-R^{B2}-V_{2'}-R^{C2}-J_A-$ is:

; wherein

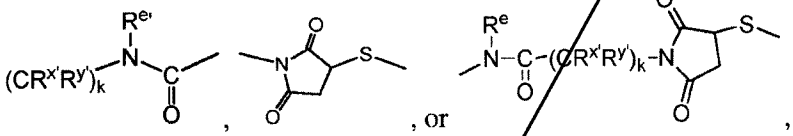
R^{A2} is $-(CR^xR^y)_l-$ or -Cy-$(CR^xR^y)_l-$;

Cy is a cycloalkyl (*e.g.*, cyclohexyl);

V_2 is $-(CH_2-CH_2-O)_{p2}-$ or $-(O-CH_2-CH_2)_{p2}-$;

Z_1 is -C(=O)-N(R^e)-, -N(R^e)-C(=O)-, or , provided that Z_1-

V_2 does not contain an N-O bond;

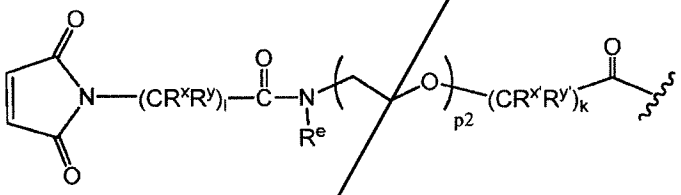
Z_2 is , provided that V_2-Z_2 does not contain an O-N bond;

l is an integer from 0 to 10;

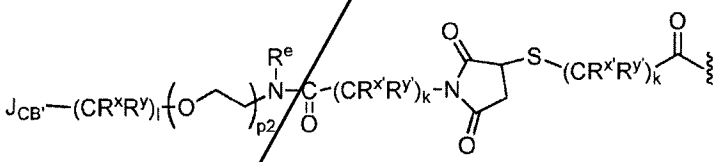
p2 is an integer from 0 to 200;

Z is -NH-.

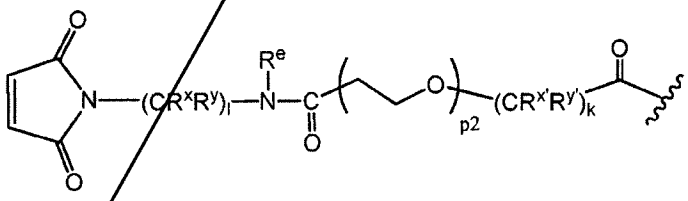
146. The compound of claim 145, wherein L_2' is represented by:



(L2'd);



(L2'r); or



(L2's);

wherein:

p2 is an integer from 0 to 200;

J_{CB}' is -COOH or -COE, wherein -COE is a reactive ester selected from N-hydroxysuccinimde ester, N-hydroxy sulfosuccinimide ester, nitrophenyl (*e.g.*, 2 or 4-nitrophenyl) ester, dinitrophenyl (*e.g.*, 2,4-dinitrophenyl) ester, sulfo-tetraflurophenyl (*e.g.*, 4-sulfo-2,3,5,6-tetrafluorophenyl) ester, and pentafluorophenyl ester;

R^x, R^y, $R^{x'}$, and $R^{y'}$, for each occurrence, are each independently H, -OH, halogen, -O-(C_{1-4} alkyl), $-SO_3^-$, $-NR_{40}R_{41}R_{42}^+$, or a C_{1-4} alkyl optionally substituted with -OH, halogen, SO_3^- or $NR_{40}R_{41}R_{42}^+$, wherein R_{40}, R_{41} and R_{42}

are each independently H or a C_{1-4} alkyl;

l and k, for each occurrence, are independently an integer from 1 to 10.

147. The compound of claim 145 or 146, wherein A is Val-Cit or Gly-Gly-Gly.

148. The compound of any one of claims 145-147, wherein X is phenyl.

149. The compound of any one of claims 126-148, wherein A is a peptide cleavable by a protease.

150. The compound of claim 149, wherein A is a peptide cleavable by a protease expressed in tumor tissue.

151. The compound of claim 149, wherein A is a peptide cleavable by a lysosomal protease.

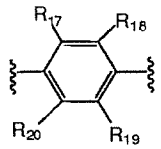
152. The compound of any one of claims 126-148, wherein A is a peptide having an amino acid that is covalent linked with –NH-X-L_1-D selected from the group consisting of Ala, Arg, Asn, Asp, Cit, Cys, selino-Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr and Val, each independently as L or D isomers.

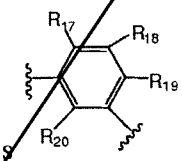
153. The compound of any one of claims 126-148, wherein A is selected from the group consisting of Val-Cit, Val-Lys, Phe-Lys, Lys-Lys, Ala-Lys, Phe-Cit, Leu-Cit, Ile-Cit, Trp, Cit, Phe-Ala, Phe-N^9-tosyl-Arg, Phe-N^9-nitro-Arg, Phe-Phe-Lys, D-Phe-Phe-Lys, Gly-Phe-Lys, Leu-Ala-Leu, Ile-Ala-Leu, Val-Ala-Val, Ala-Leu-Ala-Leu (SEQ ID NO: 1), β-Ala-Leu-Ala-Leu (SEQ ID NO: 2) and Gly-Phe-Leu-Gly (SEQ ID NO: 3), 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, D-Ala-Ala, Ala-D-Ala, and D-Ala-D-Ala.

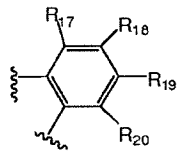
154. The compound of claim 153, wherein A is Val-Cit, Val-Lys, Val-D-Cit or Val-D-Lys.

155. The compound of any one of claims 126-148, wherein A is Cys or a peptide comprising a Cys that is covalently linked to L_2, wherein the Cys is linked to L_2 through the –SH group of Cys side chain.

156. The compound of any one of claims 126-155, wherein X is an optionally substituted phenyl.

157. The compound of claim 156, wherein X is , wherein R_{17}, R_{18}, R_{19} and R_{20} each are independently H, an alkyl, halogen, -OH, -O-alkyl, -C(=O)OR_{30}, -C(=O)R_{30}, -NO_2, or –CN, wherein R_{30} is H or an alkyl.

158. The compound of claim 156, wherein X is , wherein R_{17}, R_{18}, R_{19} and R_{20} each are independently H, an alkyl, halogen, -OH, -O-alkyl, -C(=O)OR_{30}, -C(=O)R_{30}, -NO_2, or –CN, wherein R_{30} is H or an alkyl.

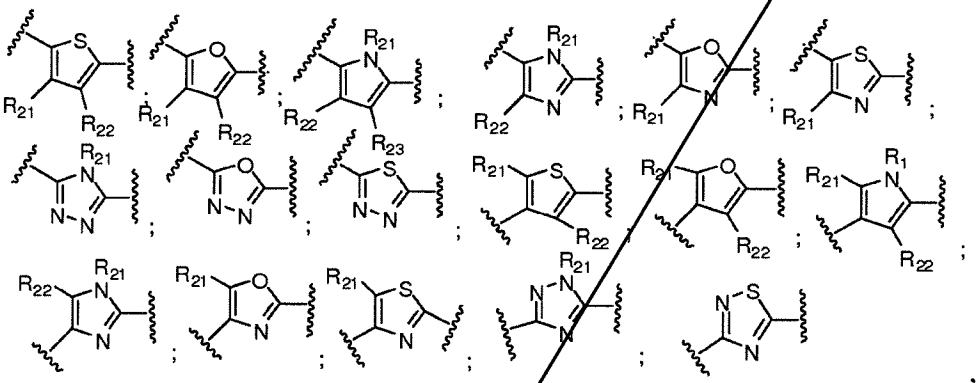
159. The compound of claim 156, wherein X is , wherein R_{17}, R_{18}, R_{19} and R_{20} each are independently H, an alkyl, halogen, -OH, -O-alkyl, -C(=O)OR_{30}, -C(=O)R_{30}, -NO_2, or –CN, wherein R_{30} is H or an alkyl.

160. The compound of any one of claims 157-159, wherein R_{17}, R_{18}, R_{19} and R_{20} are all H.

161. The compound of claim 126-155, wherein X is a six-membered heteroaryl selected from the group consisting of pyridine, pyridazine, pyrimidine, pyrazine, a triazine, and tetrazine.

162. The compound of claim 126-155, wherein X is a six-membered heteroaryl selected from the group consisting of pyridine, pyridazine, pyrimidine, pyrazine, a triazine, and tetrazine, wherein the heteroaryl is connected to the –NH group and -L_1 group at carbon atoms of the heteroaryl.

163. The compound of claims 126-155, wherein X is selected from the group consisting of:



wherein R_{21}, R_{22} and R_{23} are each independently H or an alkyl.

164. The compound of any one of claims 126-163, wherein L_1 is represented by:

$$-(CR_9R_{10})_m-W_1-R^{B1}-J_D-$$

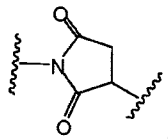
wherein:

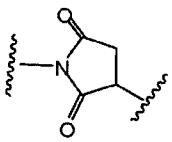
R_9 and R_{10} are each independently H or a C_{1-4} alkyl;

m is an integer from 1 to 10;

W_1 is absent, -NH-C(=O)-, -C(=O)-NH-, -C(=O)-O- or –O-C(=O)-, provided when m is 1, then W_1 is not –O-C(=O)-;

R^{B1} is absent or a C_{1-10} alkyl; and

J_D is absent, -C(=O)-, , -NH-C(=O)-CH_2-, or -S-.

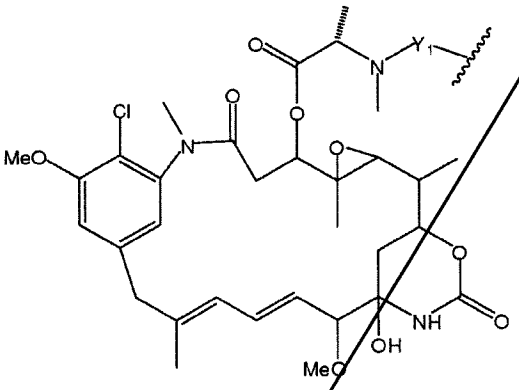
165. The compound of claim 164, wherein J_D is absent, -C(=O)- or .

166. The compound of claim 164 or 165, wherein R_9 and R_{10} are both H; and m is 1, 2 or 3.

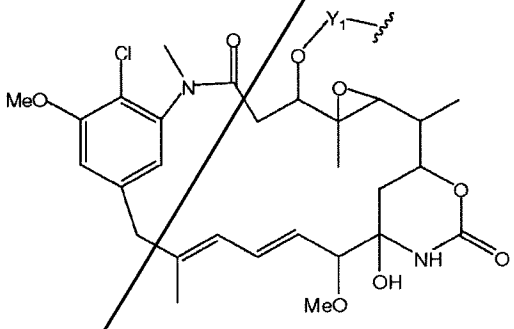
167. The compound of any one of claims 164-166, wherein W_1 is absent or -NH-C(=O)- and R^{B1} is absent or -$(CH_2)_{m'}$-, wherein m' is 1, 2, or 3.

168. The compound of any one of claims 126-167, wherein D is a maytansinoid.

169. The compound of claim 168, wherein D is represented by the following structural formula:



(A), or



(B),

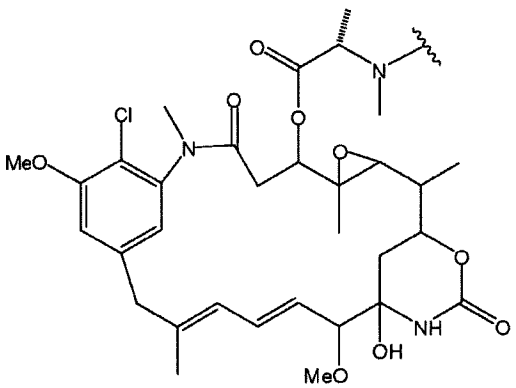
wherein:

Y_1 is absent or $-C(=O)(CR_7R_8)_{l1}(CR_5R_6)_{m1}(CR_3R_4)_{n1}CR_1R_2S-$,

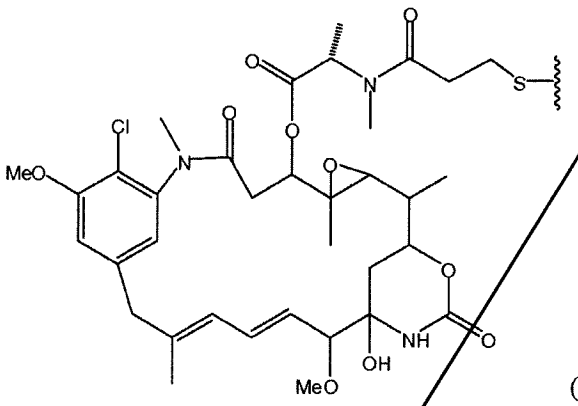
R_1, R_2, R_3, R_4, R_5, R_6, R_7 and R_8 are each independently H, an alkyl, an alkenyl, a cycloalkyl, a heteroaryl, a heterocyclyl, or an aryl;

l1, m1 and n1 are each independently 0 or an integer from 1 to 5.

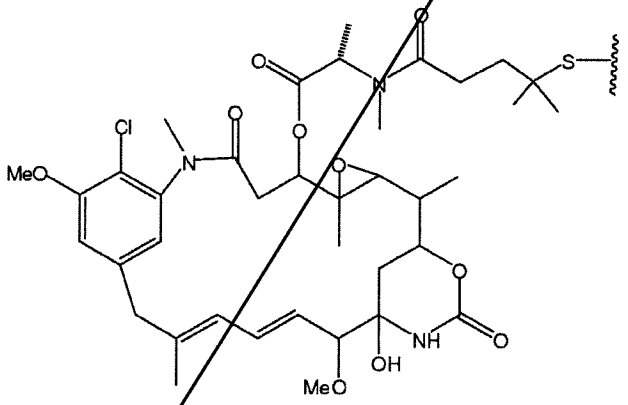
170. The compound of claim 169, wherein D is represented by any one of the following structural formulas:



(A1);

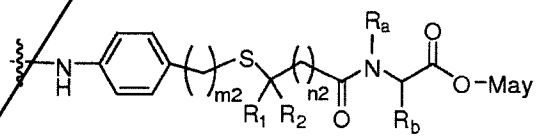


(A2); or

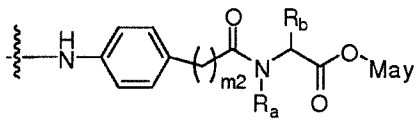


(A3).

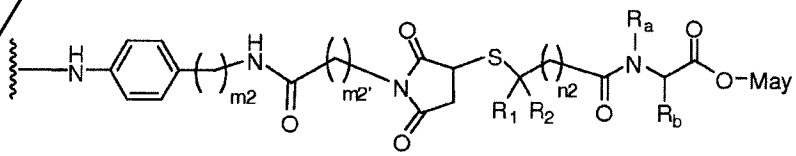
171. The compound of any one of claims 126-155, wherein -Z-X-L_1-D is represented by one of the following structural formulas:



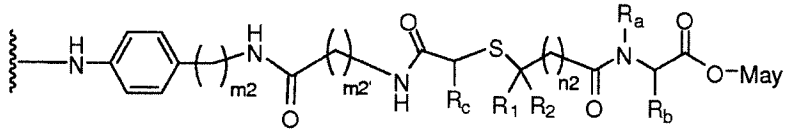
(L1a);



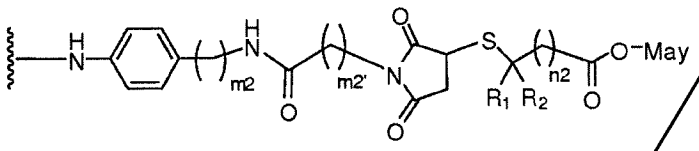
(L1b);



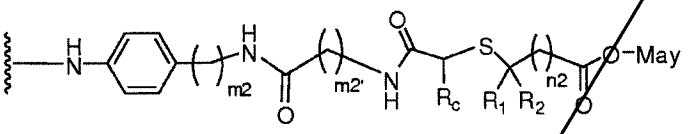
(L1c);



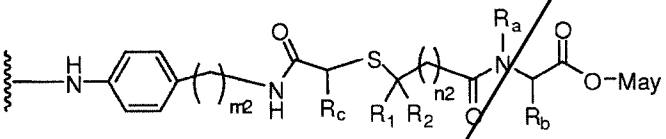
(L1d);



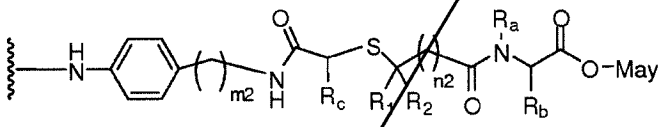
(L1e);



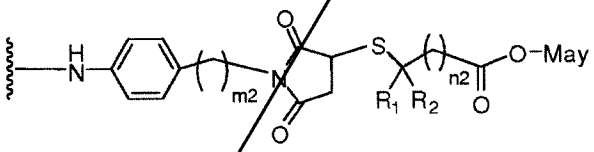
(L1f);



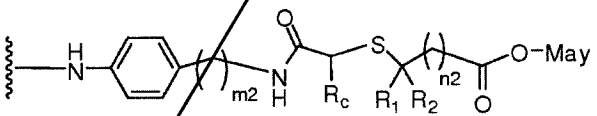
(L1g);



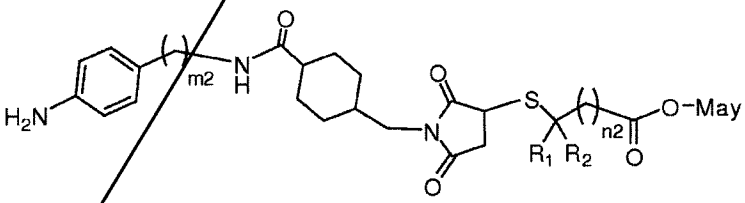
(L1h);



(L1i);

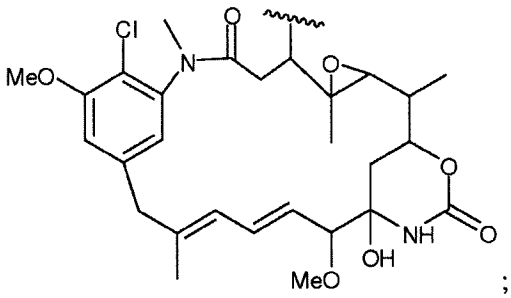


(L1j); or



(L1k)

wherein May is represented by the following structural formula:



;

R_1, R_2, R_a, R_b and R_c are each independently H or a C_{1-4} alkyl; and

m2, m2' and n2 are each independently an integer from 1 to 10.

172. The compound of claim 171, wherein R_a and R_b are both methyl; and R_c is H.

173. The compound of claim 172, wherein R_1 and R_2 are both H and n is 1.

174. The compound of claim 172, wherein R_1 and R_2 are both methyl and n is 2.

175. The compound of any one of claims 171-174, wherein m2 and m2' are each independently an integer from 1 to 3.

176. A pharmaceutical composition comprising the conjugate of any one of claims 1-55 and a pharmaceutically acceptable carrier.

177. A method of inhibiting abnormal cell growth or treating a proliferative disorder, an autoimmune disorder, destructive bone disorder, infectious disease, viral disease, fibrotic disease, neurodegenerative disorder, pancreatitis or kidney disease in a mammal comprising administering to said mammal a therapeutically effective amount of the conjugate of any one of claims 1-55 and, optionally, a chemotherapeutic agent.

178. The method of claim 177, wherein said second chemotherapeutic agent is administered to said mammal sequentially or consecutively.

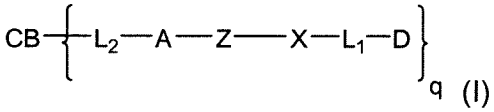
179. The method of claim 177, wherein the method is for treating a condition selected from cancer, rheumatoid arthritis, multiple sclerosis, graft versus host disease (GVHD), transplant rejection, lupus, myositis, infection, and immune deficiency.

180. The method of claim 177, wherein the method is for treating a cancer.

181. The method of claim 177, wherein the cancer is selected from breast cancer, colon cancer, brain cancer, prostate cancer, kidney cancer, pancreatic cancer, ovarian cancer, head and neck cancer, melanoma, colorectal cancer, gastric cancer, squamous cancer, small-cell lung cancer, non small-cell lung cancer, testicular cancer, Merkel cell carcinoma, glioblastoma, neuroblastoma, and cancers of lymphatic organs.

CLAIMS

1. A cell-binding agent-cytotoxic agent conjugate represented by the following structural formula:



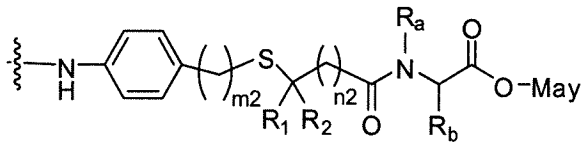
(I)

or a pharmaceutically acceptable salt thereof, wherein:

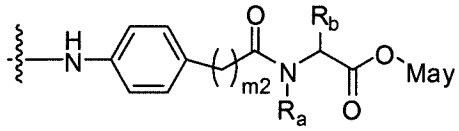
CB is an antibody and comprises a -NH- group that is covalently linked to L_2;

A is a peptide comprising 2 to 20 amino acids that is cleavable by a protease expressed in tumor tissue;

-Z-X-L_1-D is represented by the following structural formula:



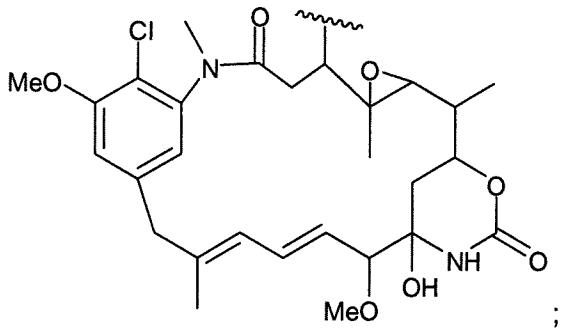
(L1a); or



(L1b);

wherein:

May is represented by the following structural formula:

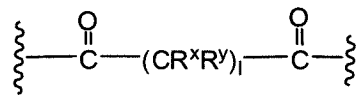


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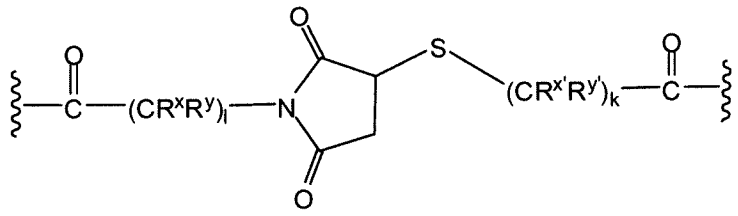
R_1, R_2, R_a, and R_b are each independently H or a C_{1-4} alkyl; and

m2 and n2 are each independently an integer from 1 to 10;

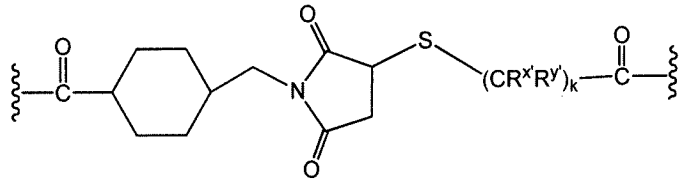
L_2 is represented by the following structural formula:



(L2a);



(L2b); or



(L2b');

wherein:

R^x, R^y, $R^{x'}$ and $R^{y'}$ are all H; and

l and k are each independently an integer from 1 to 6; and

q is an integer from 1 to 20.

2. The conjugate of claim 1, wherein A is selected from the group consisting of Val-Cit, Val-Lys, Phe-Lys, Lys-Lys, Ala-Lys, Phe-Cit, Leu-Cit, Ile-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) and Gly-Phe-Leu-Gly (SEQ ID NO: 3), Val-Arg, Arg-Val, Arg-Arg, Val-D-Cit, Val-D-Lys, Val-D-Arg, D-Val-Cit, D-Val-Lys, D-Val-Arg, Ala-Ala, Ala-D-Ala, and D-Ala-Ala.

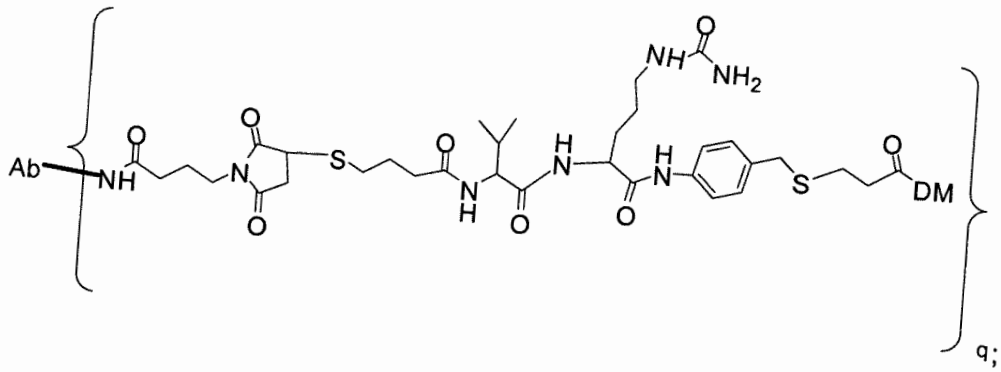
3. The conjugate of claim 1, wherein R_a and R_b are both methyl;

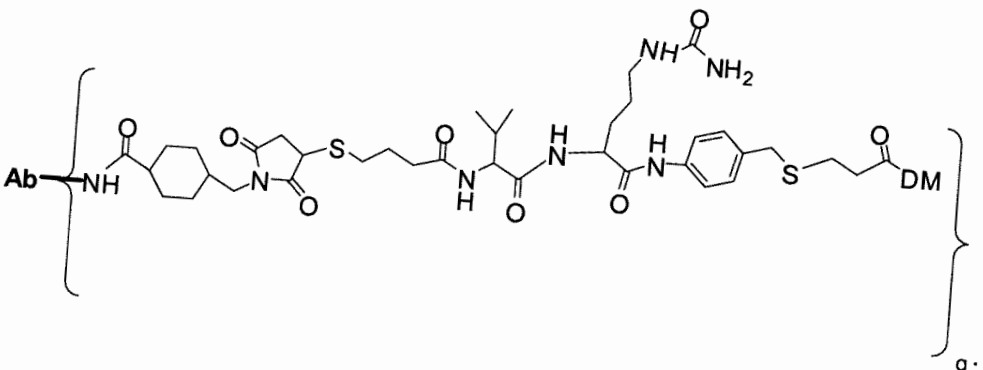
R_1 and R_2 are both H and n2 is 1; or

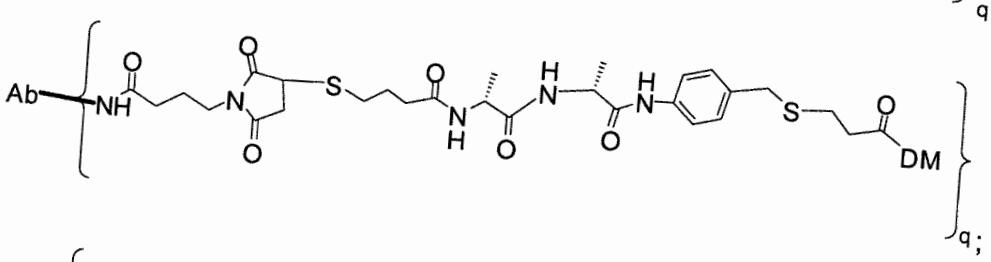
R_1 and R_2 are both methyl and n2 is 2, and m2 is an integer from 1 to 3.

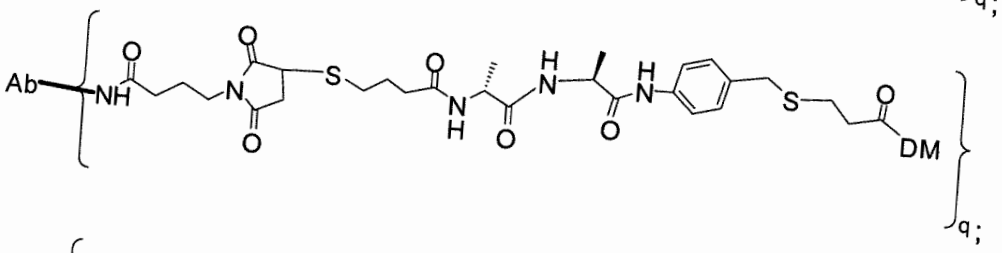
4. A pharmaceutical composition comprising the conjugate of claim 1 and a pharmaceutically acceptable carrier.

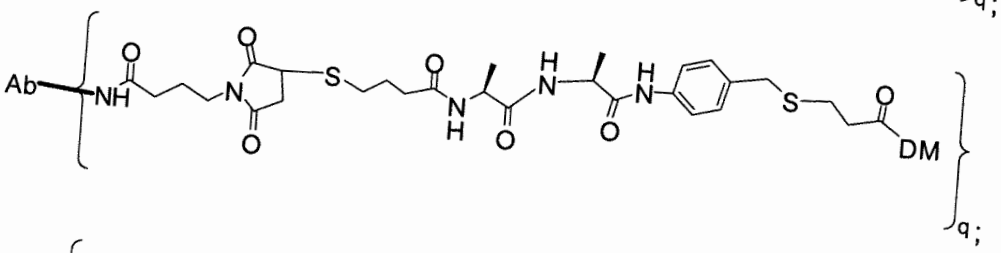
5. The conjugate of claim 1, wherein the conjugate is represented by the following structural formula:

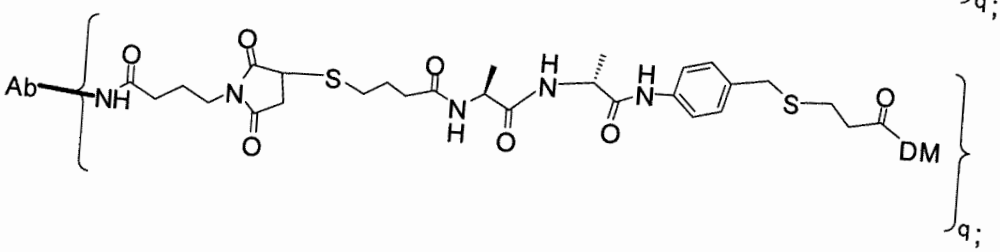


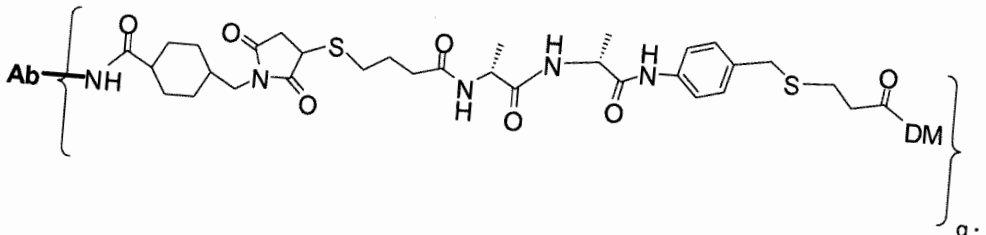


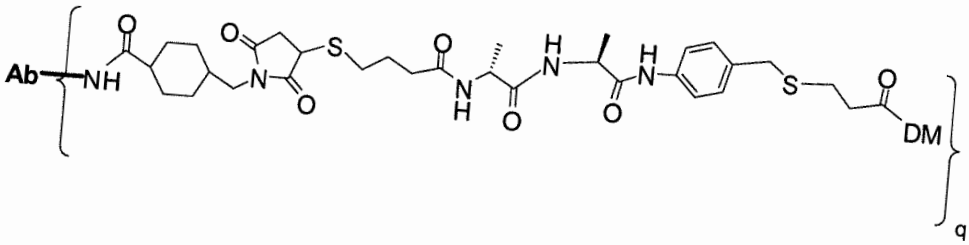


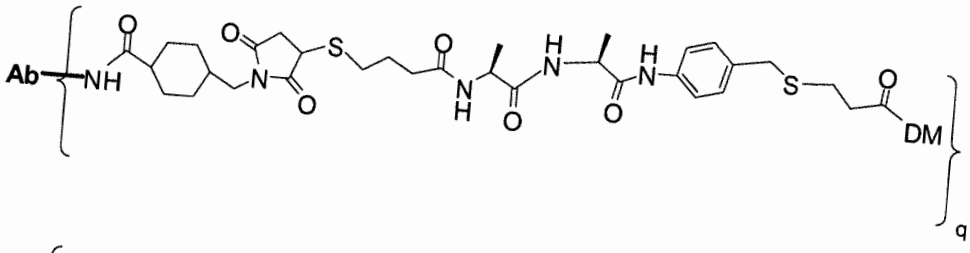


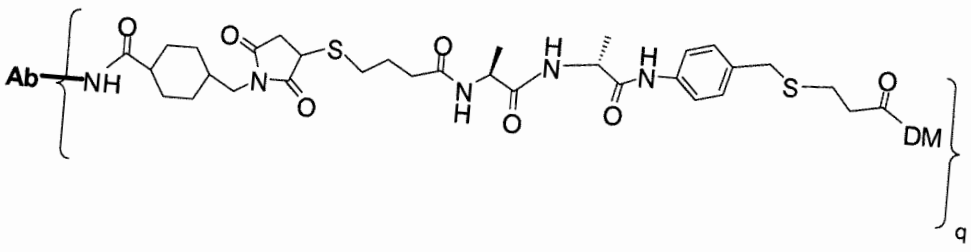


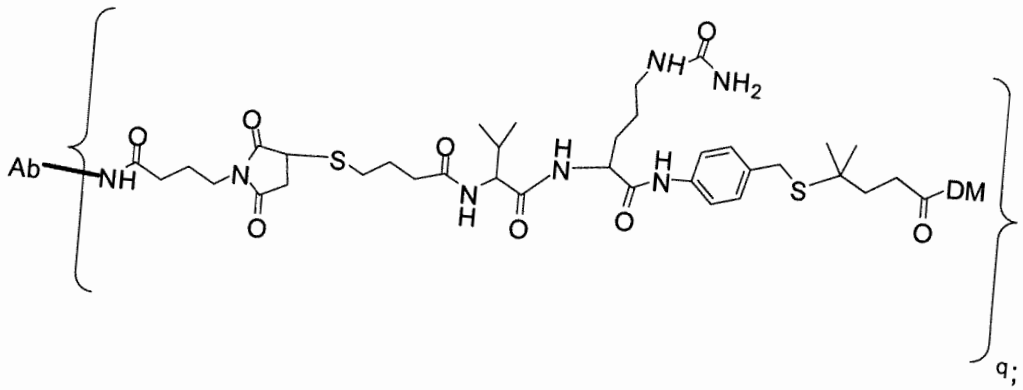


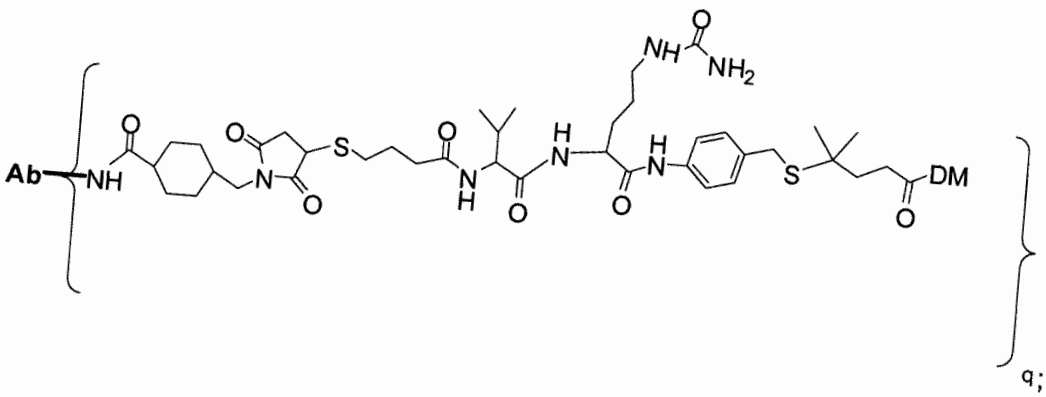

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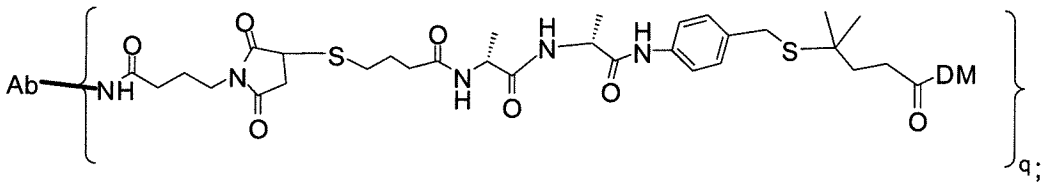

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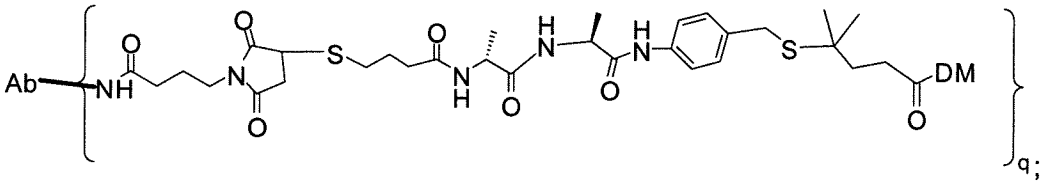

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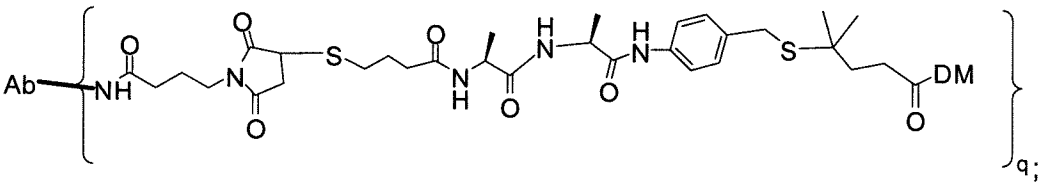

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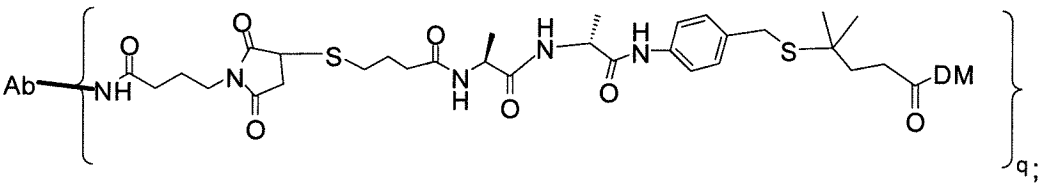

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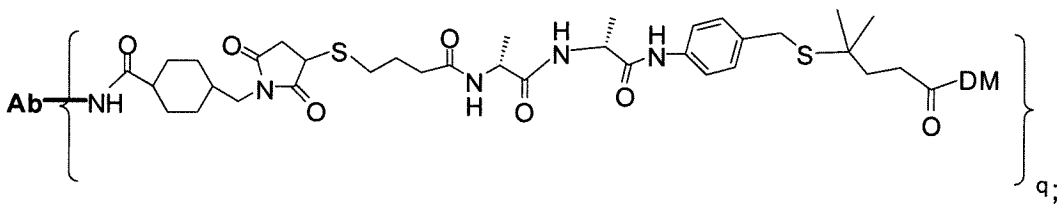

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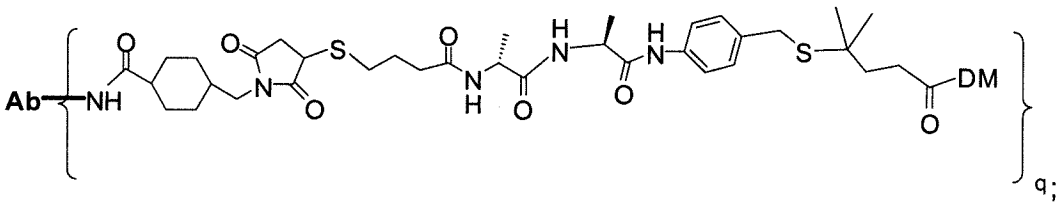

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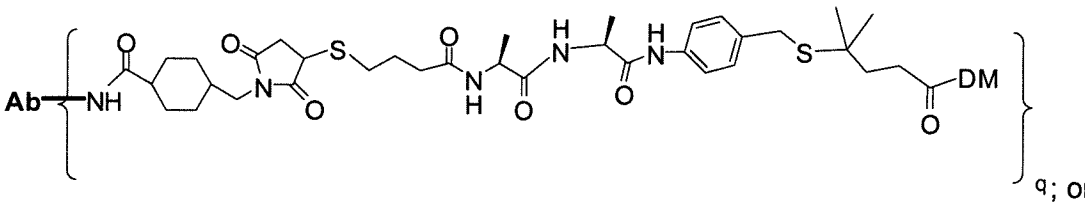

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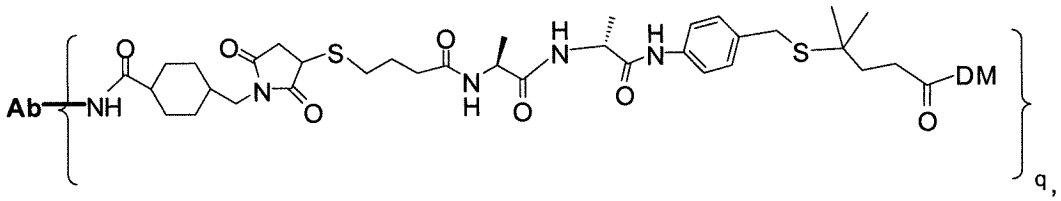

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wherein DM is represented by the following structural formula:

