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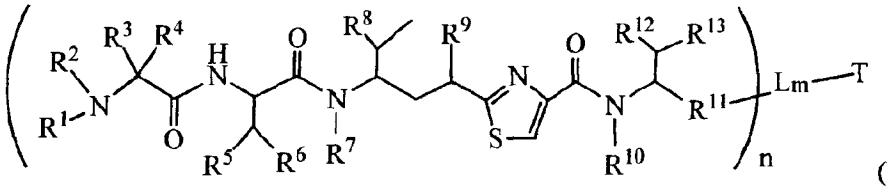
(54) Titre : CONJUGUES DE MOLECULE DE LIAISON CELLULAIRE ET D'AGENTS CYTOTOXIQUES
(54) Title: CONJUGATES OF CELL BINDING MOLECULES WITH CYTOTOXIC AGENTS



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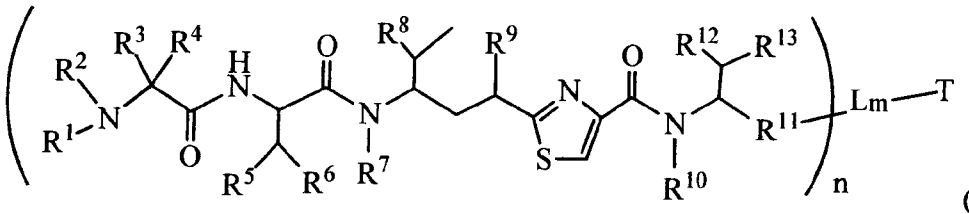
(57) Abrégé/Abstract:
A conjugate of a potent cytotoxic agent with a cell-surface receptor binding molecule having a structure represented by Formula (V), wherein T, L, m, n,-----, R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{12}, and R^{13} are defined herein, can be used for targeted treatment of cancer, autoimmune disease, and infectious disease.

(see formula V)

ABSTRACT

A conjugate of a potent cytotoxic agent with a cell-surface receptor binding molecule having a structure represented by Formula (V), wherein T, L, m, n,-----, R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{12}, and R^{13} are defined herein, can be used for targeted treatment of cancer, autoimmune disease, and infectious disease.



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CONJUGATES OF CELL BINDING MOLECULES WITH CYTOTOXIC AGENTS

This is a divisional application of Canadian Patent Application Serial No. 2,878,733 filed on July 12, 2012.

1. FIELD OF THE INVENTION

This invention relates to a conjugate of a potent cytotoxic agent with a cell-surface receptor binding molecule for targeted therapy. The invention also relates to use of the compositions comprising the cell-surface receptor binding molecule-antimitotic agent conjugates for treating cancer, autoimmune disease, and infectious disease.

It should be understood that the expression "the invention" and the like used herein may refer to subject matter claimed in either the parent or the divisional applications.

2. BACKGROUND OF THE INVENTION

There are many articles appeared on the attempted specific targeting of pathogenic cells, in particular, cancer cells utilizing cytotoxic drug conjugated to cell-surface receptor binding agents, such as antibodies (Sela et al, in *Immunoconjugates* 189-216 (C. Vogel, ed. 1987); Ghose et al, in *Targeted Drugs* 1-22 (E. Goldberg, ed. 1983); Diener et al, in *Antibody mediated delivery systems* 1-23 (J. Rodwell, ed. 1988); Silverstein, *Nat. Immunol.* 2004, 5, 1211–7; Fanning et al, *Clin. Immunol. Immunopathol.* 1996, 79, 1–14; Ricart A.D., et al., *Nature Clinical Practice Oncology* 2007, *4*, 245-255 ; Singh R. et Rickson H.K., *Therapeutic Antibodies : Methods and Protocols*, 2009, 525, 445-467), **folic acids** (Sudimack, J. et al, Adv. Drug Delivery Rev. 2000, 41, 147-162; Reddy, et al, Mol. Pharm. 2009, 6, 1518-25); **PMSA** (prostate specific membrane antigen) **binding ligands** (Low, et al, WO 2009/026177 A1); **albumin** with peptides (Temming, et al, Bioconjugate Chem. 2006, 17, 1385-1394); **cobalamin** and **proteins** (Gupta, et al, Crit. Rev. Therap. Drug Carrier Syst. 2008, 25, 347-79; Petrus, et al, Angew. Chem. Int. Ed. 2009, 48, 1022-8); carbohydrate (Darbre, et al, Curr. Top. Med. Chem.. 2008, 8, 1286-93); **bioactive polymers** (Dhar, et al, Proc. Natl. Acad. Sci. 2008, 105, 17356-61); **dendrimers** (Lee, et al, Nat. Biotechnol. 2005, 23, 1517-26; Almutairi, et al; Proc. Natl. Acad. Sci. 2009, 106, 685-90); **nanoparticles** with binding ligands (Liong, et al, ACS Nano, 2008, 19, 1309-12; Medarova, et al, Nat. Med. 2007, 13, 372-7; Javier, et al, Bioconjugate Chem. 2008, 19, 1309-12); **liposomes** (Medinai, et al, Curr. Phar. Des. 2004, 10, 2981-9); **viral capsides** (Flenniken, et al, Viruses Nanotechnol. 2009, 327, 71-93), etc.

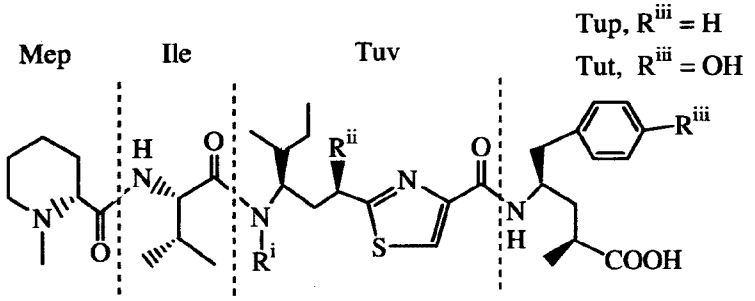
Different families of cytotoxic agents like calicheamicin derivative (Giles, et al *Cancer* 2003, 98, 2095-104; Hamann, et al, *Bioconjug Chem* 2002, 13, 47-58), maytansin derivative (Widdison, et al, *J Med Chem* 2006, 49, 4392-408; Ikeda, et al, *Clin Cancer Res* 2009, 15, 4028-

37; Xie, et al, *Expert Opin Biol Ther* 2006, 6, 281-91), auristatins (Sutherland, et al, *J Biol Chem* 2006, 281, 10540-7; Doronina, et al, *Bioconjug Chem* 2006, 17, 114-24), taxane derivatives (Miller, et al, *J Med Chem* 2004, 47, 4802-5; WO 06061258), leptomycine derivatives (WO 07144709), CC-1065 and analogues (Suzawa, et al, *J Control Release* 2002, 79, 229-42; Suzawa, et al, *Bioorg Med Chem* 2000, 8, 2175-84; WO 2007102069), doxorubicin (Trail, et al, *Science*

1993, 261, 212-5; Saleh et al, *J Clin Oncol* 2000, 18, 2282-92), daunorubicin, vincristine, vinblastine, mitomycin C, or chlorambucil have been used for the conjugation with a cell-surface receptor binding agent, in particular with antibodies (Wu, et al, *Nat. Biotechnol. 2005, 23,* 1137–1146. Ricart, et al, *Nat. Clin. Pract. Oncol. 2007, 4,* 245–255).

The use of a cell-surface receptor binding agent, particularly a targeting antibody having an affinity for the pathogenic cells makes it possible to deliver the cytotoxic agent directly in the vicinity or directly in the pathogenic cell, thus increasing the efficiency of the cytotoxic agent while minimizing the side-effects commonly associated with the cytotoxic agents.

Several short peptidic compounds that found to have biological activity have been isolated from natural sources. One of them, Tubulysins (structures shown below), which were original isolated by Hofle and Reichenbach et al. (GBF Braunschweig) from a culture browth of the



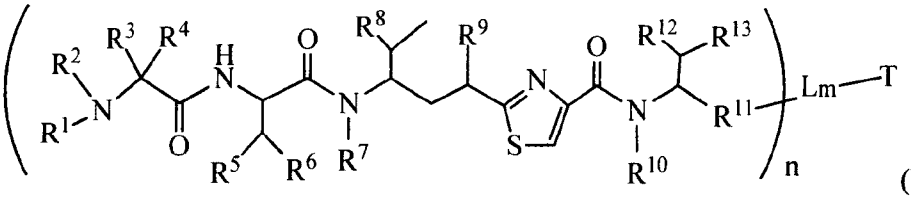
Tubulysin	R^i	R^{ii}	R^{iii}
A	$CH_2OCOCH_2CH(CH_3)_2$	$OCOCH_3$	OH
B	$CH_2OCOCH_2CH_2CH_3$	$OCOCH_3$	OH
C	$CH_2OCOCH_2CH_3$	$OCOCH_3$	OH
D	$CH_2OCOCH_2CH(CH_3)_2$	$OCOCH_3$	H
E	$CH_2OCOCH_2CH_2CH_3$	$OCOCH_3$	H
F	$CH_2OCOCH_2CH_3$	$OCOCH_3$	H
G	$CH_2OCOCH=CH_2$	$OCOCH_3$	OH
H	CH_2OCOCH_3	$OCOCH_3$	H
I	CH_2OCOCH_3	$OCOCH_3$	OH
U	H	$OCOCH_3$	H
V	H	OH	H
Z	H	OH	OH
Pretubulysin	CH_3	H	H

(The structures of existing tubulysin compounds)

myxobacterial strains of Archangium gephyra (F. Sasse et al. J. Antibiot. 2000, 53, 879-885; WO9813375), are members of group of antimitotic peptides that inhibit tubulin polymerization in dividing cells, and thus inducing apoptosis. With the exceptional potency exceeding that of vinblastine, taxol and epothilones (Wipf, et al, *Org. Lett.* 2004, *6*, 4057–60; Peltier, et al, J. Am. Chem. Soc. 2006, 128, 16018-9; Wipf, et al, *Org. Lett.*, 2007, *9*, 1605–1607; Wang, et al, Chem. Biol. Drug Des. 2007, 70, 75-86; Pando, et al, Org. Lett. 2009, 11, 5567-9), these antimitotic peptides are exciting leads for targeted therapies. Structurally, the tetrapeptide tubulysins comprise of *N*-methylpipecolinic acid (Mep) at the N-terminus, isoleucine (Ile) as the second residue, the unique thiazole-containing tubuvaline (Tuv) as the third residue, and two possible γ-amino acids at the C-terminus (tubutyrosine (Tut) or tubuphenylalanine (Tup)). Despite several tubulysins have recently been synthesized, significant general toxicities (>15% animal body weight loss) of the existing tubulysins at doses required for achieving a therapeutic effect compromise their efficacy (US Patent appl. 2010/0048490). We have been interested in the art of a conjugate of a cell surface binding ligand, particularly using an antibody to conjugate with tubulysin derivatives for having significantly lower general toxicity, yet useful therapeutic efficiency. However, the tubulysins are hardly soluble in a buffer solution, resulting in significant amount of antibody aggregation when conjugated with the tubulysins. A simpler analog, such as using phenyl alanine (Phe) and tyrosine (Tyr) to replace Tup and Tut components respectively for the antibody conjugation leads to the hydrolysis of Phe and Tyr in the animal blood circulation and generates the much less potent Mep-Ile-Tuv moiety (over 200 fold less potency than tubulysin A and D). Here this patent discloses conjugates of a cell surface binding ligand with water soluble and stable, as well as in lower systematical toxicity, tubulysin derivatives and using these conjugates for treating cancer and immune disorders.

3. SUMMARY OF THE INVENTION

In one illustrative embodiment of the invention, there is provided a conjugate of a cell binding molecule with cytotoxic agents having a structure of Formula (V):



(V)

and pharmaceutical acceptable salts and solvates thereof,

wherein T is a targeting or binding ligand; L is a releasable linker that connects to a molecule inside the bracket independently; n is 1-20 and m is 1-10;

the portion inside the round bracket is a potent antimitotic agent wherein R^1, R^2, R^3, and R^4 are independently H, a C_1-C_8 alkyl or heteroalkyl; a C_2-C_8 heterocyclic, carbocyclic, cycloalkyl, alkylcycloalkyl, or heterocycloalkyl; a C_3-C_8 of aryl, Ar-alkyl, heteroalkylcycloalkyl, or alkylcarbonyl; or two R's, that include R^1R^2, R^2R^3, R^3R^4, R^5R^6, and $R^{12}R^{13}$ are 3-7 members of a carbocyclic, cycloalkyl, heterocyclic, or heterocycloalkyl ring system;

wherein R^5, R^6, R^8 and R^{10} are independently H, a C_1-C_4 alkyl or heteroalkyl;

wherein R^7 is independently H, R^{14}, $-R^{14}C(=O)X^1R^{15}$ or $-R^{14}X^1R^{15}$, wherein R^{14} and R^{15} are independently a C_1-C_8 alkyl or heteroalkyl; a C_2-C_8 alkenyl, alkynyl, heterocyclic, carbocyclic, or cycloalkyl; a C_3-C_8 aryl, heterocycloalkyl, heteroalkylcycloalkyl, heteroaralkyl, or alkylcarbonyl; X^1 is O, S, S-S, NH, or NR^{14};

wherein R^9 is independently H, -O-, $-OR^{14}$, $-OC(=O)R^{14}$-, $-OC(=O)NHR^{14}$-, $-OC(=O)R^{14}SSR^{15}$-, $OP(=O)(OR^{14})$, or $OR^{14}OP(=O)(OR^{15})$, wherein R^{14}, R^{15} are independently a C_1-C_8 alkyl or heteroalkyl; a C_2-C_8 alkenyl, alkynyl, heterocyclic, or carbocyclic; a C_3-C_8 aryl, cycloalkyl, alkylcycloalkyl, heterocycloalkyl, heteroalkylcycloalkyl, heteroaralkyl, or alkylcarbonyl;

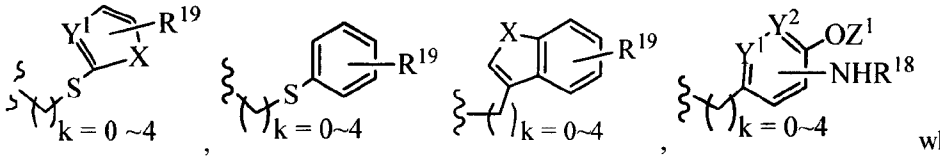
wherein R^{11} is independently $-R^{14}$-, $-R^{14}C(=O)R^{17}$-, $-R^{14}X^2R^{17}$-, or $-R^{14}C(=O)X^2$-, wherein R^{17} is independently H, OH, a C_1-C_8 alkyl; a C_2-C_8 alkenyl, alkynyl, or heteroalkyl; a C_3-C_8 aryl, arylene, heterocyclic, carbocyclic, heterocycloalkyl, or an amino acid, or two amino acids; X^2 is -O-, -S-, -NH-, $-N(R^{14})$-, $-O-R^{14}$-, $-S-R^{14}$-, $-S(=O)-R^{14}$-, or $-NHR^{14}$-; R^{14} is a C_1-C_8 alkyl or heteroalkyl; a C_2-C_8 alkenyl, alkynyl, heterocyclic, carbocyclic; C_3-C_8 aryl, cycloalkyl, alkylcycloalkyl, heterocycloalkyl, heteroalkylcycloalkyl, heteroaralkyl, or alkylcarbonyl;

wherein R^{12} is independently R^{14}, -OH, -SH, $-NH_2$, =NH, $=NNH_2$, $-NH(R^{14})$, $-OR^{14}$, $-COR^{16}$, $-COOR^{14}$-, $C(O)NH_2$, $C(O)NHR^{14}$, $-SR^{14}$, $-S(=O)R^{14}$, $-P(=O)(OR^{16})_2$, $-OP(=O)(OR^{16})_2$, $-CH_2OP(=O)(OR^{16})_2$, $-SO_2R^{16}$; R^{14} is independently a C_1-C_8 alkyl or heteroalkyl; a C_2-C_8 alkenyl, alkynyl, hetero-cyclic, or carbocyclic; a C_3-C_8 aryl, cycloalkyl, alkylcycloalkyl, heterocycloalkyl, heteroaralkyl, heteroalkylcycloalkyl, or alkylcarbonyl; R^{16} is H, OH, R^{14} or one to four amino acid units;

wherein R^{13} is C_1-C_{10} alkyl, heteroalkyl, alkyl acid, alkyl amide, alkyl amine, or Ar; Ar refers to an aromatic or hetero aromatic group, composed of one or several rings, comprising four to ten carbon atoms; the term hetero aromatic group refers to an aromatic group that has one or several carbon atoms replaced by hetero atoms which are O, N, Si, Se, P or S; the term aryl or Ar also refers to an aromatic group, wherein one or several H atoms are replaced independently by R^{17}, F, Cl, Br, I, OR^{16}, SR^{16}, $NR^{16}R^{17}$, $N=NR^{16}$, $N=R^{16}$, $NR^{16}R^{17}$, NO_2, $SOR^{16}R^{17}$, SO_2R^{16}, SO_3R^{16}, OSO_3R^{16}, $PR^{16}R^{17}$, $POR^{16}R^{17}$, $PO_2R^{16}R^{17}$, $OP(O)(OR^{17})_2$, $OCH_2OP(O)(OR^{17})_2$, $OC(O)OP(O)(OR^{17})_2$, $PO(OR^{16})(OR^{17})$, $OP(O)(OR^{17})OP(O)(OR^{17})_2$, $OC(O)R^{17}$ or $OC(O)NHR^{17}$; wherein R^{16} and R^{17} are independently H, a C_1-C_8 alkyl or heteroalkyl; a C_2-C_8 alkenyl, alkynyl, heterocyclic, carbocyclic; a

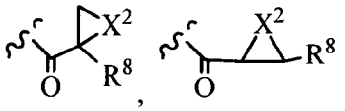
C_3-C_8 aryl, cycloalkyl, alkylcycloalkyl, heterocycloalkyl, heteroalkylcycloalkyl, heteroaralkyl, alkylcarbonyl or a C_4-C_{12} glycoside, or pharmaceutical salt;

in addition, R^{12} can be H when R^{10} is not H, or when R^{13} is:

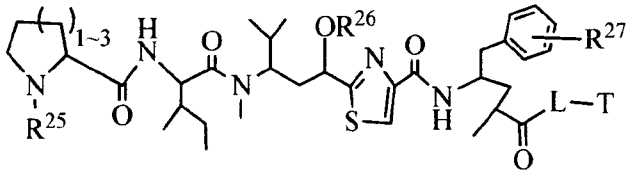


wherein Z^1 is H, $CH_2OP(O)(OR^{18})_2$, $C(O)OP(O)(OR^{18})_2$, $PO(OR^{18})_2$, $P(O)(OR^{18})OP(O)(OR^{18})_2$, $C(O)R^{18}$, $C(O)NHR^{18}$, $SO_2(OR^{18})$, a C_4-C_{12} glycoside or a C_1-C_8 alkyl, carboxyalkyl, or heterocyclic group; R^{18} is H, a C_1-C_8 alkyl, carboxyalkyl, heteroalkyl; a C_2-C_8 alkenyl, alkynyl, or heterocyclic group; a C_3-C_8 aryl or alkylcarbonyl; R^{19} is H, OH, NH_2, $OSO_2(OR^{18})$, $XCH_2OP(O)(OR^{18})_2$, $XPO(OR^{18})_2$, $XC(O)OP(O)(OR^{18})_2$, $XC(O)R^{18}$, $XC(O)NHR^{18}$, a C_1-C_8 alkyl, carboxyalkyl, or carboxylic acid derivative; a C_2-C_8 alkenyl, alkynyl, or heterocyclic group; a C_3-C_8 aryl, alkylcarbonyl; or pharmaceutical salt; X is O, S, or NH; Y^1 and Y^2 are N or CH respectively;

or R^{12} can be H when R^{11} is:

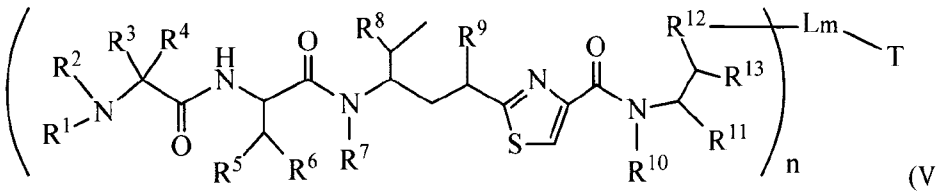


wherein X^2 is O, S, or N-R^8; R^8 is H, a C_1-C_6 alkyl or heteroalkyl group; wherein said conjugate of formula (V) specifically excludes the following structures:



wherein R^{25} is an alkyl, C(O)alkyl or C(O)aryl; R^{26} is H, a C_1-C_5 alkyl, C_2-C_5 alkenyl, $C(=O)C_1$-C_5 alkyl, or $C(=O)C_2$-C_5 alkenyl; R^{27} is H, 1 to 3 substituents which are a halogen, nitrogen, carboxylate or a derivative thereof, cyno, hydroxyl, alkyl, haloalkyl, alkoxy, haloalkoxy, a phenol protecting group, or a prodrug moiety, or OR^{29}, wherein R^{29} is a substituted aryl, $C(O)R^{30}$, $P(O)(OR^{30})_2$, or SO_3R^{30}, wherein R^{30} is H, alkyl, alkenyl, cycloalkyl, heterocyclyl, aryl, arylalkyl or a metal cation; T is a targeting or binding ligand; and L is a releasable linker.

In another illustrative embodiment of the invention, there is provided the conjugate of a cell binding molecule with cytotoxic agents having a structure of Formula (VI):



(VI)

and pharmaceutical acceptable salts and solvates thereof,

wherein T is a targeting or binding ligand; L is a releasable linker that connects to a molecule inside the bracket independently; n is 1-20 and m is 1-10;

inside the bracket is a potent antimitotic agent wherein R^1, R^2, R^3, and R^4 are independently H, a C_1-C_8 alkyl or heteroalkyl; a C_2-C_8 heterocyclic, carbocyclic, cycloalkyl, alkylcycloalkyl, or heterocycloalkyl; a C_3-C_8 aryl, Ar-alkyl, heteroalkylcycloalkyl, or alkylcarbonyl; or two R's, that include R^1R^2, R^2R^3, R^3R^4, R^5R^6, and $R^{12}R^{13}$ which can be 3-7 members of a carbocyclic, cycloalkyl, heterocyclic, or heterocycloalkyl ring system;

wherein R^5, R^6, R^8 and R^{10} are independently H, C_1-C_4 alkyl or heteroalkyl;

wherein R^7 is independently H, R^{14}, $-R^{14}C(=O)X^1R^{15}$ or $-R^{14}X^1R^{15}$, wherein R^{14} and R^{15} are independently a C_1-C_8 alkyl or heteroalkyl; a C_2-C_8 alkenyl, alkynyl, heterocyclic, carbocyclic, or cycloalkyl; a C_3-C_8 aryl, heterocycloalkyl, heteroalkylcycloalkyl, heteroaralkyl, or alkylcarbonyl; X^1 is O, S, S-S, NH, or NR^{14};

wherein R^9 is independently H, -O-, $-OR^{14}$, $-OC(=O)R^{14}$-, $-OC(=O)NHR^{14}$-, $-OC(=O)\ R^{14}SSR^{15}$-, $OP(=O)(OR^{14})$, or $OR^{14}OP(=O)(OR^{15})$, wherein R^{14} and R^{15} are independently a C_1-C_8 alkyl or heteroalkyl; a C_2-C_8 alkenyl, alkynyl, heterocyclic, or carbocyclic; a C_3-C_8 aryl, cycloalkyl, alkylcycloalkyl, heterocycloalkyl, heteroalkylcycloalkyl, heteroaralkyl, or alkylcarbonyl;

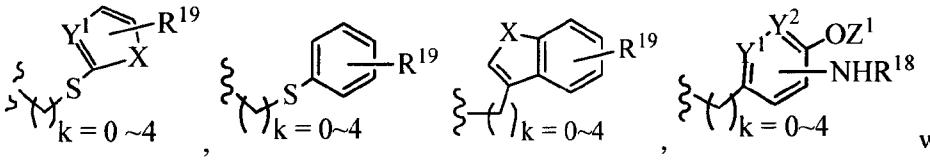
wherein R^{11} is independently H, R^{14}, $-R^{14}C(=O)R^{16}$, $-R^{14}X^2R^{16}$, or $-R^{14}C(=O)X^2$, wherein X^2 is -O-, -S-, -NH-, $-N(R^{14})$-, $-O-R^{14}$-, $-S-R^{14}$-, $-S(=O)-R^{14}$-, or $-NHR^{14}$-; R^{14} is a C_1-C_8 alkyl or heteroalkyl; a C_2-C_8 alkenyl, alkynyl, heterocyclic, or carbocyclic; a C_3-C_8 aryl, cycloalkyl, alkylcycloalkyl, heterocycloalkyl, heteroalkylcycloalkyl, heteroaralkyl, or alkylcarbonyl; R^{16} is H, OH, R^{14}, or one to four amino acid units;

wherein R^{12} is independently R^{14}, -O-, -S-, -NH-, =N-, =NNH-, $-N(R^{14})$-, $-OR^{14}$-, -C(O)O-, -C(O)NH-, $C(O)NR^{14}$-, $-SR^{14}$-, $-S(=O)R^{14}$-, $-NHR^{14}$-, $-CH_2OP(=O)(OR^{16})$-, $-P(=O)(OR^{16})$ -, $-C(O)OP(=O)(OR^{16})$-, $-OP(=O)(OR^{16})O$-, or $-SO_2R^{14}$, wherein R^{14} is a C_1-C_8 alkyl or heteroalkyl; a C_2-C_8 alkenyl, alkynyl, hetero-cyclic, or carbocyclic; a C_3-C_8 aryl, cycloalkyl, alkylcycloalkyl, heterocycloalkyl, heteroaralkyl, heteroalkylcycloalkyl, or alkylcarbonyl; R^{16} is H, OH, R^{14} or one to four amino acid units;

wherein R^{13} is C_1-C_{10} alkyl, heteroalkyl, alkyl acid, alkyl amide, alkyl amine, or Ar; Ar is an aromatic or hetero aromatic group, composed of one or several rings, comprising four to ten carbon

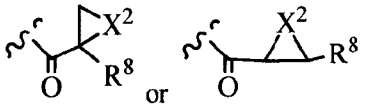
atoms; the hetero aromatic group is an aromatic group that has one or several carbon atoms replaced by hetero atoms which is O, N, Si, Se, P or S; the term aryl or Ar also includes an aromatic group, wherein one or several H atoms are replaced independently by R^{17}, F, Cl, Br, I, OR^{16}, SR^{16}, $NR^{16}R^{17}$, $N{=}NR^{16}$, $N{=}R^{16}$, $NR^{16}R^{17}$, NO_2, $SOR^{16}R^{17}$, SO_2R^{16}, SO_3R^{16}, OSO_3R^{16}, $PR^{16}R^{17}$, $POR^{16}R^{17}$, $PO_2R^{16}R^{17}$, $OP(O)(OR^{17})_2$, $OCH_2OP(O)(OR^{17})_2$, $OC(O)OP(O)(OR^{17})_2$, $PO(OR^{16})(OR^{17})$, $OP(O)(OR^{17})OP(O)(OR^{17})_2$, $OC(O)R^{17}$ or $OC(O)NHR^{17}$, wherein R^{16} and R^{17} are independently H, a C_1-C_8 alkyl or heteroalkyl; a C_2-C_8 alkenyl, alkynyl, heterocyclic, or carbocyclic; a C_3-C_8 aryl, cycloalkyl, alkylcycloalkyl, heterocycloalkyl, heteroalkylcycloalkyl, heteroaralkyl, alkylcarbonyl or a C_4-C_{12} glycoside, or pharmaceutical salt;

in addition, R^{12} can be H when R^{10} is not H, or when R^{13} is:



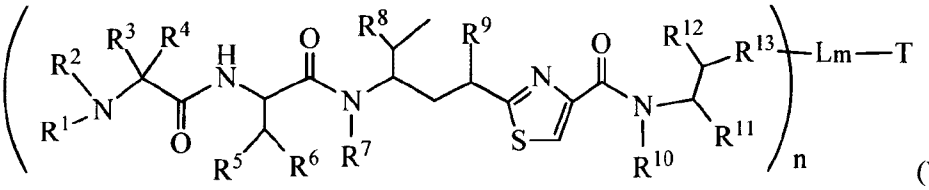
wherein Z^1 is H, $CH_2OP(O)(OR^{18})_2$, $C(O)OP(O)(OR^{18})_2$, $PO(OR^{18})_2$, $P(O)(OR^{18})OP(O)(OR^{18})_2$, $C(O)R^{18}$, $C(O)NHR^{18}$, $SO_2(OR^{18})$, a C_4-C_{12} glycoside or a C_1-C_8 alkyl, carboxyalkyl, or heterocyclic; R^{18} is H, C_1-C_8 alkyl, carboxyalkyl, heteroalkyl; C_2-C_8 alkenyl, alkynyl, or heterocyclic; a C_3-C_8 aryl or alkylcarbonyl; R^{19} is H, OH, NH_2, $OSO_2(OR^{18})$, $XCH_2OP(O)(OR^{18})_2$, $XPO(OR^{18})_2$, $XC(O)OP(O)(OR^{18})_2$, $XC(O)R^{18}$, or $XC(O)NHR^{18}$, a C_1-C_8 alkyl, carboxyalkyl, or carboxylic acid derivative; a C_2-C_8 alkenyl, alkynyl, or heterocyclic; a C_3-C_8 aryl or alkylcarbonyl; or a pharmaceutical salt; X is O, S, or NH; Y^1 and Y^2 are N and CH respectively;

or R^{12} can be H when R^{11} is:



wherein X^2 is O, S, or N-R^8; R^8 is H, a C_1-C_6 alkyl or heteroalkyl.

In yet another illustrative embodiment of the invention, there is provided a conjugate of cell binding molecule with cytotoxic agents having a structure of Formula (VII):



(VII)

and pharmaceutical acceptable salts and solvates thereof,

wherein T is a targeting or binding ligand; L is a releasable linker that connects to a molecule inside the bracket independently; n is 1-20 and m is 1-10;

inside the bracket is a potent antimitotic agent wherein R^1, R^2, R^3, and R^4 are independently H, a C_1-C_8 alkyl or heteroalkyl; a C_2-C_8 heterocyclic, carbocyclic, cycloalkyl, alkylcycloalkyl, or heterocycloalkyl; a C_3-C_8 aryl, Ar-alkyl, heteroalkylcycloalkyl, or alkylcarbonyl; or two R's, that include R^1R^2, R^2R^3, R^3R^4, R^5R^6, and $R^{12}R^{13}$ are 3-7 members of a carbocyclic, cycloalkyl, heterocyclic, or heterocycloalkyl ring system;

wherein R^5, R^6, R^8 and R^{10} are independently H, C_1-C_4 alkyl or heteroalkyl;

wherein R^7 is independently H, R^{14}, $-R^{14}C(=O)X^1R^{15}$ or $-R^{14}X^1R^{15}$, wherein R^{14} and R^{15} are independently a C_1-C_8 alkyl or heteroalkyl; a C_2-C_8 alkenyl, alkynyl, heterocyclic, carbocyclic, or cycloalkyl; a C_3-C_8 aryl, heterocycloalkyl, heteroalkylcycloalkyl, heteroaralkyl, or alkylcarbonyl; X^1 is O, S, S-S, NH, or NR^{14};

wherein R^9 is independently H, -O-, $-OR^{14}$, $-OC(=O)R^{14}$-, $-OC(=O)NHR^{14}$-, $-OC(=O)\ R^{14}SSR^{15}$-, $OP(=O)(OR^{14})$, or $OR^{14}OP(=O)(OR^{15})$, wherein R^{14} and R^{15} are independently C_1-C_8 alkyl or heteroalkyl; a C_2-C_8 alkenyl, alkynyl, heterocyclic, or carbocyclic; a C_3-C_8 aryl, cycloalkyl, alkylcycloalkyl, heterocycloalkyl, heteroalkylcycloalkyl, heteroaralkyl, or alkylcarbonyl;

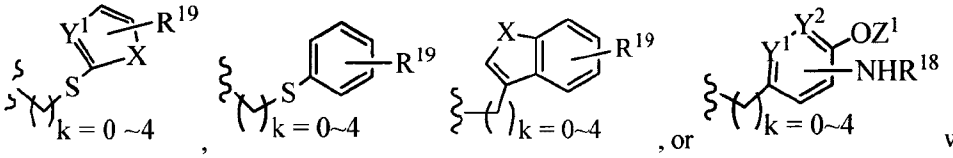
wherein R^{11} is independently H, R^{14}, $-R^{14}C(=O)R^{16}$, $-R^{14}X^2R^{16}$, or $-R^{14}C(=O)X^2$, wherein X^2 is -O-, -S-, -NH-, $-N(R^{14})$-, $-O-R^{14}$-, $-S-R^{14}$-, $-S(=O)-R^{14}$-, or $-NHR^{14}$-; R^{14} is a C_1-C_8 alkyl or heteroalkyl; a C_2-C_8 alkenyl, alkynyl, heterocyclic, or carbocyclic; a C_3-C_8 aryl, cycloalkyl, alkylcycloalkyl, heterocycloalkyl, heteroalkylcycloalkyl, heteroaralkyl, or alkylcarbonyl; R^{16} is H, OH, R^{14}, or one to four amino acid units;

wherein R^{12} is independently R^{14}, -OH, -SH, $-NH_2$, =NH, $=NNH_2$, $-NH(R^{14})$, $-OR^{14}$, $-COR^{16}$, $-COOR^{14}$-, $C(O)NH_2$, $C(O)NHR^{14}$, $-SR^{14}$, $-S(=O)R^{14}$, $-P(=O)(OR^{16})_2$, $-OP(=O)(OR^{16})_2$, $-CH_2OP(=O)(OR^{16})_2$, or $-SO_2R^{16}$; R^{14} is independently a C_1-C_8 alkyl or heteroalkyl; a C_2-C_8 alkenyl, alkynyl, hetero-cyclic, or carbocyclic; a C_3-C_8 aryl, cycloalkyl, alkylcycloalkyl, heterocycloalkyl, heteroaralkyl, heteroalkylcycloalkyl, or alkylcarbonyl; R^{16} is H, OH, R^{14} or one to four amino acid units;

wherein R^{13} is a C_1-C_{10} alkyl, heteroalkyl, alkyl acid, alkyl amide, alkyl amine, or Ar; Ar is an aromatic or hetero aromatic group, composed of one or several rings, comprising four to ten carbon atoms; the hetero aromatic group is an aromatic group that has one or several carbon atoms replaced by hetero atoms which are O, N, Si, Se, P or S; Ar can also be an aromatic group, wherein one or several H atoms are replaced independently by R^{18}, F, Cl, Br, I, OR^{16}, SR^{16}, $NR^{16}R^{17}$, $N{=}NR^{16}$, $N{=}R^{16}$, $NR^{16}R^{18}$, NO_2, $SOR^{16}R^{18}$, SO_2R^{16}, SO_3R^{16}, OSO_3R^{16}, $PR^{16}R^{18}$, $POR^{16}R^{18}$, $PO_2R^{16}R^{18}$, $OPO_3OR^{16}R^{18}$, $CH_2OPO_3R^{16}R^{18}$, $OP(O)(OR^{16})OP(O)(OR^{18})_2$, $C(O)OPO_3R^{16}R^{18}$, or $PO_3R^{16}R^{18}$, wherein R^{16} and R^{18} are independently H, a C_1-C_8 alkyl; a C_2-C_8 alkenyl, alkynyl, or

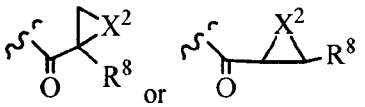
heteroalkyl; a C_3-C_8 aryl, heterocyclic, carbocyclic, cycloalkyl, alkylcycloalkyl, heterocycloalkyl, heteroaralkyl, heteroalkylcycloalkyl, or alkylcarbonyl; or a C_4-C_{12} glycoside; or pharmaceutical salts;

in addition, R^{12} can be H when R^{10} is not H, or when R^{13} is:



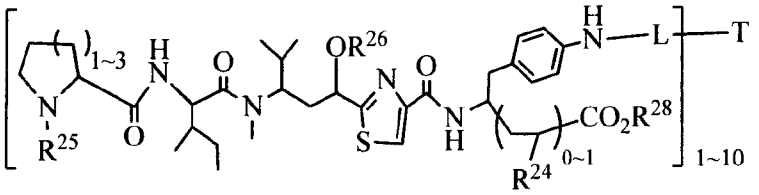
wherein Z^1 is H, $CH_2OP(O)(OR^{18})_2$, $C(O)OP(O)(OR^{18})_2$, $PO(OR^{18})_2$, $P(O)(OR^{18})OP(O)(OR^{18})_2$, $C(O)R^{18}$, $C(O)NHR^{18}$, $SO_2(OR^{18})$, a C_4-C_{12} glycoside or a C_1-C_8 alkyl, carboxyalkyl, or heterocyclic; R^{18} is H, a C_1-C_8 alkyl, carboxyalkyl, or heteroalkyl; a C_2-C_8 alkenyl, alkynyl, or heterocyclic; a C_3-C_8 aryl or alkylcarbonyl; R^{19} is H, OH, NH_2, $OSO_2(OR^{18})$, $XCH_2OP(O)(OR^{18})_2$, $XPO(OR^{18})_2$, $XC(O)OP(O)(OR^{18})_2$, $XC(O)\ R^{18}$, $XC(O)NHR^{18}$, a C_1-C_8 alkyl, a carboxyalkyl, or carboxylic acid derivative; a C_2-C_8 alkenyl, alkynyl, or heterocyclic; a C_3-C_8 aryl or alkylcarbonyl; or pharmaceutical salts; X is O, S, or NH; Y^1 and Y^2 are N and CH respectively;

or R^{12} can be H when R^{11} is:



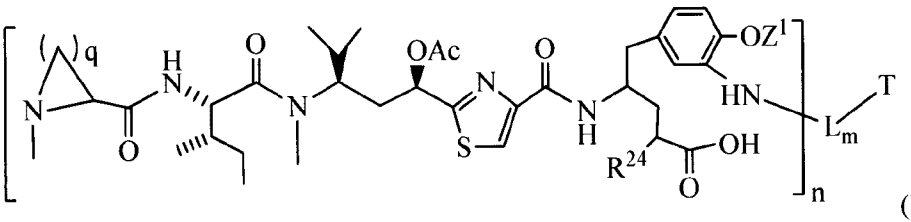
wherein X^2 is O, S, or N-R^8; R^8 is H, C_1-C_6 alkyl or heteroalkyl;

wherein said conjugate of the formula (VII) specifically excludes the following structures:



wherein R^{24} is H or CH_3; R^{25} is alkyl, C(O)alkyl or C(O)aryl; R^{26} is H, C_1-C_5 alkyl, C_2-C_5 alkenyl, $C(=O)C_1$-C_5 alkyl, or $C(=O)C_2$-C_5 alkenyl; R^{28} is H or C_1-C_5 alkyl; T is a targeting or binding ligand; and L is a releasable linker.

In a further illustrative embodiment of the invention, there is provided a conjugate of cell binding molecule with cytotoxic agents having a structure of Formula (VIII):



(VIII)

and pharmaceutical acceptable salts and solvates thereof,

wherein T is a targeting or binding ligand; L is a releasable linker that connects to a molecule inside the bracket independently; n is 1~20 and m is 1~10; q = 1~5;

wherein Z^1 is H, $CH_2OP(O)(OR^{18})_2$, $PO(OR^{18})_2$, $C(O)OP(O)(OR^{18})_2$, $P(O)(OR^{18})OP(O)(OR^{18})_2$, $C(O)R^{18}$, $C(O)NHR^{18}$, $SO_2(OR^{18})$, C_4~C_{12} of glycosides, or C_1~C_8 of alkyl, carboxyalkyl, heterocyclic; R^{18} is H, C_1~C_8 of alkyl, carboxyalkyl, heteroalkyl; C_2~C_8 of alkenyl, alkynyl, heterocyclic; C_3~C_8 of aryl, alkylcarbonyl; R^{24} is H or CH_3.

In yet another aspect, a compound of formula V to VIII or a pharmaceutically acceptable salt or solvate thereof is used for treating cancer, an autoimmune disease or an infectious disease in a human or an animal.

4. BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the synthesis of branched linkers for conjugation of drugs to cell binding agents.

Figure 2 shows the synthesis of maleimido linkers and their use for the drug-binding molecule conjugation.

Figure 3 shows the synthesis of bromomaleimido and dibromomaleimido linkers and their uses for the drug-binding molecule conjugation.

Figure 4 shows the synthesis of amino acid (Val-Cit) linkers for the conjugation antimitotic agents with a cell surface binding ligand.

Figure 5 shows the synthesis of Tuv component of the antimitotic agents.

Conditions: a. $CuSO_4$, H_2SO_4, Acetone, 95%; b. DIBAL-H, THF/Tol, -78°C, 95%; c. NH_2OH, $NaHCO_3$, CH_3OH/H_2O; (**74**), 40°C, 48h, CH_2Cl_2 83%; f. LiOH, THF/H_2O, 86%; g. $HClO_4$, CH_3CN/H_2O, 98%; h. BOC_2O, Na_2CO_3, THF/H_2O, 95%; i. L-(S)-Tr-cysteine methyl ester, EDC, CH_2Cl_2, 85%; j. $Ph_3P{=}O$, Tf_2O, CH_2Cl_2; k: MnO_2, CH_2Cl_2, 76% (2 steps); 1. $Mo(CO)_6$, CH_3CN/H_2O, 87%; m. Ac_2O, Pyr., 95%; n. 1). NaH, THF, CH_3I, 85%; 2). $HOSnMe_3$, $ClCH_2CH_2Cl$, 80°C, 95%, 3). Ac_2O/Pyr, 86%.

Figure 6 shows the synthesis of Tuv components of the antimitotic agents.

Conditions: o): Fmoc-Cl, $NaHCO_3$, THF/H_2O, 95%; p): L-(S)-Tr-cysteine methyl ester, EDC, CH_2Cl_2, 87%; q): $Ph_3P{=}O$, Tf_2O, CH_2Cl_2; r): MnO_2, CH_2Cl_2, 76% (2 steps); s): $Mo(CO)_6$, CH_3CN/H_2O, 87%; t): TES-Cl, Pyr., 95%; u): NaH, THF, CH_3I, 90%; v): NaH, THF, $BrCH_2COOtBu$, 0°C, 87%; w): $HOSnMe_3$, $ClCH_2CH_2Cl$, 80°C; ~90%; x): Bu4NF, THF; y): Ac_2O, Pyr. 81%.

Figure 7 shows the synthesis of Boc-Tuv moieties and a conjugatable antimitotic agent.

Conditions: a): NaH, THF, N-(4-Bromobutyl)phthalimide, NaI, ~83%; b): NaH, DMF, CH_3I, 90%; c): $HOSnMe_3$, $ClCH_2CH_2Cl$, 80°C, ~85%; d): Ac_2O, Pyr.; e): (R)-(+)-b-Methylphenethylamine, EDC, DMA, 85%; f): 4M HCl, dioxane; g); Boc-Ile-OH, PyBroP, DMAP, DMA, 78%; h): D-Mep, $PyBroP/CH_2Cl_2$, 81%; i): NH_2NH_2, DMA; j); **58** (n=3), EDC, DMA, k): Ac_2O, Pyr. 56%.

Figure 8 shows the synthesis of Tuv, Ile-Tuv and Mep-Ile-Tuv moieties of the antimitotic agents.

Figure 9 shows the synthesis of Ile-Tuv, Mep-Leu-Tuv, Val-Ile-Tuv and Val-Ile-Tuv (O-alkyl) moieties of the antimitotic agents.

Figure 10 shows the synthesis of a conjugatable antimitotic agent and its conjugation to an antibody.

Figure 11 shows the synthesis of amino acid (Phe-(D)Lys) linkers for conjugation of the antimitotic agents.

Figure 12 shows the synthesis of antibody-antimitotic agent conjugates.

Figure 13 shows the synthesis of antibody-antimitotic agent conjugates.

Figure 14 shows the synthesis of the binding molecule-antimitotic agent conjugates.

Figure 15 shows the synthesis of a binding molecule-antimitotic agent conjugate.

Figure 16 shows the synthesis of an antibody-antimitotic agent conjugate.

Figure 17 shows the synthesis of an antibody-antimitotic agent conjugate.

Figure 18 shows the Figure 18. Synthesis of hydrophilic Tut analogs for the synthesis of the hydrophilic (phosphate prodrug of) antimitotic agents

Figure 19 shows the synthesis of the conjugates of the antimitotic agents with an antibody.

Figure 20 shows the Boc solid-phase synthesis of the conjugatable antimitotic agents. Conditions: a): Piperazine (5 ~ 20 eq), CH_2Cl_2, 4h; b): Boc-Aa2-OH (2 ~ 5 eq), PyBroP (2 ~ 5 eq), DIPEA (3 ~ 10 eq), DMF, 4 h; c): 4M HCl/Dioxane, 0.5h; then washed with DIPEA (2 ~ 3 eq), DMF; d): Boc-Aa1-OH (2 ~ 5 eq), TBTU (2 ~ 5 eq), DIPEA (3 ~ 10 eq), DMF, 4 h; e): (2 ~ 5 eq) BocNMe-Phe-OH, or Boc-Trp-OH, or BocNMe-Tyr(PO(OBz)OH)-OH, or BocNMe-(Pyr)Ala-OH, or Boc-(Thienyl)-Ser-OH, or Boc-(Thiazolyl)-Ala-OH), Boc-Hyp-OH, PyBroP (2 ~ 5 eq), DIPEA (3 ~ 10 eq), DMF, 4 h; f): Boc-N(Me)-Tuv-OH (1.5 ~ 3 eq), PyBroP (2 ~ 5 eq), DIPEA (3 ~ 10 eq), DMF, 2 h; g): Boc-Ile-OH (2 ~ 5 eq), PyBroP (2 ~ 5 eq), DIPEA (3 ~ 10 eq), DMF, 3 h; h): NMe_2-Ile-OH, TBTU (2 ~ 5 eq), DIPEA (3 ~ 10 eq), DMF, 2h; i): TFA, anisole; j): 4-maleimido butyric acid NHS ester (1.5 ~ 2 eq), DIPEA (3 ~ 10 eq), DMF, 2 h; k): 4-(methyldisulfanyl)butanoic acid NHS ester (1.5 ~ 2 eq), or 4,4-dimethyl 4-(methyldisulfanyl)-butanoic acid NHS ester (1.5 ~ 2 eq), DIPEA (3 ~ 10 eq), DMF, 2 h; l): TCEP (3 ~ 10 eq), Dioxane/buffer pH 7,0, then solid supported guanidine.

Figure 21 shows the Fmoc solid-phase synthesis of the conjugatable antimitotic agents. Conditions: a): $(MeNCH_2)_2$ (5 ~ 20 eq), DCM, 4h; b): Fmoc-Aa2-OH (2 ~ 5 eq), PyBroP (2 ~ 5 eq), DIPEA (3 ~ 10 eq), DMF, 4 h; e): 20% piperidine, DMF, 2h; d): Fmoc-Aal-OH (2 ~ 5 eq),

TBTU (2 ~ 5 eq), DIPEA (3 ~ 10 eq), DMF, 4 h; e): (2 ~ 5 eq) FmocNMe-Tyr(SO_3H)-OH, or Fmoc-TrpOH-OH, or FmocNMe-Tyr(PO(OBz)-OH)-OH or FmocNMe-Tyr(Glucose)-OH, or Boc-(quinolyl)Ala-OH, or Fmoc-(thienyl)Ser-OH, (phenyl)-Cys-OH, PyBroP (2 ~ 5 eq), DIPEA (3 ~ 10 eq), DMF, 4 h; f): Fmoc- N(Me)-Tuv-OH (1.5 ~ 3 eq), PyBroP (2 ~ 5 eq), DIPEA (3 ~ 10 eq), DMF, 4 h; g): Fmoc-Ile-OH (2 ~ 5 eq), PyBroP (2 ~ 5 eq), DIPEA (3 ~ 10 eq), DMF, 4 h; h): Mep-OH (2 ~ 4 eq), or (R)-1-methylaziridine-2-carboxylate, TBTU (2 ~ 5 eq), DIPEA (3 ~ 10 eq), DMF, 2h; i): TFA, DCM, anisole; j): 4-maleimido butyric acid NHS ester (1.5 ~ 2 eq), DIPEA (3 ~ 10 eq), DMF, 4 h; k): 20% TFA, DCM; 1): 4-(methyldisulfanyl)butanoic acid NHS ester (1.5 ~ 2 eq), or 4,4-dimethyl 4-(methyldisulfanyl)butanoic acid NHS ester (1.5 ~ 2 eq), DIPEA (3 ~ 10 eq), DMF, 4 h; m): TCEP (8 eq), Dioxane, buffer pH 7.0. then solid supported guanidine.

Figure 22 shows the synthesis of the hydrophilic antimitotic drugs and their conjugates with an antibody.

Figure 23 shows the synthesis of Tuv derivatives and the solid phase synthesis of components of Mep-Ile-Tuv and NMe_2-Val-Ile-Tuv.

Conditions: a): compound 72, DIPEA, CsI, DMF, 2 h; b): 20% TFA/DCM, 0.5 h, then washed with DIPEA, MeOH, DCM; c): Boc-Ile-OH (3 ~ 5 eq), PyBroP (3 ~ 5 eq), DIPEA (3 ~ 10 eq), DMF, 6 h; d): Mep-OH (2 ~ 4 eq), or NMe_2-Leu-OH, TBTU (2 ~ 5 eq), DIPEA (3 ~ 10 eq), DMF, 6 h, e); 95%TFA/anisole/DCM.

Figure 24 shows the synthesis of the antimitotic agents and their conjugation with an antibody.

Conditions: a): $(COCl)_2$ 6 eq, DMF (cat), DCM, 1h; b): D-(+)-Boc-norephedrine 4 eq, DIPEA, DCM, 4h; c): 20% TFA/DCM, 0.5 h, then washed with DIPEA, MeOH, DCM; d): compound 274 (1.2 eq), TBTU (5 eq), DMF, 6h; e): Boc-Ile-OH (3 ~ 5 eq), PyBrop (3~5 eq), DIPEA (3 ~ 10 eq), DMF, 4 h; f): Mep-OH (2 ~ 4 eq), or NMe_2-Leu-OH, TBTU (2 ~ 5 eq), DIPEA (3 ~10 eq), DMF, 2h; g): $HOSnMe_3$, $ClCH_2CH_2Cl$, 80°C, 8h.

Figure 25 shows the solid phase synthesis of antimitotic agents and their conjugates with an antibody.

Conditions: a): 20% TFA/DCM, 0.5 h, then washed with DIPEA, MeOH, DCM; b): Boc-(2-thiazolyl)-Cys-OH (2 eq), or 2-Thienyl)-L-Cys-OH (2 eq) PyBroP (4 eq), DIPEA (4 eq), DMF, 6 h; c): compound 276, TBTU (4 eq), DIPEA (4 eq), DMF, 6 h; d): Boc-Ile-OH (4 eq), PyBroP (4 eq), DIPEA (4 eq), DMF, 6h;

Conditions: e): Mep-OH (2 ~ 4 eq), or NMe_2-Leu-OH, TBTU (2 ~ 5 eq), DIPEA (3 10 eq), DMF, 2h, f): TFA/DCM/anisole/p-thiolcresole (95:4:0.5:0.5)

Figure 26 shows the solid-phase synthesis of antimitotic drugs and their conjugates with an antibody.

Conditions: a): DMF/piperidine (4:1); b): 331/DMF/PyBroP (2~5 eq); c): Fmoc-Tuv-OH (1.2 eq), TBTU (5 eq), DMF: d): Fmoc-Ile-OH (4 eq), PyBroP (4 eq), DIPEA (4 eq), DMF; e): N, N(methyl, Maleimido-pentanoic)-bal-OH (2 eq), TBTU, DMF; f): N,N-(methyl, 2'-pyridinyl-disufanylbutyric)-Val-OH, TBTU (4 eq), DMF; g): 5% TFA/DCM/1%TIS; i) DTT/pH 7.0 PBS buffer/DMF, then HPLC; j): N, N (methyl, Maleimido-pentanoic)-Mep-OH (2 eq), TBTU, DMF; f): N, N-(methyl, 2'-pyridinyl-disufanylbutyric)-Mep-OH, TBTU (4 eq), DMF;

Figure 27 shows the synthesis of *trans*-2-arylcyclopropylamines, *trans*-2-arylcyclopropyl-carboxyl acids and *trans*-2-arylethylepoxidyl carboxyl acids.

Figure 28 shows the synthesis of alkene amino acids and alkyl epoxidyl amino acids

Figure 29 shows the synthesis of hydrophilic Tut analogs for the synthesis of the hydrophilic prodrugs of antimitotic agents

Figure 30 shows the synthesis of the hydrophilic prodrugs of antimitotic agents for the conjugation with a cell binding agent.

Figure 31 shows the synthesis of the hydrophilic prodrugs of antimitotic agents for the conjugation with an antibody.

Figure 32 shows the synthesis of the hydrophilic prodrugs of antimitotic agents for the conjugation with an antibody.

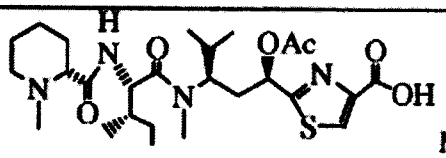
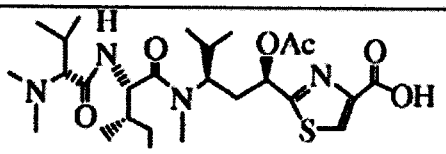
Figure 33 shows the in vitro cytotoxic effects of antiCD22 antibody-antimitotic agent (TZ01~TZ09) conjugates with drug /antibody ratio (D/A) 3.0 ~ 4.3 on Ramos (Burkitt lymphoma cell line). The cells were incubated with the conjugates for 5 days and the IC_{50} values are indicated on the figure.

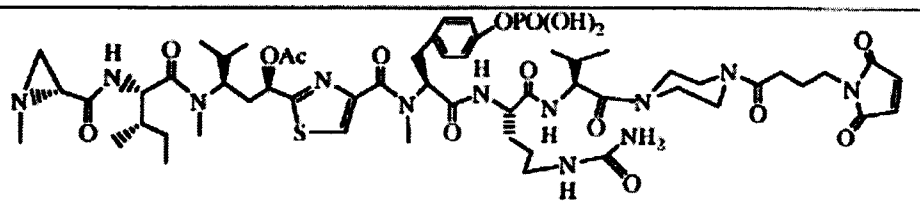
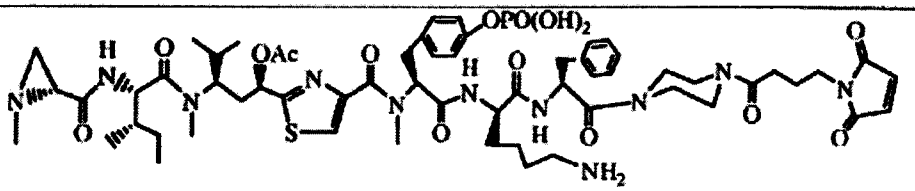
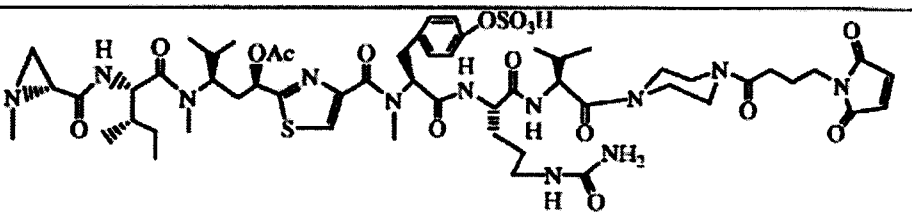
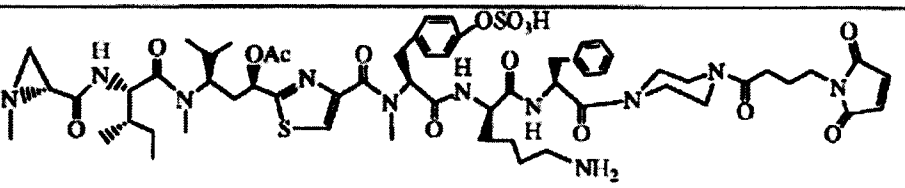
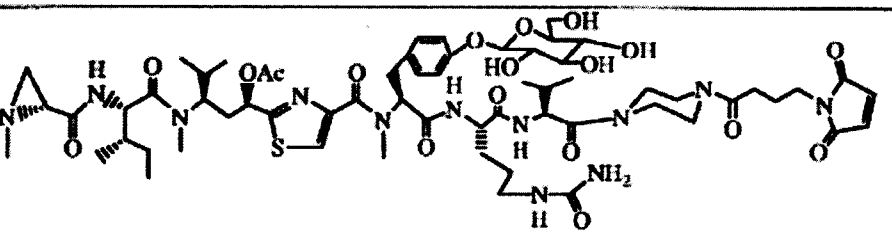
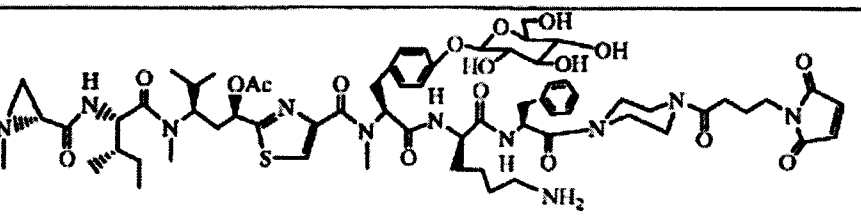
Figure 34 shows the cytotoxic effects of Trastuzumab-antimitotic agent (TZ03, TZ04, and TZ07) conjugates with drug /antibody ratio (DAR) 3.5 ~ 4.0 on KPL-4 (breast cancer cell line). It also shows that Trastuzumab-TZ03 conjugate induces a specifically potent antiproliferative effect - with IC_{50} = 90 pM in the absence of unconjugated Trastuzumab and IC_{50} >20 nM in the presence of 1 micromole concentration of Trastuzumab (to saturate the antigen binding) respectively. The specificity window is > 222 (IC_{50} = 20 nM/ IC_{50} =0.09 nM)

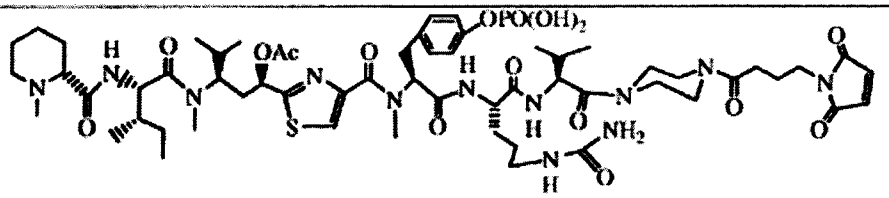
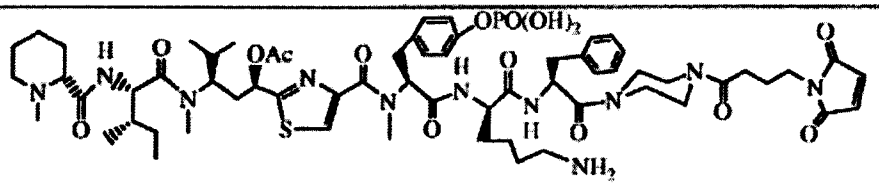
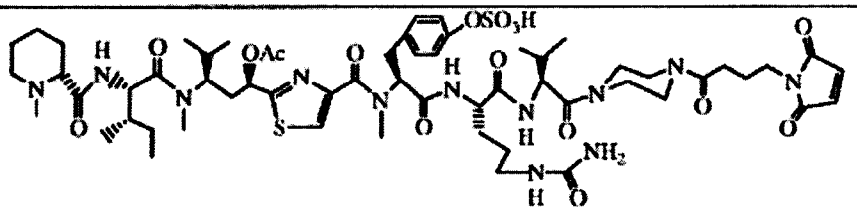
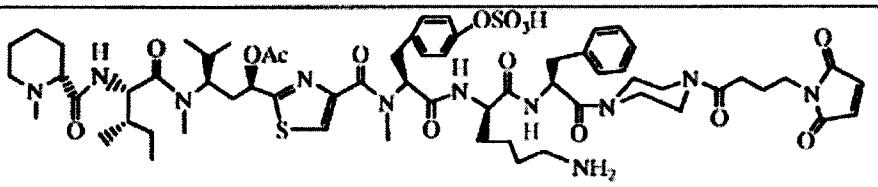
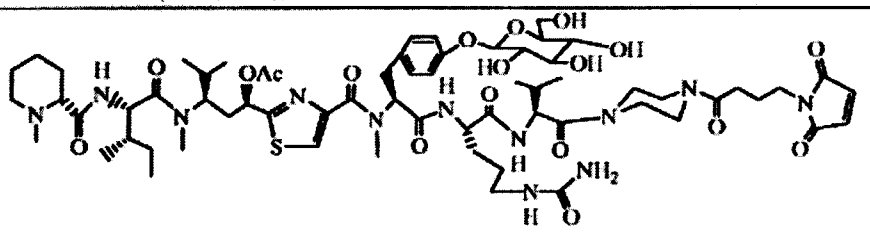
Figure 35 shows the cytotoxic effects of antiCD22 antibody-antimitotic agent (TZ03, TZ04, and TZ07) conjugates with drug /antibody ratio (D/A) 3.8 ~ 4.2 and of unconjugated CD22 antibody as well as of CD20 antibody (Rituximab) on BJAB (Burkitt lymphoma cell line). It shows that the conjugate induces much more potent antiproliferative effect -with IC_{50} =5 ~19 pM than the unconjugated antibodies with IC_{50} > 20 nM. The specificity windows when used 1 micromole concentration of unconjugated CD22 antibody to saturate the antigen binding are > 660 (IC_{50} =

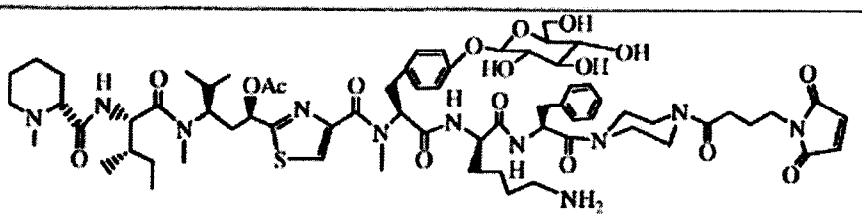
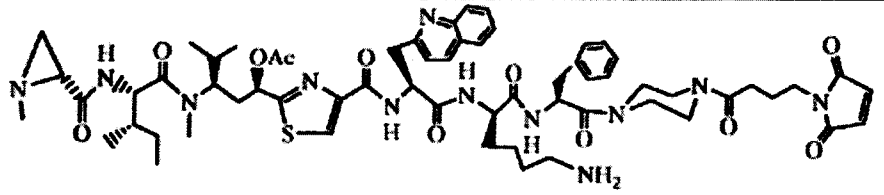
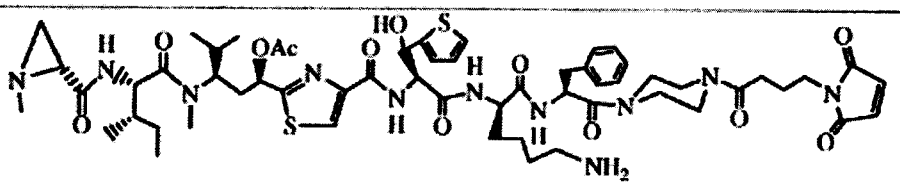
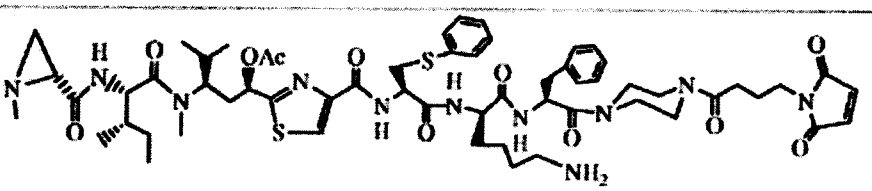
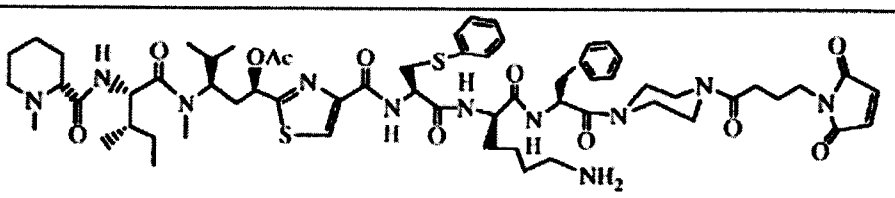
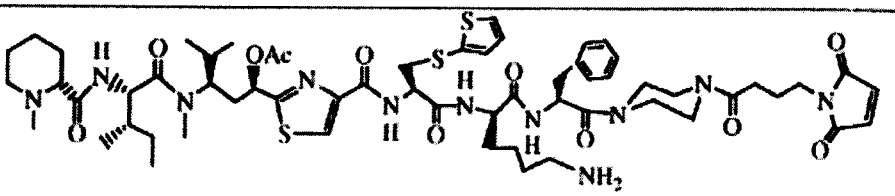
3.3 nM/IC_{50} =0.005 nM) for huCD22-TZ03 conjugate and > 790 (IC_{50} =0.019 nM) for huCD-TZ07 conjugate.

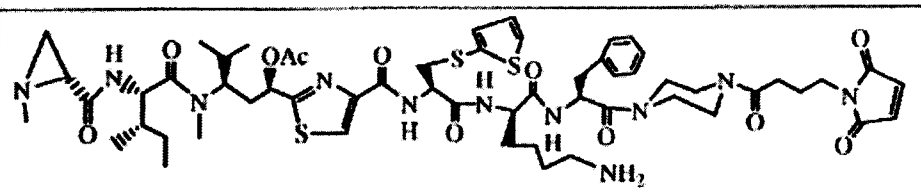
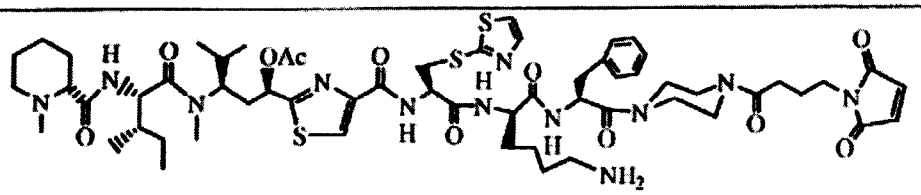
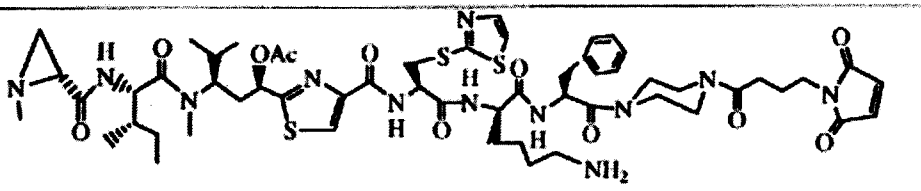
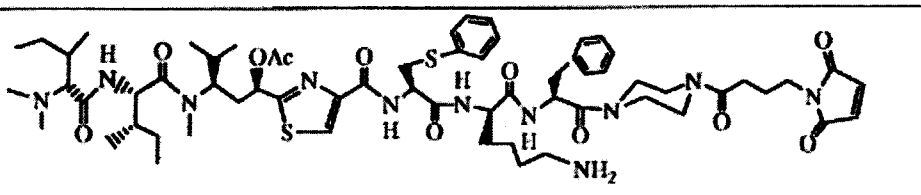
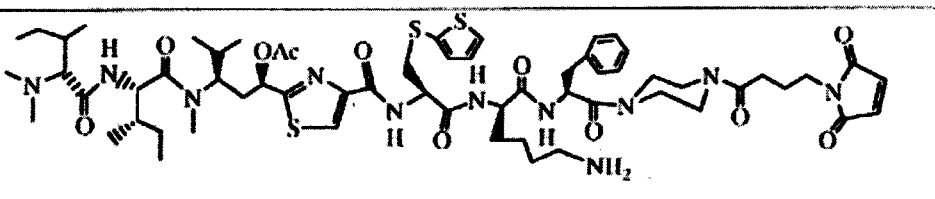
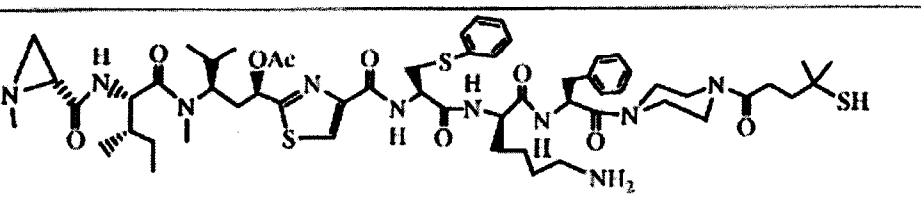
Table 1: The structure of the antimitotic drugs made through solid phase synthesis, their molecular ion of mass spectra and in vitro cytotoxicity of these drugs against Ramos cell (ATCC, a human Burkitt's lymphoma cell)

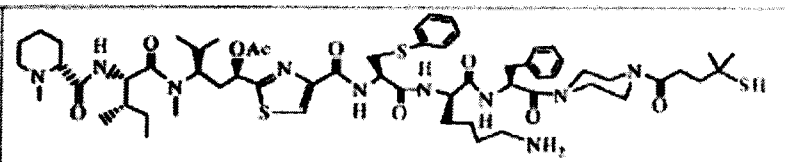
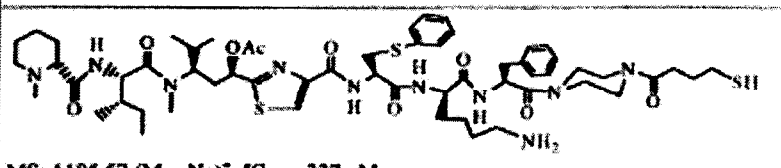
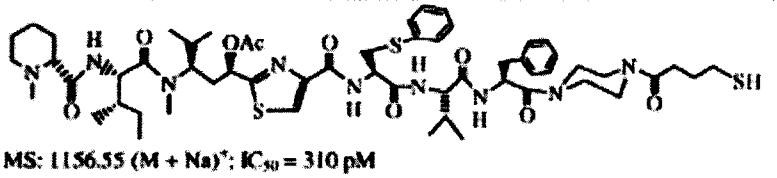
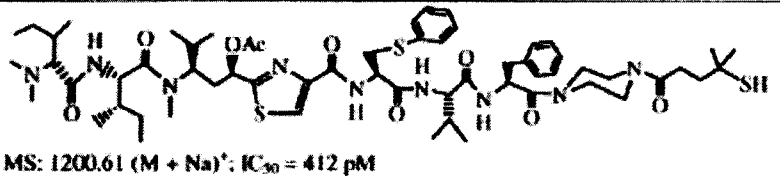
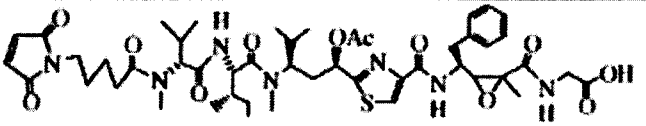
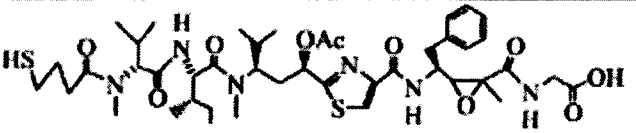
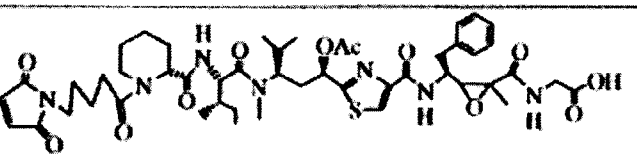
106	 MS 537.28 $(M-H)^-$; IC_{50} > 500 nM
283	 MS 539.30 $(M-H)^-$; IC_{50} > 500 nM

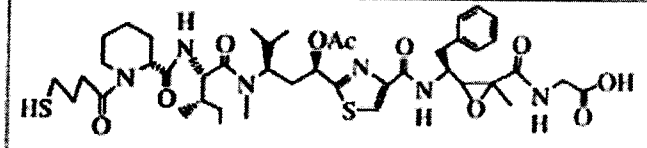
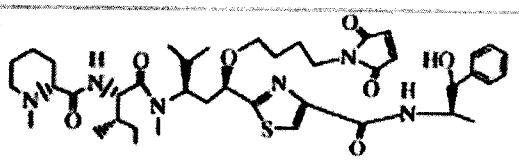
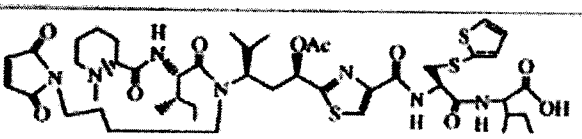
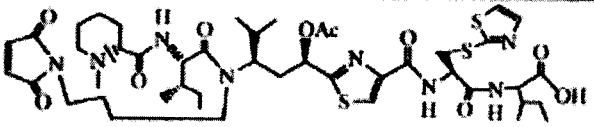
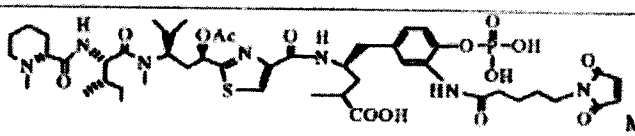
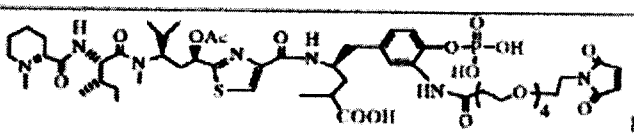
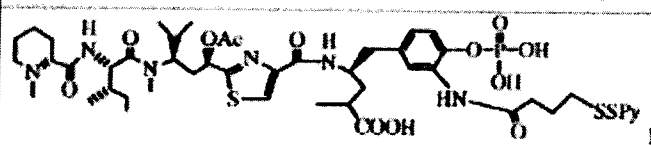
232a	 MS: 1241.55 $(M-H)^-$, IC_{50} = 27 nM, or 321 pM (in presence of 1 u of acid phosphatase)
232b	 MS: 1260.56 $(M-H)^-$; IC_{50} = 47 nM, or 375 pM (in presence of 1 u of acid phosphatase)
232c	 MS: 1241.54 $(M-H)^-$; IC_{50} = 90 nM.
232d	 MS: 1260.55 $(M-H)^-$; IC_{50} = 107 nM
232e	 MS: 1347.64 $(M+Na)^+$; IC_{50} = 77 nM, or 121 pM (in presence of 1 u of glycosidase)
232f	

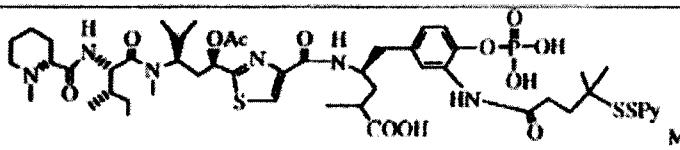
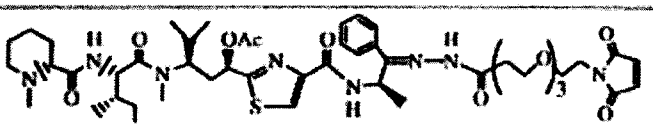
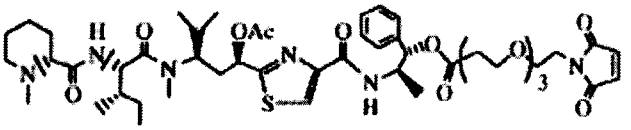
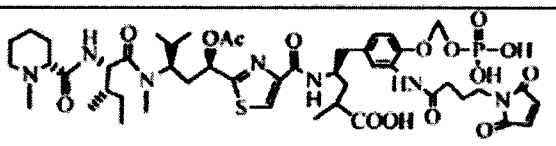
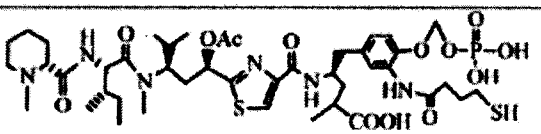
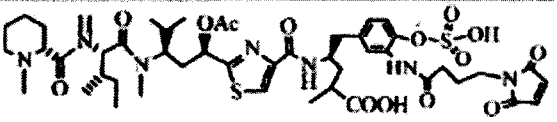
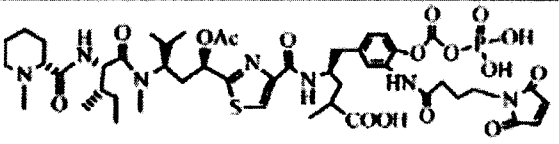
	MS: 1366.65 $(M+Na)^+$; IC_{50} = 99 nM, or 115 pM (in presence of 1 u of glycosidase)
232g	 MS: 1283.60 $(M-H)^-$; IC_{50} = 37 nM, or 101 pM (in presence of 1 u of acid phosphatase)
232h	 MS: 1302.61 $(M-H)^-$; IC_{50} = 56 nM, or 116 pM (in presence of 1 u of acid phosphatase)
232i	 MS: 1283.59 $(M-H)^-$; IC_{50} = 7.4 nM,
232j	 MS: 1302.60 $(M-H)^-$; IC_{50} = 9.7 nM,
232k	 MS: 1389.68 $(M + Na)^+$; IC_{50} = 7.9 nM, or 161 pM (in presence of 1 u of glycosidase)

232l	 MS: 1408.69 (M + Na)$^+$; IC_{50} = 7.1 nM, or 142 pM (in presence of 1 u of glycosidase)
232m	 MS: 1225.59 (M + Na)$^+$; IC_{50} = 1.1 nM
232n	 MS: 1196.54 (M + Na)$^+$; IC_{50} = 1.6 nM
232o	 MS: 1206.56 (M + Na)$^+$; IC_{50} = 127 pM
232p	 MS: 1248.60 (M + Na)$^+$; IC_{50} = 191 pM
232q	 MS: 1254.56 (M + Na)$^+$; IC_{50} = 387 pM

232r	 MS: 1212.51 (M + Na)$^+$; IC_{50} = 219 pM
232s	 MS: 1255.55 (M + Na)$^+$; IC_{50} = 268 pM
232t	 MS: 1213.51 (M + Na)$^+$; IC_{50} = 391 pM
221a	 MS: 1264.63 (M + Na)$^+$; IC_{50} = 167 pM
221b	 MS: 1270.60 (M + Na)$^+$; IC_{50} = 317 pM
233a	 MS: 1171.56 (M + Na)$^+$; IC_{50} = 242 pM

233b	 MS: 1213.61 (M + Na)$^+$; IC_{50} = 236 pM
233c	 MS: 1185.57 (M + Na)$^+$; IC_{50} = 337 pM
233d	 MS: 1156.55 (M + Na)$^+$; IC_{50} = 310 pM
222a	 MS: 1200.61 (M + Na)$^+$; IC_{50} = 412 pM
308a	 MS: 988.46 (M + Na)$^+$; IC_{50} = 167 pM
309a	 MS: 911.41 (M + Na)$^+$; IC_{50} = 257 pM
308b	 MS: 986.44 (M + Na)$^+$; IC_{50} = 19 pM

309b	 MS: 909.40 (M + Na)$^+$; IC_{50} = 119 pM
290	 MS: 817.44 (M + Na)$^+$; IC_{50} = 0.62 nM
299a	 MS: 996.41 (M + Na)$^+$; IC_{50} = 1.1 nM
299b	 MS: 997.41 (M + Na)$^+$; IC_{50} = 637 pM
241	 MS: 1017.43 (M-H)$^-$; IC_{50} = 0.8 nM, or 21 pM (in presence of 1 u of acid phosphatase)
242	 MS: 1164.50 (M-H)$^-$; IC_{50} = 1.5 nM, or 78 pM (in presence of 1 u of acid phosphatase).
247	 MS: 1048.38 (M-H)$^-$; IC_{50} = 1.1 nM, or 68 pM (in presence of 1 u of acid phosphatase).

248	 MS: 1076.40 (M-H)$^-$; IC_{50} = 1.0 nM, or 19 pM (in presence of 1 u of acid phosphatase)
206	 MS: 1033.50 (M+Na)$^+$, IC_{50} = 307 pM
211	 MS: 977.47 (M+Na)$^+$, IC_{50} = 201 pM
346	 MS: 1032.42 (M - H)$^-$, IC_{50} = 1.0 nM, or 28 pM (in presence of an acid phosphatase)
350	 MS: 969.40 (M - H)$^-$, IC_{50} = 1.8 nM, or 110 pM (in presence of an acid phosphatase)
354	 MS: 1002.40 (M - H)$^-$, IC_{50} = 2.6 nM,
357	 MS: 1046.40 (M - H)$^-$, IC_{50} = 1.3 nM, or 98 pM (in presence of an acid phosphatase)

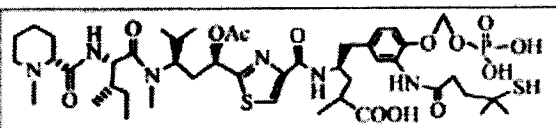
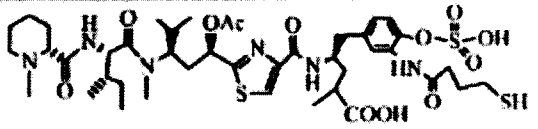
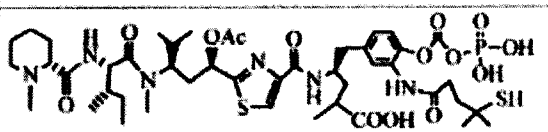
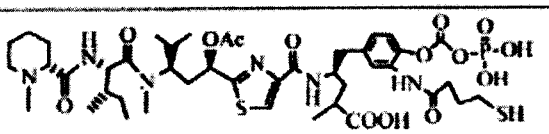
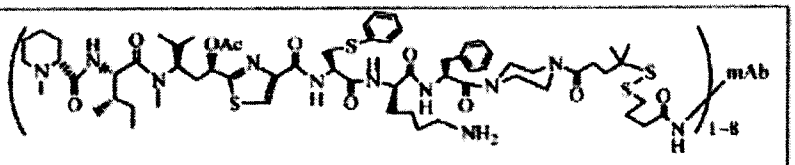
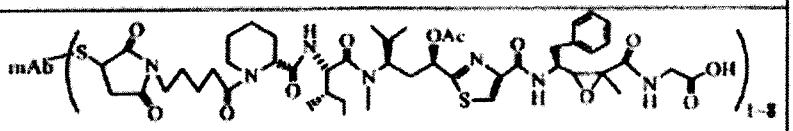
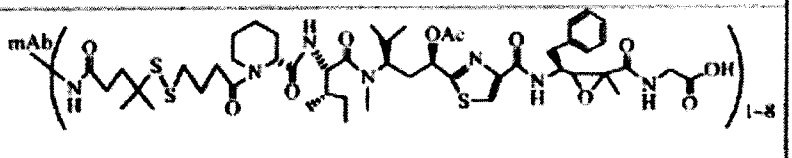
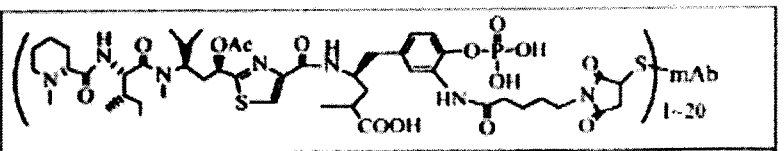
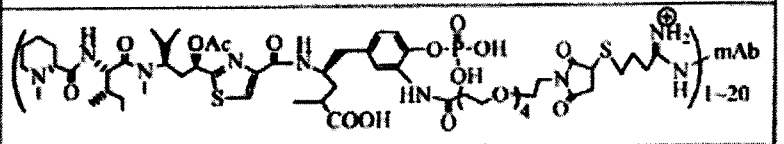
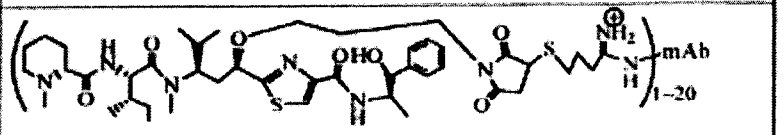
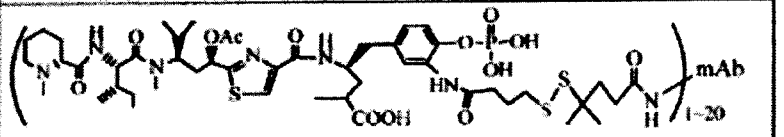
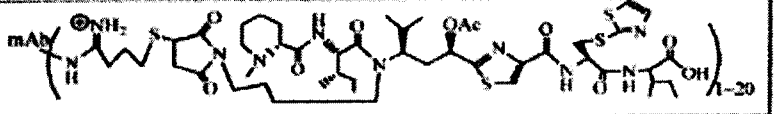
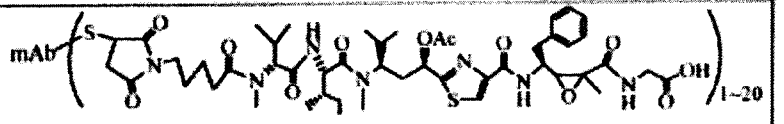
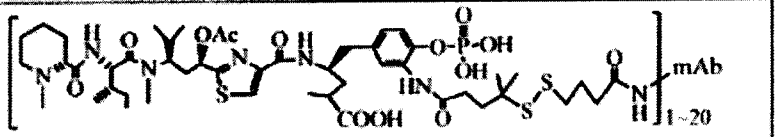
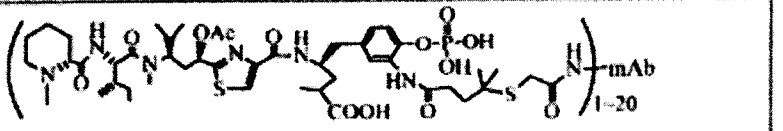
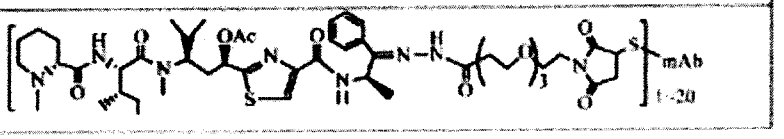
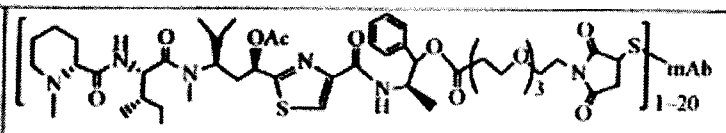
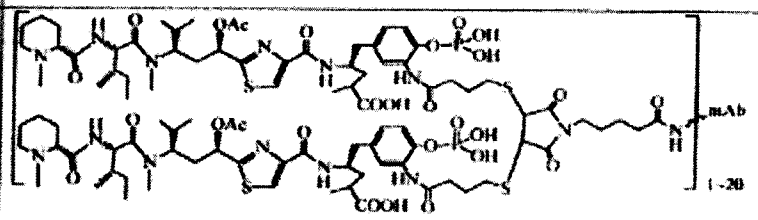
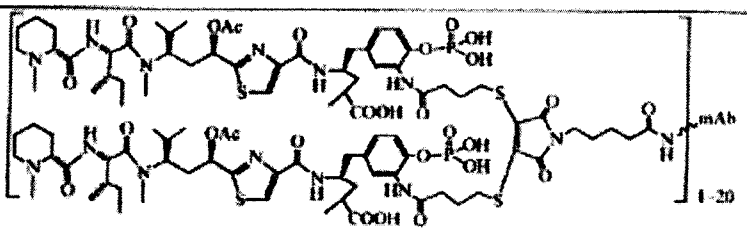
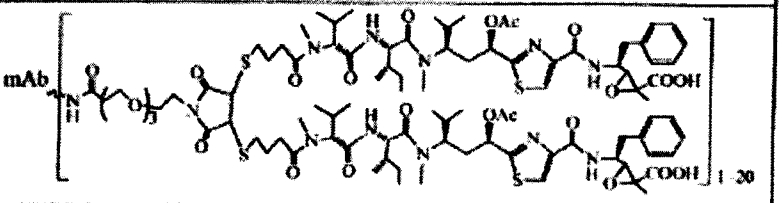
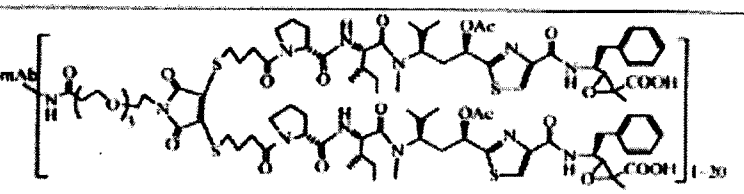
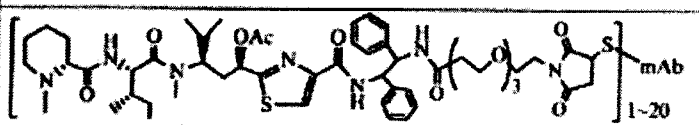
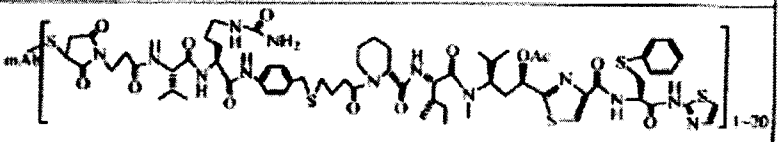
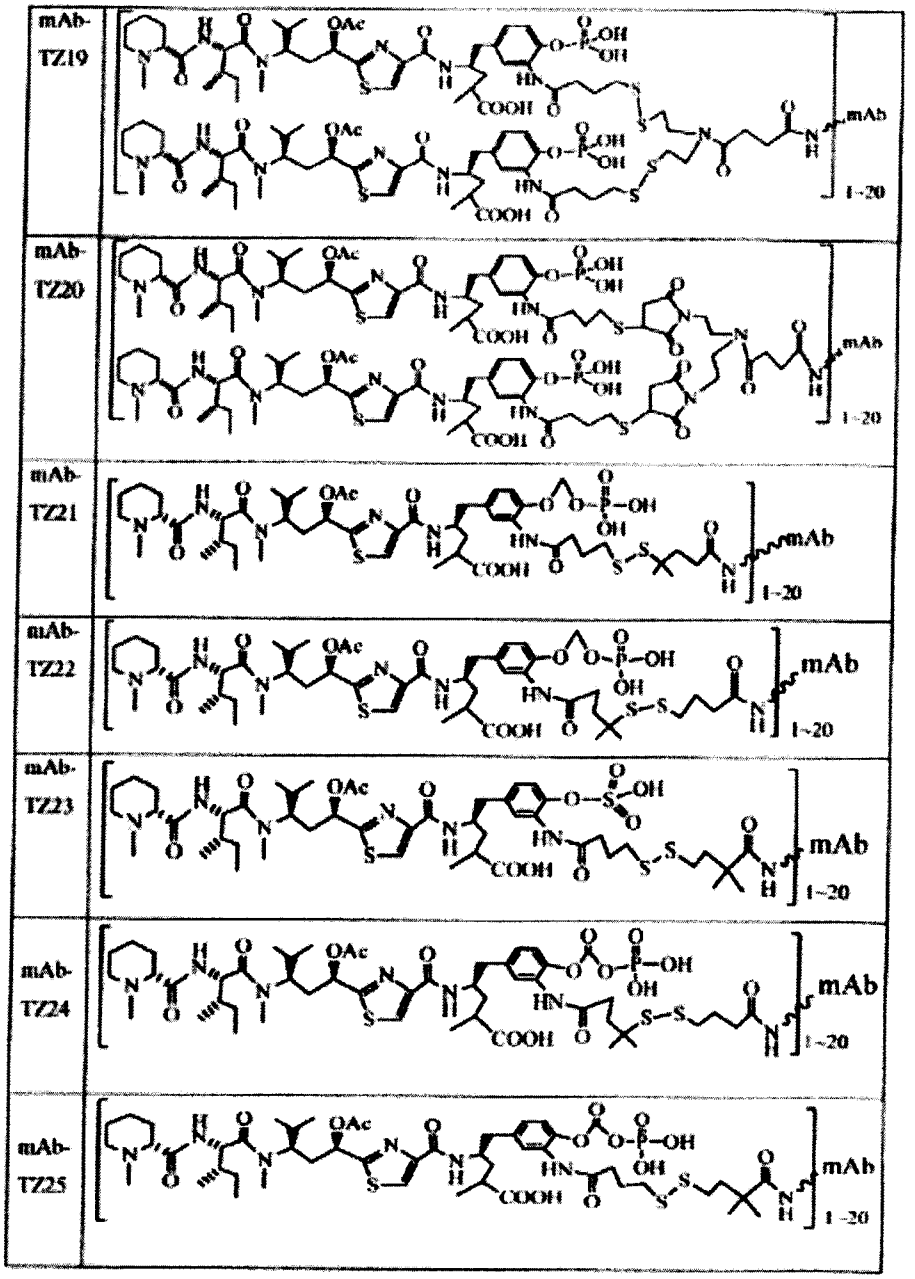
361	 MS: 997.43 (M - H)⁻, IC_{50} = 1.5 nM, or 142 pM (in presence of an acid phosphatase)
366	 MS: 939.37 (M - H)⁻, IC_{50} = 1.9 nM
371	 MS: 1011.41 (M - H)⁻, IC_{50} = 1.4 nM, or 95 pM (in presence of an acid phosphatase)
376	 MS: 983.37 (M - H)⁻, IC_{50} = 1.5 nM, or 191 pM (in presence of an acid phosphatase)

Table 2. Structures of some experimental antibody-antimitotic agent conjugates.

mAb-TZ01	
mAb-TZ02	
mAb-TZ03	

mAb-TZ04	
mAb-TZ05	
mAb-TZ06	
mAb-TZ07	
mAb-TZ08	
mAb-TZ09	
mAb-TZ10 (a)	
mAb-TZ10 (b)	
mAb-TZ11	

mAb-TZ12	
mAb-TZ13	
mAb-TZ14	
mAb-TZ15	
mAb-TZ16	
mAb-TZ17	
mAb-TZ18	



5. DETAILED DESCRIPTION OF THE INVENTION

5.1 Definitions

"Alkyl" means an aliphatic hydrocarbon group which may be straight or branched having 1 to 8 carbon atoms in the chain or cyclic. "Branched" means that one or more lower alkyl groups such as methyl, ethyl or propyl are attached to a linear alkyl chain. Exemplary alkyl groups include methyl, ethyl, *n*-propyl, *i*-propyl, *n*-butyl, *t*-butyl, *n*-pentyl, 3-pentyl, octyl,

nonyl, decyl, cyclopentyl, cyclohexyl, 2,2-dimethylbutyl, 2,3-dimethylbutyl, 2,2-dimethylpentyl, 2,3-dimethylpentyl, 3,3-dimethylpentyl, 2,3,4-trimethylpentyl, 3-methylhexyl, 2,2-dimethylhexyl, 2,4-dimethylhexyl, 2,5-dimethylhexyl, 3,5-dimethylhexyl, 2,4-dimethylpentyl, 2-methylheptyl, 3-methylheptyl, n-heptyl, isoheptyl, n-octyl, and isooctyl. A C_1-C_8 alkyl group can be unsubstituted or substituted with one or more groups including, but not limited to, -C_1~C_8 alkyl, -O-(C_1~C_8 alkyl), -aryl, -C(O)R', -OC(O)R', -C(O)OR', -C(O)NH_2, -C(O)NHR', -C(O)N(R')$_2$-NHC(O)R', -$S(O)_2$R', -S(O)R', -OH, -halogen (F, Cl, Br or I), -N_3, -NH_2, -NH(R'), -N(R')$_2$ and -CN; where each R' is independently selected from -C_1~C_8 alkyl and aryl.

A "C_3~C_8 carbocycle" means a 3-, 4-, 5-, 6-, 7- or 8-membered saturated or unsaturated non-aromatic carbocyclic ring. Representative C_3~C_8 carbocycles include, but are not limited to, -cyclopropyl, -cyclobutyl, -cyclopentyl, -cyclopentadienyl, -cyclohexyl, -cyclohexenyl, -1,3-cyclohexadienyl, -1,4-cyclohexadienyl, -cycloheptyl, -1,3-cycloheptadienyl, -1,3,5-cycloheptatrienyl, -cyclooctyl, and -cyclooctadienyl. A C_3~C_8 carbocycle group can be unsubstituted or substituted with one or more groups including, but not limited to, --C_1~C_8 alkyl, -O-(C_1~C_8 alkyl), -aryl, -C(O)R', -OC(O)R', -C(O)OR', -C(O)NH_2, -C(O)NHR', -C(O)N(R')$_2$-NHC(O)R', -$S(O)_2$R', -S(O)R', -OH, -halogen, -N_3, -NH_2, -NH(R'), -N(R')$_2$ and -CN; where each R' is independently selected from -C_1~C_8 alkyl and aryl.

A "C_3~C_8carbocyclo" refers to a C_3~C_8 carbocycle group defined above wherein one of hydrogen atoms on the carbocycle is replaced with a bond.

"Alkenyl" means an aliphatic hydrocarbon group containing a carbon-carbon double bond and which may be straight or branched having 2 to 8 carbon atoms in the chain. Exemplary

alkenyl groups include ethenyl, propenyl, *n*-butenyl, *i*-butenyl, 3-methylbut-2-enyl, *n*-pentenyl, hexylenyl, heptenyl, octenyl.

"Alkynyl" means an aliphatic hydrocarbon group containing a carbon-carbon triple bond and which may be straight or branched having 2 to 8 carbon atoms in the chain. Exemplary alkynyl groups include ethynyl, propynyl, *n*-butynyl, 2-butynyl, 3-methylbutynyl, pentynyl, *n*-pentynyl, hexylynyl, heptynyl, and octynyl.

"Heteroalkyl" is C_2~C_8 alkyl in which one to four carbon atoms are independently replaced with a heteroatom from the group consisting of O, S and N.

"Aryl" or Ar refers to an aromatic or hetero aromatic group, composed of one or several rings, comprising three to fourteen carbon atoms, preferentially six to ten carbon atoms. The term of hetero aromatic group refers one or several carbon on aromatic group, preferentially one, two, three or four carbon atoms are replaced by O, N, Si, Se, P or S, preferentially by O, S, and N. The term aryl or Ar also refers to a aromatic group, wherein one or several H atoms are replaced independently by R^{13}, F, Cl, Br, I, OR^{13}, or SR^{13}, $NR^{13}R^{14}$, $N{=}NR^{13}$, $N{=}R^{13}$, $NR^{13}R^{14}$, NO_2, $SOR^{13}R^{14}$, SO_2R^{13}, SO_3R^{13}, OSO_3R^{13}, $PR^{13}R^{14}$, $POR^{13}R^{14}$, $PO_2R^{13}R^{14}$, $OPO_3R^{13}R^{14}$, or $PO_3R^{13}R^{14}$ wherein R^{13}, R^{14} are independently H, alkyl, alkenyl, alkynyl, heteroalkyl, aryl, arylalkyl, carbonyl, or pharmaceutical salts.

"Halogen atom" refers to fluorine, chlorine, bromine or iodine atom; preferably fluorine and chlorine atom.

"Heterocycle" refers to an aromatic or non-aromatic C_2~C_8 heterocycle in which one to four of the ring carbon atoms are independently replaced with a heteroatom from the group of O, N, S Se, and P. Preferable heteroatoms are oxygen, nitrogen and sulfur. Suitable heterocycles are also disclosed in *The Handbook of Chemistry and Physics*, 78th Edition, CRC Press, Inc., 1997-1998, p. 2-25 to 2-26. Preferred non aromatic heterocyclic include, but are not limited to epoxy, aziridinyl, thiiranyl, pyrrolidinyl, pyrazolidinyl, imidazolidinyl, oxiranyl, tetrahydrofuranyl, dioxolanyl, tetrahydro-pyranyl, dioxanyl, dioxolanyl, piperidyl, piperazinyl, morpholinyl, pyranyl, imidazolinyl, pyrrolinyl, pyrazolinyl, thiazolidinyl, tetrahydrothiopyranyl, dithianyl, thiomorpholinyl, dihydro-pyranyl, tetrahydropyranyl, dihydropyranyl, tetrahydro-pyridyl, dihydropyridyl, tetrahydropyrinidinyl, dihydrothiopyranyl, azepanyl, as well as the fused systems resulting from the condensation with a phenyl group.

The term "heteroaryl" or aromatic heterocycles refers to a 5 to 14, preferably 5 to 10 membered aromatic hetero, mono-, bi- or multicyclic ring. Examples include pyrrolyl, pyridyl, pyrazolyl, thienyl, pyrimidinyl, pyrazinyl, tetrazolyl, indolyl, quinolinyl, purinyl, imidazolyl, thienyl, thiazolyl, benzothiazolyl, furanyl, benzofuranyl, 1,2,4-thiadiazolyl, isothiazolyl, triazoyl, tetrazolyl,

isoquinolyl, benzothienyl, isobenzofuryl, pyrazolyl, carbazolyl, benzimidazolyl, isoxazolyl, pyridyl-N-oxide, as well as the fused systems resulting from the condensation with a phenyl group.

"Alkyl", "cycloalkyl", "alkenyl", "alkynyl", "aryl", "heteroaryl", "heterocycle" and the like refer also to the corresponding "alkylene", "cycloalkylene", "alkenylene", "alkynylene", "arylene", "heteroarylene", "heterocyclene" and the likes which are formed by the removal of two hydrogen atoms.

"Pharmaceutically" or "pharmaceutically acceptable" refer to molecular entities and compositions that do not produce an adverse, allergic or other untoward reaction when administered to an animal, or a human, as appropriate.

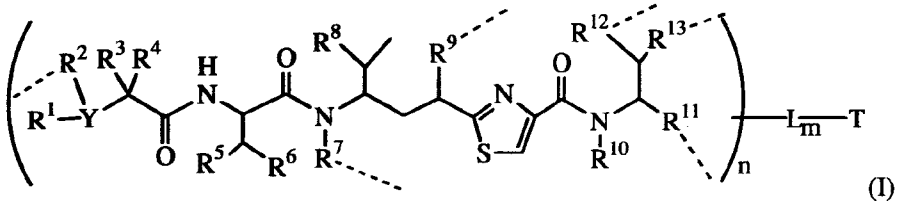
"Pharmaceutically acceptable excipient" includes any carriers, diluents, adjuvants, or vehicles, such as preserving or antioxidant agents, fillers, disintegrating agents, wetting agents, emulsifying agents, suspending agents, solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions as suitable therapeutic combinations.

As used herein, "pharmaceutical salts" refer to derivatives of the disclosed compounds wherein the parent compound is modified by making acid or base salts thereof. The pharmaceutically acceptable salts include the conventional non-toxic salts or the quaternary ammonium salts of the parent compound formed, for example, from non-toxic inorganic or organic acids. For example, such conventional non-toxic salts include those derived from inorganic acids such as hydrochloric, hydrobromic, sulfuric, sulfamic, phosphoric, nitric and the like; and the salts prepared from organic acids such as acetic, propionic, succinic, tartaric, citric, methanesulfonic, benzenesulfonic, glucuronic, glutamic, benzoic, salicylic, toluenesulfonic, oxalic, fumaric, maleic, lactic and the like. Further addition salts include ammonium salts such as tromethamine, meglumine, epolamine, etc., metal salts such as sodium, potassium, calcium, zinc or magnesium.

The pharmaceutical salts of the present invention can be synthesized from the parent compound which contains a basic or acidic moiety by conventional chemical methods. Generally, such salts can be prepared by reaction of the free acid or base forms of these compounds with a stoichiometric amount of the appropriate base or acid in water or in an organic solvent, or in a mixture of the two. Generally, non-aqueous media like ether, ethyl acetate, ethanol, isopropanol, or acetonitrile are preferred. Lists of suitable salts are found in *Remington's Pharmaceutical Sciences*, 17th ed., Mack Publishing Company, Easton, PA, 1985, p. 1418.

5.2 Drug-Linker- Binding Ligand Conjugates

As stated above, this invention provides a cell surface binding molecule - antimitotic agent conjugate of formula (I):



(I)

and pharmaceutical acceptable salts and solvates thereof

Wherein T is a targeting or binding ligand; L is a releasable linker; ----- is a linkage bond that L connects to a molecule inside the bracket independently; n is 1~20 and m is 1~10.

Inside the round bracket is a potent antimitotic agent/drug wherein R^1, R^2, R^3, and R^4 are independently C_1~C_8 of alkyl, heteroalkyl; C_2~C_8 of heterocyclic, carbocyclic, alkylcycloalkyl, heterocycloalkyl, C_3~C_8 of aryl, Ar-alkyl, heteroalkylcycloalkyl, alkylcarbonyl; or two R's, such as R^1R^2, R^2R^3, R^3R^4, R^5R^6 and $R^{12}R^{13}$ can be 3~7 members of a carbocyclic, cycloalkyl, or heterocyclic, heterocycloalkyl ring system; Y is N or CH; In addition, R^1, R^3, and R^4 can be H; and R^2 can be absent.

Wherein R^5, R^6, R^8 and R^{10} are independently H, C_1~C_4 of alkyl or heteroalkyl.

Wherein R^7 is independently selected from H, R^{14}, or $-R^{14}C(=O)X^1R^{15}$ or $-R^{14}X^1R^{15}$, wherein R^{14} and R^{15} are independently selected from C_1~C_8 of alkyl, or heteroalkyl; C_2~C_8 of alkenyl, alkynyl; heterocyclic, carbocyclic, cycloalkyl; C_3~C_8 of aryl, heterocycloalkyl, heteroaralkyl, heteroalkylcycloalkyl, alkylcarbonyl; X^1 is selected from O, S, S-S, NH, or NR^{14}.

Wherein R^9 is independently H, -O-, $-OR^{14}$-, $-OC(=O)R^{14}$-, $-OC(=O)NHR^{14}$-, $-OC(=O)R^{14}SSR^{15}$-, $OP(=O)(OR^{14})$-, or $OR^{14}OP(=O)(OR^{15})$, wherein R^{14}, R^{15} are independently C_1~C_8 of alkyl, heteroalkyl; C_2~C_8 of alkenyl, alkynyl, heterocyclic, carbocyclic; C_3~C_8 of aryl, cycloalkyl, alkylcycloalkyl, heterocycloalkyl, heteroalkylcycloalkyl, heteroaralkyl, alkylcarbonyl.

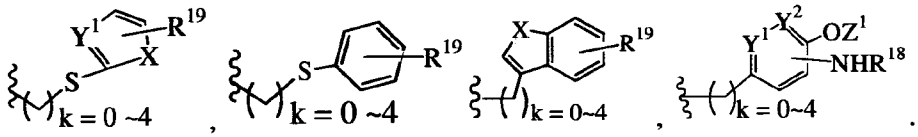
Wherein R^{11} is independently H, R^{14}, $-R^{14}C(=O)R^{16}$, $-R^{14}X^2R^{16}$, $-R^{14}C(=O)X^2$, wherein X^2 is -O-, -S-, -NH-, $-N(R^{14})$-, $-O-R^{14}$-, $-S-R^{14}$-, $-S(=O)-R^{14}$-, or $-NHR^{14}$-; R^{14} is C_1~C_8 of alkyl, heteroalkyl; C_2~C_8 of alkenyl, alkynyl, heterocyclic, carbocyclic; C_3~C_8 of aryl, cycloalkyl, alkylcycloalkyl, heterocycloalkyl, heteroalkylcycloalkyl, heteroaralkyl, alkylcarbonyl; R^{16} is H, OH, R^{14} or one to four amino acid units;

Wherein R^{12} is independently R^{14}, -O-, -S-, -N-, =N-, =NNH-, $-NH(R^{14})$-, $-OR^{14}$-, -C(O)O-, $-C(O)OR^{16}$-, C(O)NH-, $C(O)NHR^{14}$, $-SR^{14}$-, $-S(=O)R^{14}$-, $-P(=O)(OR^{16})$ -, $-OP(=O)(OR^{16})$ -, $-CH_2OP(=O)(OR^{16})$ -, $-C(O)OP(=O)(OR^{16})$ -, $-SO_2R^{16}$-. R^{14} is independently C_1~C_8 of alkyl, heteroalkyl; C_2~C_8 of alkenyl, alkynyl, hetero-cyclic, carbocyclic; C_3~C_8 of aryl, cycloalkyl,

alkylcycloalkyl, heterocycloalkyl, heteroaralkyl, heteroalkylcycloalkyl, alkylcarbonyl. R^{16} is H, OH, R^{14} or one to four amino acid units;

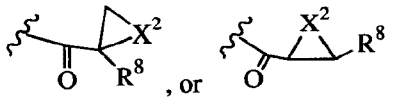
Wherein R^{13} is C_1~C_{10} of alkyl, heteroalkyl, alkyl acid, alkyl amide, alkyl amine, or Ar; Ar refers to a aromatic or hetero aromatic group, composed of one or several rings, comprising four to ten carbon, preferentially four to six carbon atoms. The term of hetero aromatic group refers one or several carbon on aromatic group, preferentially one, two or three carbon atoms are replaced by O, N, Si, Se, P or S, preferentially O, S, N. The term aryl or Ar also refers to a aromatic group, wherein one or several H atoms are replaced independently by R^{17}, F, Cl, Br, I, OR^{16}, SR^{16}, $NR^{16}R^{17}$, $N{=}NR^{16}$, $N{=}R^{16}$, $NR^{16}R^{17}$, NO_2, $SOR^{16}R^{17}$, SO_2R^{16}, SO_3R^{16}, OSO_3R^{16}, $PR^{16}R^{17}$, $POR^{16}R^{17}$, $PO_2R^{16}R^{17}$, $OP(O)(OR^{17})_2$, $OCH_2OP(O)(OR^{17})_2$, $OC(O)OP(O)(OR^{17})_2$, $PO(OR^{16})(OR^{17})$, $OP(O)(OR^{16})OP(O)(OR^{17})_2$, $OC(O)R^{17}$ or $OC(O)NHR^{17}$, wherein R^{16}, R^{17} are independently H, C_1~C_8 of alkyl, heteroalkyl; C_2~C_8 of alkenyl, alkynyl, heterocyclic, carbocyclic; C_3~C_8 of aryl, cycloalkyl, alkylcycloalkyl, heterocycloalkyl, heteroalkylcycloalkyl, heteroaralkyl, alkylcarbonyl or C_4 ~ C_{12} glycosides, or pharmaceutical salts.

In addition, R^{12} can be H when R^{10} is not H, or when R^{13} is:



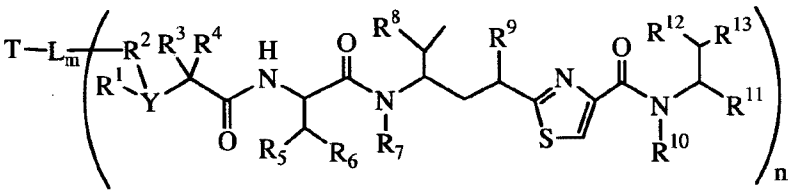
. wherein Z^1 is H, $CH_2OP(O)(OR^{18})_2$, $C(O)OP(O)(OR^{18})_2$, $PO(OR^{18})_2$, $C(O)R^{18}$, $C(O)NHR^{18}$, $SO_2(OR^{18})$, C_4 ~ C_{12} glycosides or C_1~C_8 of alkyl, carboxyalkyl, heterocyclic; R^{18} is H, C_1~C_8 of alkyl, carboxyalkyl, heteroalkyl; C_2~C_8 of alkenyl, alkynyl, heterocyclic; C_3~C_8 of aryl, alkylcarbonyl; R^{19} is H, OH, NH_2, $OSO_2(OR^{18})$, $XCH_2OP(O)(OR^{18})_2$, $XPO(OR^{18})_2$, $XC(O)R^{18}$, $XC(O)NHR^{18}$, C_1~C_8 of alkyl, carboxyalkyl, carboxylic acid derivative; C_2~C_8 of alkenyl, alkynyl, heterocyclic; C_3~C_8 of aryl, alkylcarbonyl; or pharmaceutical salts; X is O, S, NH; Y^1 and Y^2 are N or CH respectively.

Or R^{12} can be H when R^{11} is:



, or X^2 is O, S, N-R^8; R^8 is H, C_1~C_6 of alkyl or heteroalkyl

In another embodiment, conjugates of antimitotic agents have the formula (II)



(II)

and pharmaceutical acceptable salts and solvates thereof

Wherein T is a targeting or binding ligand; L is a releasable linker; ----- is a linkage bond that L connects to a molecule inside the bracket independently; n is 1~20 and m is 1~10.

Inside the round bracket is a potent antimitotic agent/drug wherein R^1, R^2, R^3, and R^4 are independently C_1~C_8 of alkyl, heteroalkyl; C_2~C_8 of heterocyclic, carbocyclic, alkylcycloalkyl, heterocycloalkyl, C_3~C_8 of aryl, Ar-alkyl, heteroalkylcycloalkyl, alkylcarbonyl; or two R's, such as R^1R^2, R^2R^3, R^3R^4, R^5R^6 and $R^{12}R^{13}$ can be 3~7 members of a carbocyclic, cycloalkyl, or heterocyclic, heterocycloalkyl ring system; Y is N or CH; In addition, R^1, R^3, and R^4 can be H; and R^2 can be absent.

Wherein R^5, R^6, R^8 and R^{10} are independently H, C_1~C_4 of alkyl or heteroalkyl.

Wherein R^7 is independently selected from H, R^{14}, or $-R^{14}C(=O)X^1R^{15}$ or $-R^{14}X^1R^{15}$, wherein R^{14} and R^{15} are independently selected from C_1~C_8 of alkyl, or heteroalkyl; C_2~C_8 of alkenyl, alkynyl; heterocyclic, carbocyclic, cycloalkyl; C_3~C_8 of aryl, heterocycloalkyl, heteroaralkyl, heteroalkylcycloalkyl, alkylcarbonyl; X^1 is selected from O, S, S-S, NH, or NR^{14}.

Wherein R^9 is independently H, -O-, $-OR^{14}$, $-OC(=O)R^{14}$, $-OC(=O)NHR^{14}$, $-OC(=O)R^{14}SSR^{15}$, $OP(=O)(OR^{14})$, or $OR^{14}OP(=O)(OR^{15})$, wherein R^{14}, R^{15} are independently C_1~C_8 of alkyl, heteroalkyl; C_2~C_8 of alkenyl, alkynyl, heterocyclic, carbocyclic; C_3~C_8 of aryl, cycloalkyl, alkylcycloalkyl, heterocycloalkyl, heteroalkylcycloalkyl, heteroaralkyl, alkylcarbonyl.

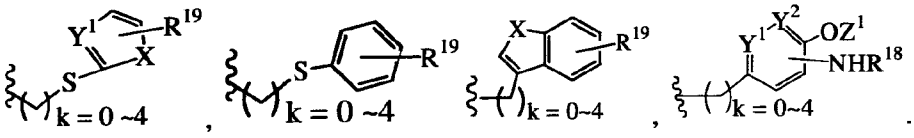
Wherein R^{11} is independently H, $-R^{14}C(=O)R^{16}$, $-R^{14}X^2R^{16}$, $-R^{14}C(=O)X^2$, wherein X^2 is -O-, -S-, -NH-, $-N(R^{14})-$, $-O-R^{14}-$, $-S-R^{14}-$, $-S(=O)-R^{14}-$, or $-NHR^{14}-$; R^{14} is C_1~C_8 of alkyl, heteroalkyl; C_2~C_8 of alkenyl, alkynyl, heterocyclic, carbocyclic; C_3~C_8 of aryl, cycloalkyl, alkylcycloalkyl, heterocycloalkyl, heteroalkylcycloalkyl, heteroaralkyl, alkylcarbonyl; R^{16} is H, OH, R^{14} or one to four amino acid units;

Wherein R^{12} is independently R^{14}, -OH, -SH, $-NH_2$, =NH, $=NNH_2$, $-NH(R^{14})$, $-OR^{14}$, COR^{16}, $COOR^{14}$, $C(O)NH_2$, $C(O)NHR^{14}$, $-SR^{14}$, $-S(=O)R^{14}$, $-P(=O)(OR^{16})_2$, $-OP(=O)(OR^{16})_2$, $-CH_2OP(=O)(OR^{16})_2$, $-SO_2R^{16}$. R^{14} is independently C_1~C_8 of alkyl, heteroalkyl; C_2~C_8 of alkenyl, alkynyl, hetero-cyclic, carbocyclic; C_3~C_8 of aryl, cycloalkyl, alkylcycloalkyl, heterocycloalkyl, heteroaralkyl, heteroalkylcycloalkyl, alkylcarbonyl. R^{16} is H, OH, R^{14} or one to four amino acid units;

Wherein R^{13} is C_1~C_{10} of alkyl, heteroalkyl, alkyl acid, alkyl amide, alkyl amine, or Ar; Ar refers to a aromatic or hetero aromatic group, composed of one or several rings, comprising four to ten carbon, preferentially four to six carbon atoms. The term of hetero aromatic group refers one or several carbon on aromatic group, preferentially one, two or three carbon atoms are replaced by O, N, Si, Se, P or S, preferentially O, S, N. The term aryl or Ar also refers to a aromatic group, wherein one or several H atoms are replaced independently by R^{17}, F, Cl, Br, I,

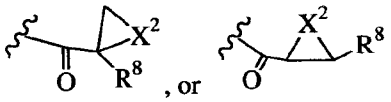
OR^{16}, SR^{16}, $NR^{16}R^{17}$, $N{=}NR^{16}$, $N{=}R^{16}$, $NR^{16}R^{17}$, NO_2, $SOR^{16}R^{17}$, SO_2R^{16}, SO_3R^{16}, OSO_3R^{16}, $PR^{16}R^{17}$, $POR^{16}R^{17}$, $PO_2R^{16}R^{17}$, $OP(O)(OR^{17})_2$, $OCH_2OP(O)(OR^{17})_2$, $OC(O)OP(O)(OR^{17})_2$, $PO(OR^{16})(OR^{17})$, $OPO(OR^{16})OPO(OR^{16})(OR^{17})$, $OC(O)R^{17}$ or $OC(O)NHR^{17}$, wherein R^{16}, R^{17} are independently H, C_1~C_8 of alkyl, heteroalkyl; C_2~C_8 of alkenyl, alkynyl, heterocyclic, carbocyclic; C_3~C_8 of aryl, cycloalkyl, alkylcycloalkyl, heterocycloalkyl, heteroalkylcycloalkyl, heteroaralkyl, alkylcarbonyl or C_4 ~ C_{12} glycosides, or pharmaceutical salts.

In addition, R^{12} can be H when R^{10} is not H, or when R^{13} is:



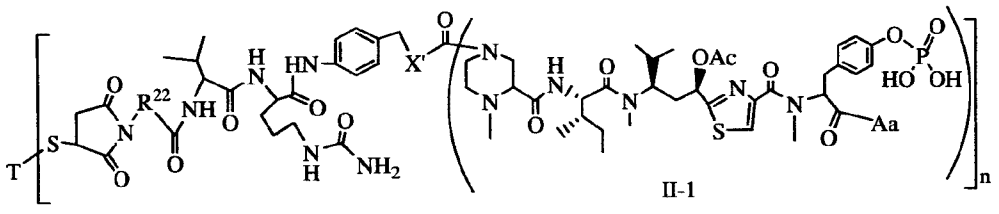
. wherein Z^1 is H, $CH_2OP(O)(OR^{18})_2$, $C(O)OP(O)(OR^{18})_2$, $PO(OR^{18})_2$, $C(O)R^{18}$, $C(O)NHR^{18}$, $SO_2(OR^{18})$, C_4 ~ C_{12} glycosides or C_1~C_8 of alkyl, carboxyalkyl, heterocyclic; R^{18} is H, C_1~C_8 of alkyl, carboxyalkyl, heteroalkyl; C_2~C_8 of alkenyl, alkynyl, heterocyclic; C_3~C_8 of aryl, alkylcarbonyl; R^{19} is H, OH, NH_2, $OSO_2(OR^{18})$, $XCH_2OP(O)(OR^{18})_2$, $XC(O)OP(O)(OR^{18})_2$, $XPO(OR^{18})_2$, $XC(O)R^{18}$, $XC(O)NHR^{18}$, C_1~C_8 of alkyl, carboxyalkyl, carboxylic acid derivative; C_2~C_8 of alkenyl, alkynyl, heterocyclic; C_3~C_8 of aryl, alkylcarbonyl; or pharmaceutical salts; X is O, S, NH; Y^1 and Y^2 are N or CH respectively.

Or R^{12} can be H when R^{11} is:

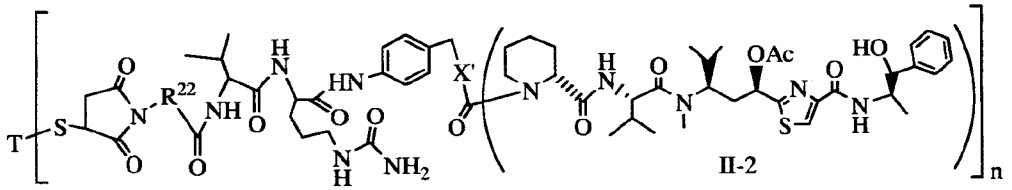


X^2 is O, S, N-R^8; R^8 is H, C_1~C_6 of alkyl or heteroalkyl

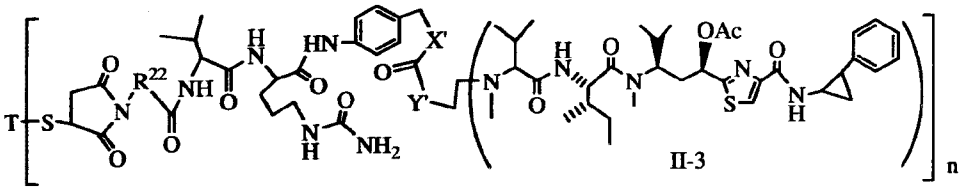
Illustrative classes of compounds of formula II have the structures:



IIa

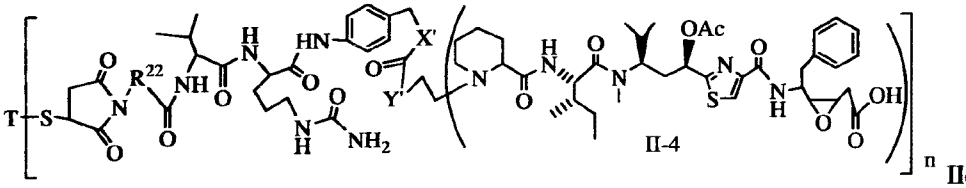


IIb



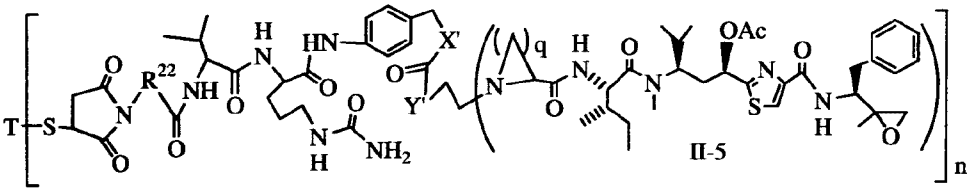
II-3

IIc



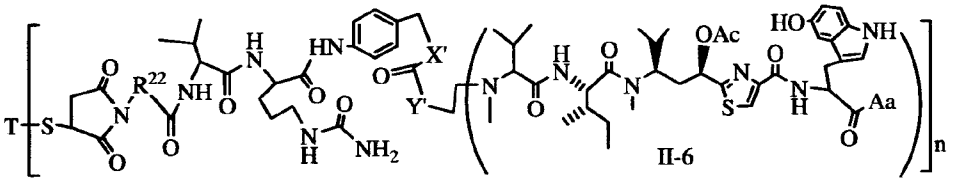
II-4

IId



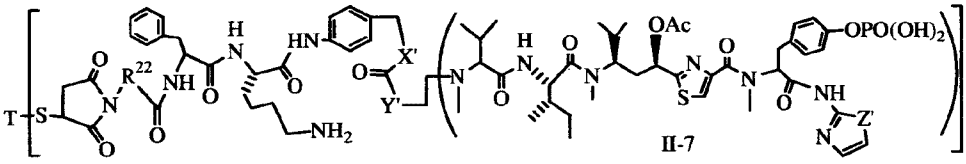
II-5

IIe



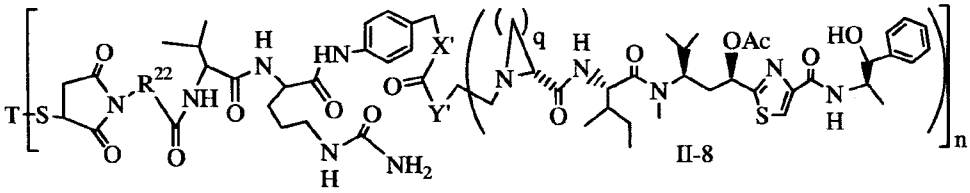
II-6

IIf



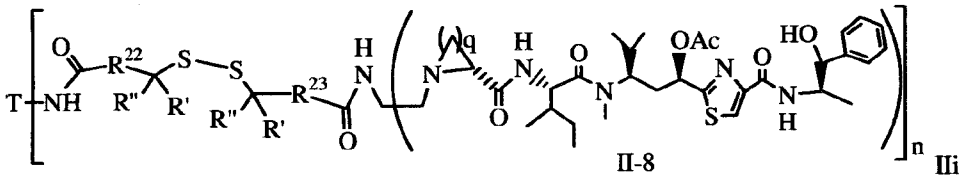
II-7

IIg



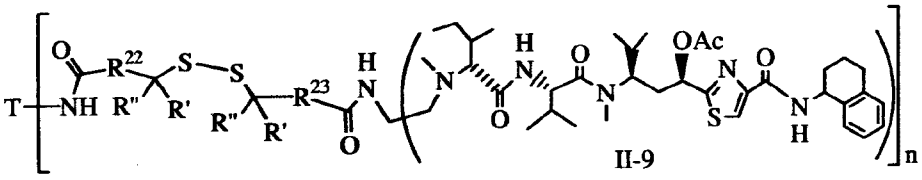
II-8

IIh

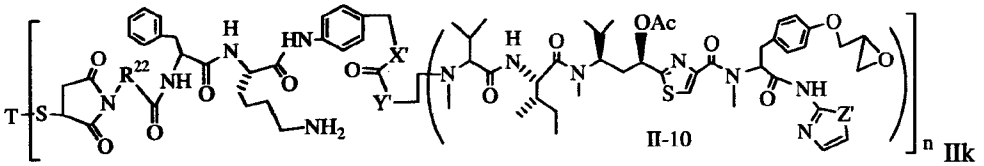


II-8

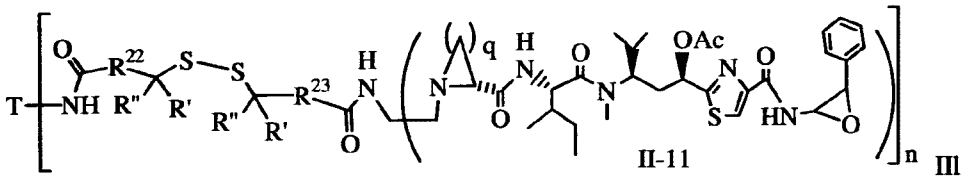
IIi



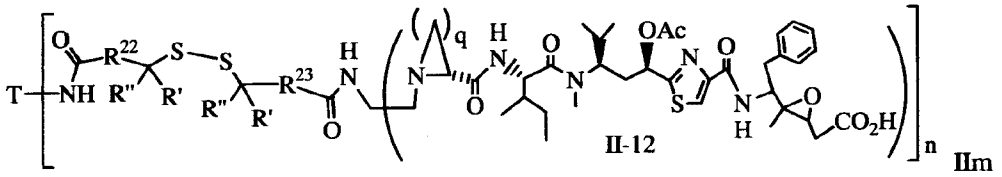
II-9 IIj



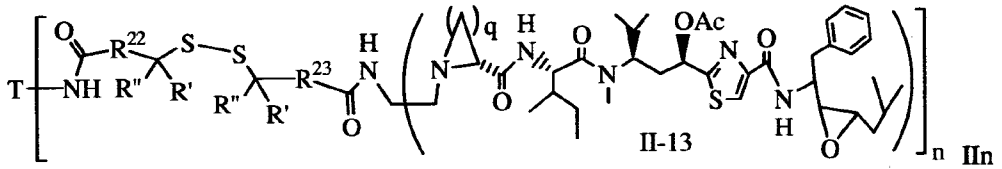
II-10 IIk



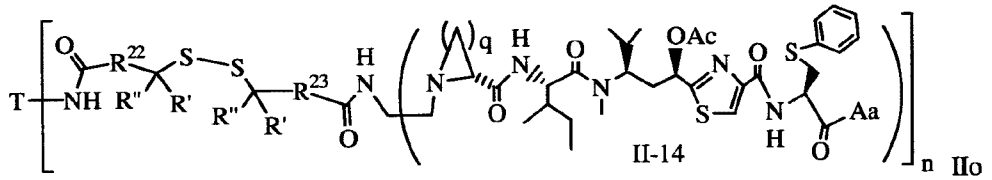
II-11 III



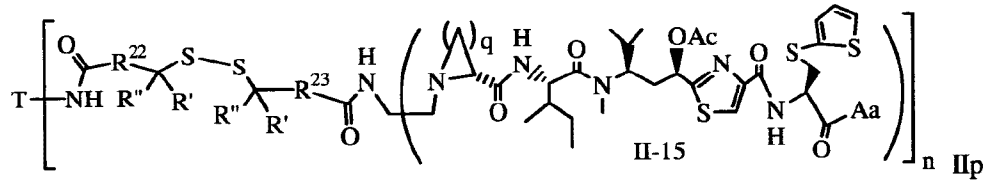
II-12 IIm



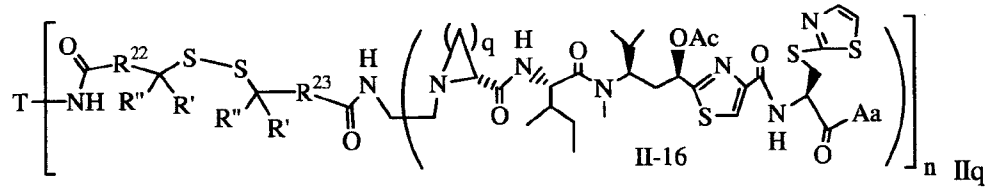
II-13 IIn



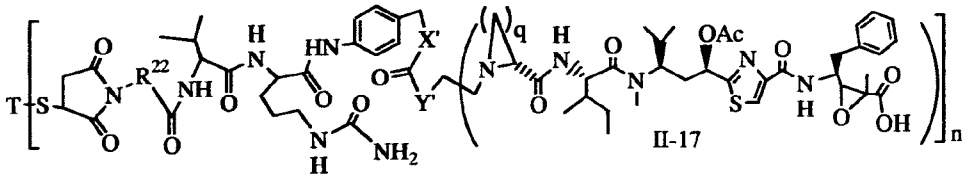
II-14 IIo



II-15 IIp



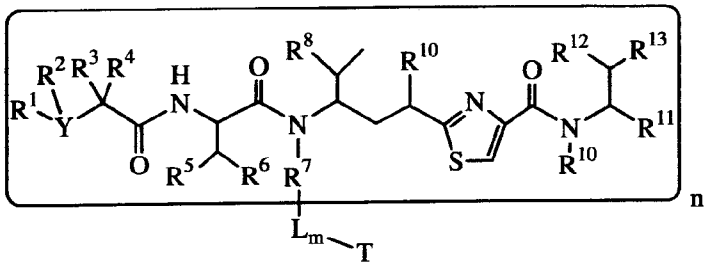
II-16 IIq



IIr.

wherein Aa is a natural or an unnatural amino acid; n is 1 ~ 20; q = 1~5; X', Y' and Z' are independently CH, O, S, NH, or NR^{22}; R^{22} and R^{23} are independently C_1~C_8 of alkyl; C_2~C_8 of alkenyl, alkynyl, heteroalkyl; C_3~C_8 of aryl, heterocyclic, carbocyclic, cycloalkyl, alkylcycloalkyl, heterocycloalkyl, Ar-alkyl, heteroalkylcycloalkyl, heteroaralkyl, or -$(OCH_2CH_2)_n$-; R' and R'' are independently H or CH_3. Inside the round brackets are the antimitotic drugs and the inside square brackets are the antimitotic drug with linkers.

In another embodiment, a conjugate of a cell binding molecule-antimitotic agent has the formula (III):

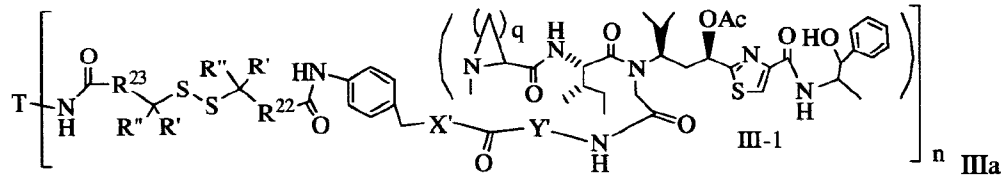


(III)

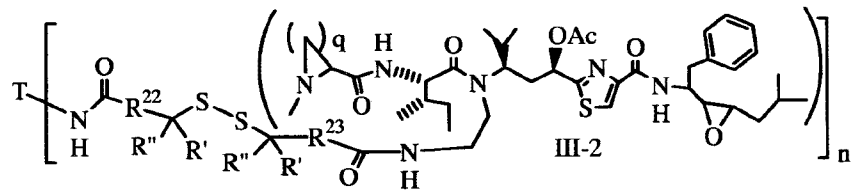
wherein T, L, m, Y, R^1, R^2, R^3, R^4, R^5, R^6, R^8, R^9, R^{10}, R^{11}, R^{12}, R^{13} and n are defined the same as in formula (II).

Wherein R^7 is independently selected from R^{14}, or -$R^{14}C(=O)X^1R^{15}$- or -$R^{14}X^1R^{15}$-. R^{14} and R^{15} are independently selected from C_1~C_8 of alkyl, or heteroalkyl; C_2~C_8 of alkenyl, alkynyl; heterocyclic, carbocyclic, cycloalkyl; C_3~C_8 of aryl, heterocycloalkyl, heteroaralkyl, heteroalkylcycloalkyl, alkylcarbonyl; X^1 is selected from O, S, S-S, NH, or NR^{14}.

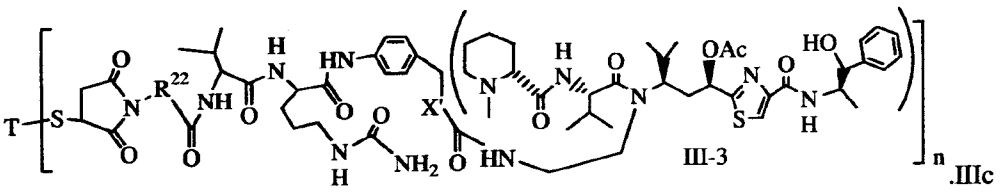
Illustrative examples of compounds of formula (III) have the structures:



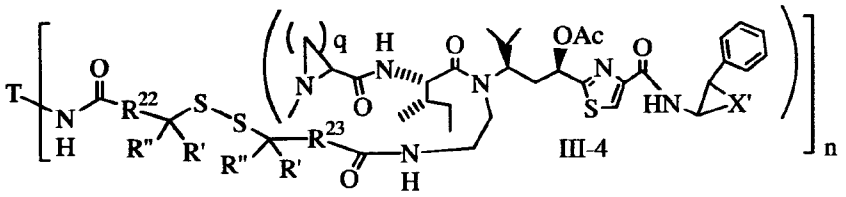
IIIa



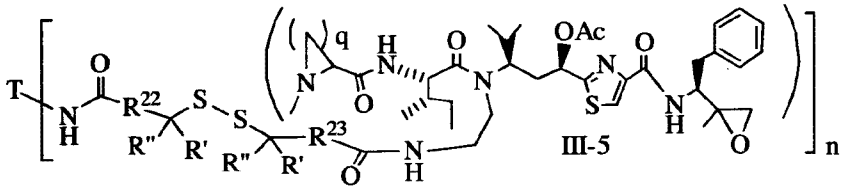
IIIb



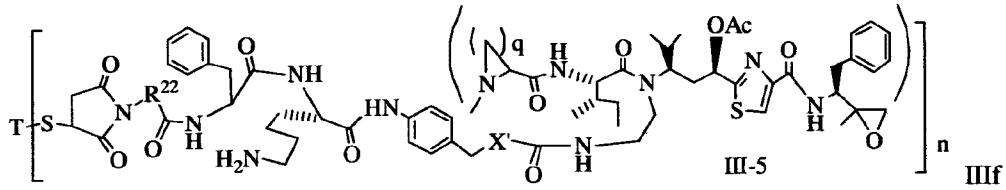
IIIc



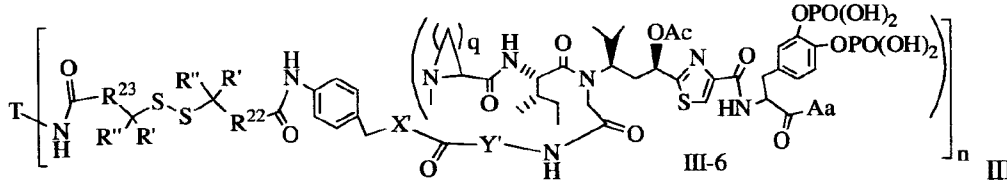
IIId



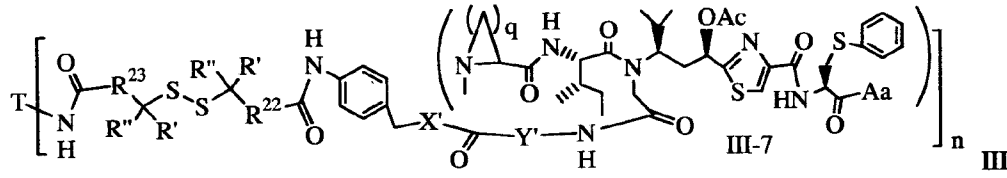
IIIe



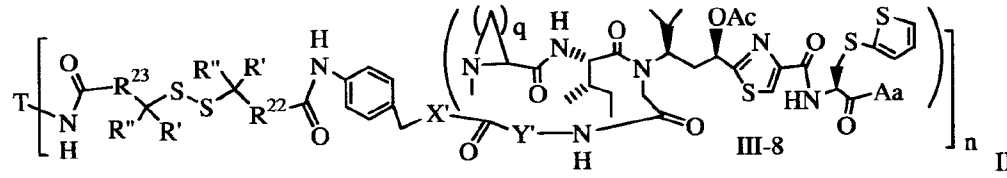
IIIf



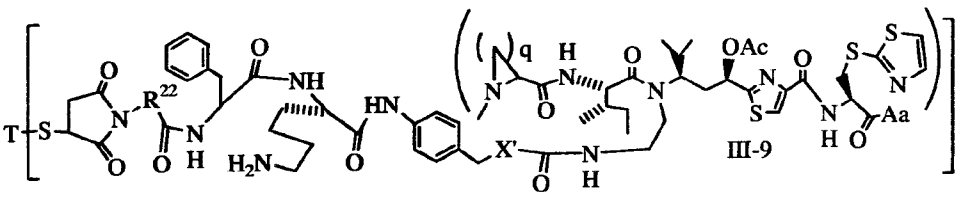
IIIg



IIIh



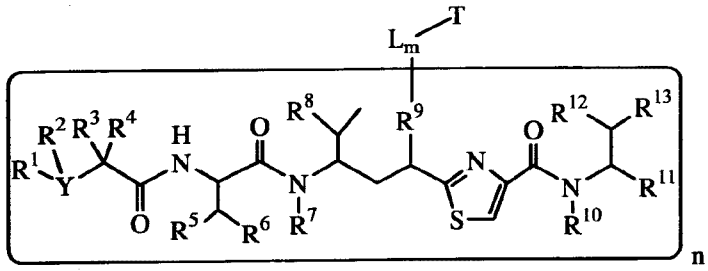
IIIi



IIIj

Wherein Ar, n, q, X', Y', Z', R^{22}, R^{23}, R' and R'' are defined the same as for formula IIa ~ IIr.

In another embodiment, a binding molecule-antimitotic agent conjugate has the formula (IV)

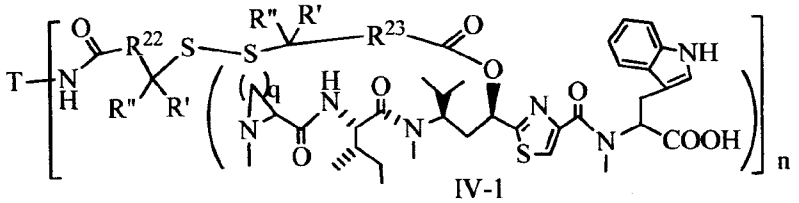


n (IV)

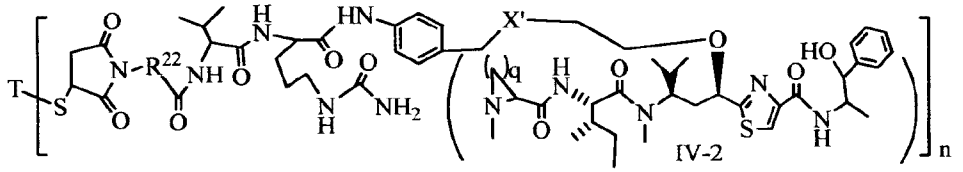
wherein T, L, m, Y, R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^{10}, R^{11}, R^{12}, R^{13} and n are defined the same as in formula (II).

Wherein R^9 is independently, -O-, -OR^{14}-, -OC(=O)R^{14}-, -OC(=O)NHR^{14}-, -OC(=O)R^{14}SSR^{15}-, -OP(=O)(OR^{14})O-, wherein R^{14}, R^{15} are independently C_1~C_8 of alkyl, heteroalkyl; C_3~C_8 of aryl, heteroaryl, heterocyclic, carbocyclic, cycloalkyl, alkylcycloalkyl, heterocycloalkyl, heteroalkylcycloalkyl, heteroaralkyl, alkylcarbonyl or pharmaceutical salts. In addition, R^9 can be absent.

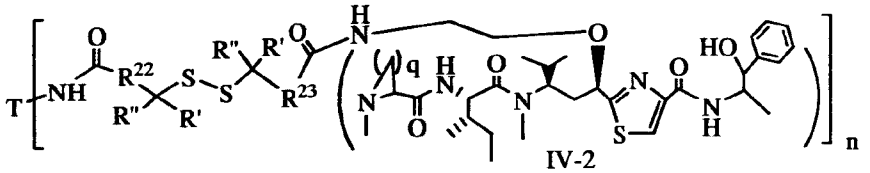
Illustrative examples of compounds of formula (IV) have the structures:



IVa

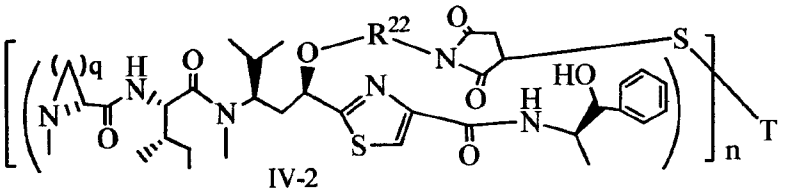


IVb



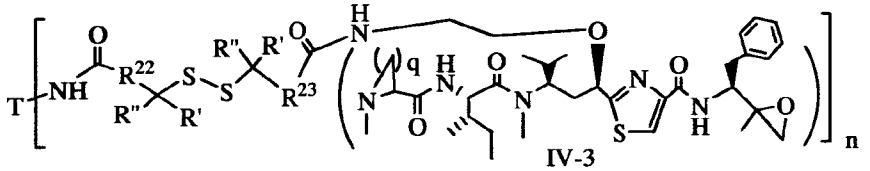
IV-2

IVc



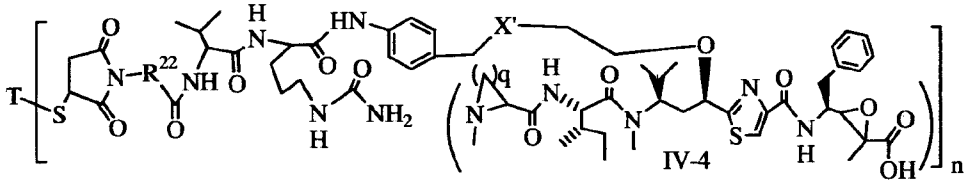
IV-2

IVd



IV-3

IVe

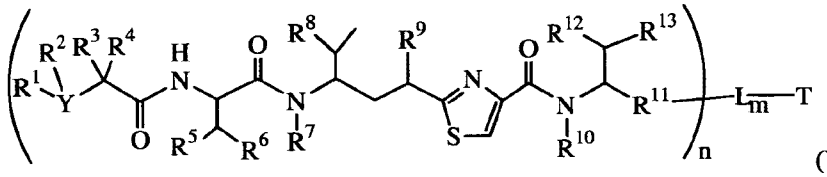


IV-4

IVf

wherein Ar, Aa, n, q, X', Y', R^{22}, R^{23}, R' and R'' are defined the same as for formula IIa ~ IIr.

In another embodiment, a binding molecule -antimitotic agent conjugate has the formula (V)

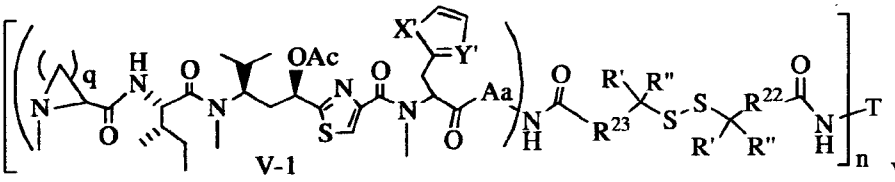


(V)

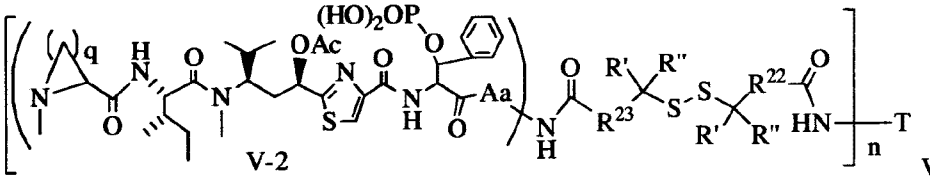
wherein T, L, m, Y, R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{12}, R^{13} and n are defined the same as in formula (II).

Wherein R^{11} is independently $-R^{14}-$, $-R^{14}C(=O)R^{17}-$, $-R^{14}X^2R^{17}-$, $-R^{14}C(=O)X^2-$, wherein R^{17} is independently H, OH, C_1~C_8 of alkyl; C_2~C_8 of alkenyl, alkynyl, heteroalkyl; C_3~C_8 of aryl, arylene, heterocyclic, carbocyclic, heterocycloalkyl; or an amino acid, or two amino acid units; X^2 is -O-, -S-, -NH-, $-N(R^{14})-$, $-O-R^{14}-$, $-S-R^{14}-$, $-S(=O)-R^{14}-$, or $-NHR^{14}-$; R^{14} is C_1~C_8 of alkyl, heteroalkyl; C_2~C_8 of alkenyl, alkynyl; C_3~C_8 of aryl, heterocyclic, carbocyclic, cycloalkyl, alkylcycloalkyl, heterocycloalkyl, heteroalkylcycloalkyl, heteroaralkyl, alkylcarbonyl.

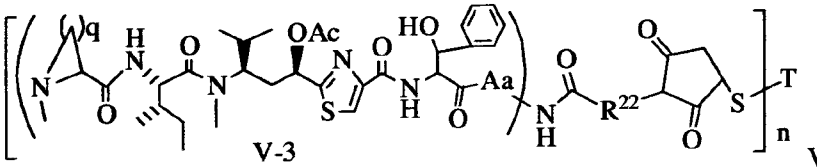
Illustrative examples of compounds of formula (V) have the structures:



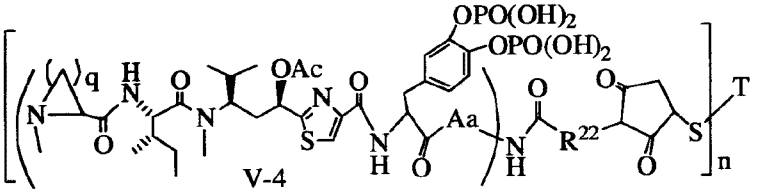
V-1 Va



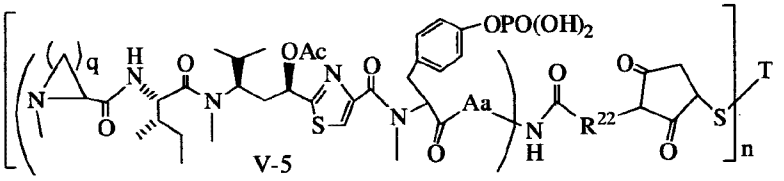
V-2 Vb



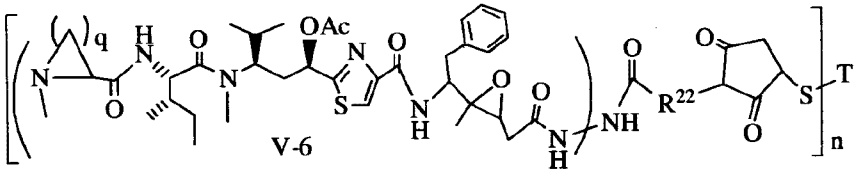
V-3 Vc



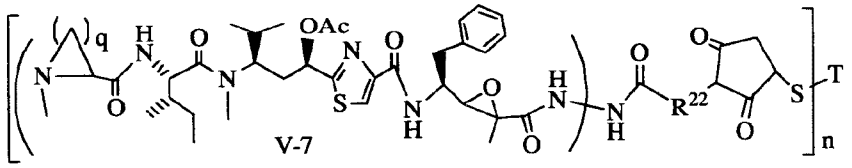
V-4 Vd



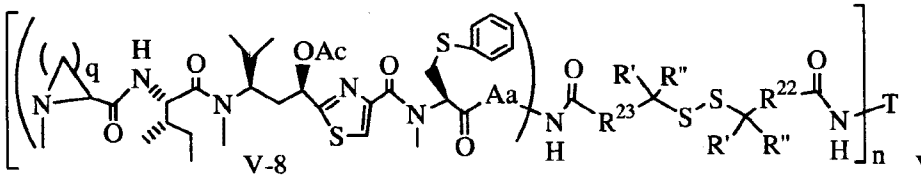
V-5 Ve



V-6 Vf



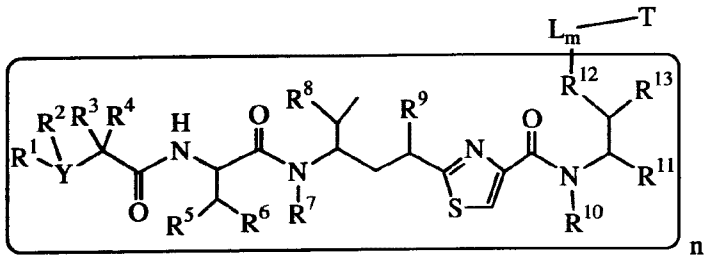
V-7 Vg



V-8 Vh

Wherein Ar, Aa, n, q, X', R^{22}, R^{23}, R' and R'' are defined the same as for formula IIa ~ IIr.

In another embodiment, a conjugates of a cell binding-antimitotic agent have the formula (VI)

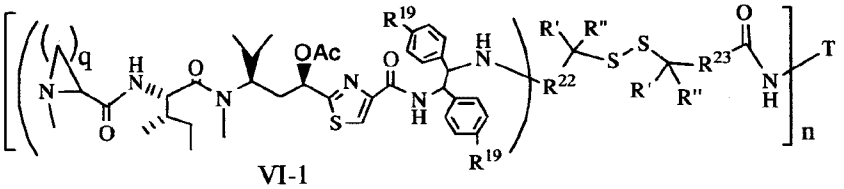


(VI)

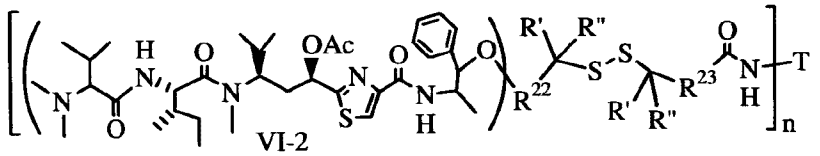
wherein T, L, m, R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{11}, R^{13} and n are defined the same as in formula (II).

Wherein R^{12} is independently R^{14}, -O-, -S-, -NH-, =N-, =NNH-, -N(R^{14})-, -OR^{14}-,C(O)O-, C(O)NH-, C(O)NR^{14}-, -SR^{14}-, -S(=O)R^{14}-, -NHR^{14}-, -CH_2OP(=O)(OR^{15})- , -P(=O)(OR^{15})-,. -OP(=O)(OR^{15})O-, -SO_2R^{14}. R^{14}, R^{15} are independently C_1~C_8 of alkyl, heteroalkyl; C_2~C_8 of alkenyl, alkynyl; C_3~C_8 of aryl, heterocyclic, carbocyclic, cycloalkyl, alkylcycloalkyl, heterocycloalkyl, heteroaralkyl, heteroalkylcycloalkyl, alkylcarbonyl.

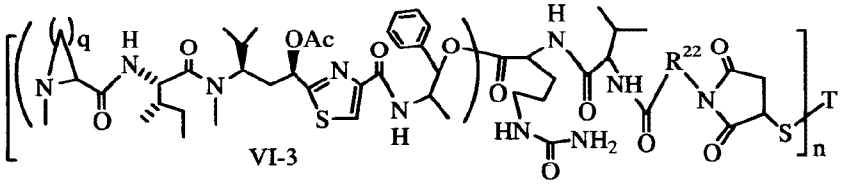
Illustrative examples of compounds of formula VI have the following structures:



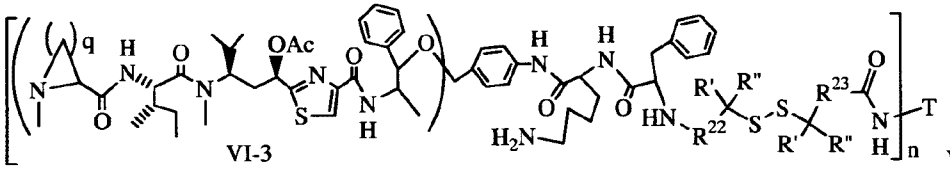
VI-1 VIa



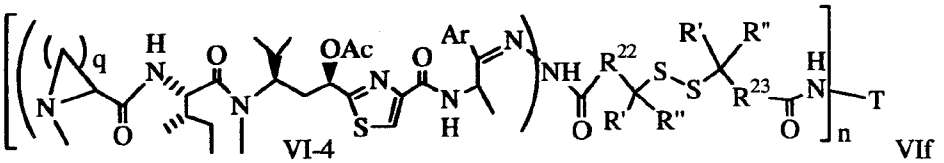
VI-2 VIb



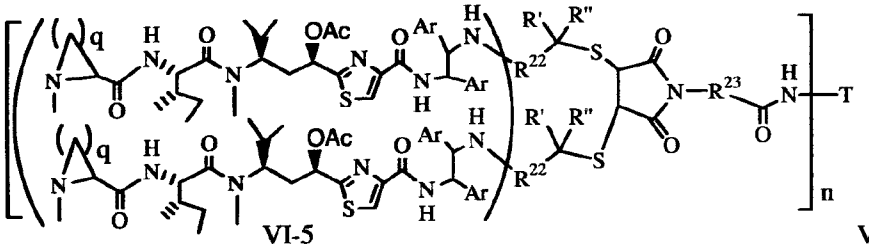
VI-3 VIc



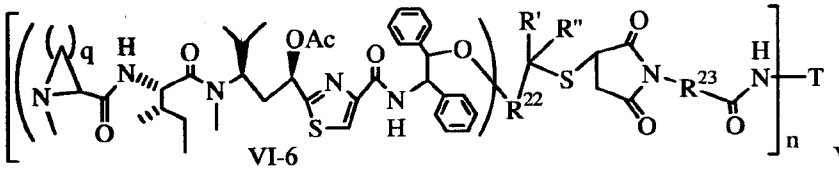
VI-3 VId



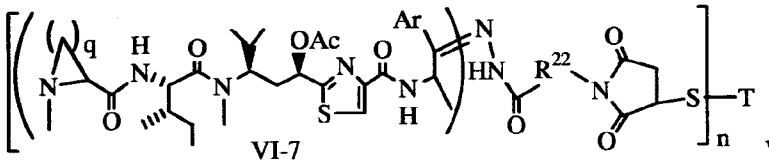
VI-4 VIf



VI-5 VIg



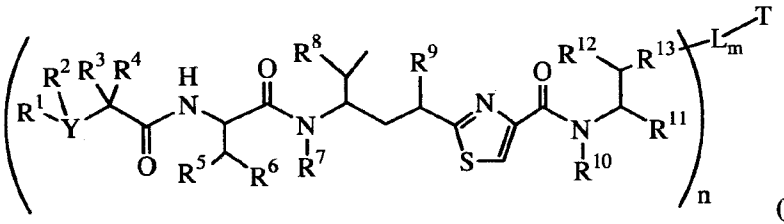
VI-6 VIh



VI-7 VIi

Wherein Ar, Aa, n, q, X', R^{22}, R^{23}, R' and R'' are defined the same as for formula IIa ~ IIr.

In another embodiment, the conjugates of the cell-surface binding molecule-antimitotic agents have the formula (VII)



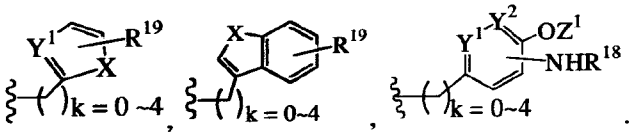
(VII)

wherein T, L, m, R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8, R_9, R_{11}, R_{12} and n are defined the same as in formula (II).

Wherein R^{13} is C_1~C_{10} of alkyl, heteroalkyl, alkyl acid, alkyl amide, alkyl amine, or Ar; Ar refers to a aromatic or hetero aromatic group, composed of one or several rings, comprising four to ten carbon, preferentially four to six carbon atoms. The term of hetero aromatic group refers one or several carbon on aromatic group, preferentially one, two or three carbon atoms are replaced by O, N, Si, Se, P or S, preferentially O, S, N. The term aryl or Ar also refers to a aromatic group, wherein one or several H atoms are replaced independently by R^{18}, F, Cl, Br, I, OR^{16}, SR^{16}, $NR^{16}R^{18}$, $N{=}NR^{16}$, $N{=}R^{16}$, $NR^{16}R^{18}$, NO_2, $SOR^{16}R^{18}$, SO_2R^{16}, SO_3R^{16}, OSO_3R^{16},

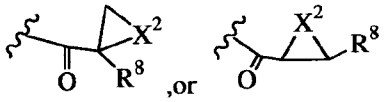
$PR^{16}R^{18}$, $POR^{16}R^{18}$, $PO_2R^{16}R^{18}$, $OPO_3R^{16}R^{18}$, $OPO_2R^{16}OPO_3R^{16}R^{18}$ or $PO_3R^{16}R^{18}$ wherein R^{16}, R^{18} are independently H, C_1~C_8 of alkyl; C_2~C_8 of alkenyl, alkynyl, heteroalkyl; C_3~C_8 of aryl, heterocyclic, carbocyclic, cycloalkyl, alkylcycloalkyl, heterocycloalkyl, heteroaralkyl, heteroalkylcycloalkyl, alkylcarbonyl; or C_4 ~ C_{12} glycosides; or pharmaceutical salts.

In addition, R^{12} can be H when R^{10} is not H, or when R^{13} is:



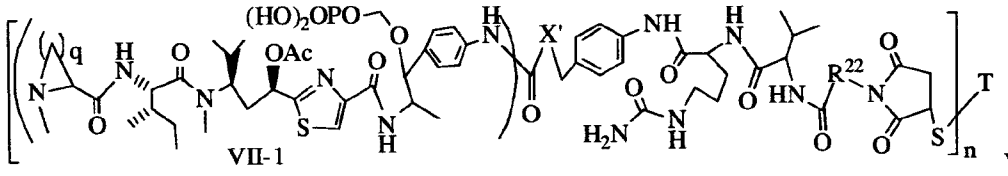
. wherein Z^1 is H, $CH_2OP(O)(OR^{18})_2$, $C(O)OP(O)(OR^{18})_2$, $PO(OR^{18})_2$, $PO(OR^{18})OPO(OR^{18})_2$, $C(O)R^{18}$, $C(O)NHR^{18}$, $SO_2(OR^{18})$, C_4 ~ C_{12} glycosides, or C_1~C_8 of alkyl, carboxyalkyl, heterocyclic; R^{18} is H, C_1~C_8 of alkyl, carboxyalkyl, heteroalkyl; C_2~C_8 of alkenyl, alkynyl, heterocyclic; C_3~C_8 of aryl, alkylcarbonyl; R^{19} is H, OH, NH_2, $OSO_2(OR^{18})$, $XCH_2OP(O)(OR^{18})_2$, $XC(O)OP(O)(OR^{18})_2$, $XPO(OR^{18})_2$, $XC(O)R^{18}$, $XC(O)NHR^{18}$, C_1~C_8 of alkyl, carboxyalkyl, carboxylic acid derivative; C_2~C_8 of alkenyl, alkynyl, heterocyclic; C_3~C_8 of aryl, alkylcarbonyl; or pharmaceutical salts; X is O, S, NH; Y^1 and Y^2 are N or CH respectively.

Or R^{12} can be H when R^{11} is:

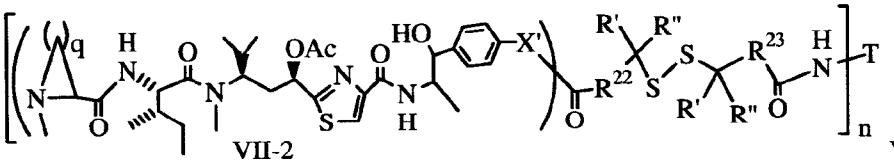


,or X^2 is O, S, N-R^8; R^8 is H, C_1~C_4 of alkyl or heteroalkyl.

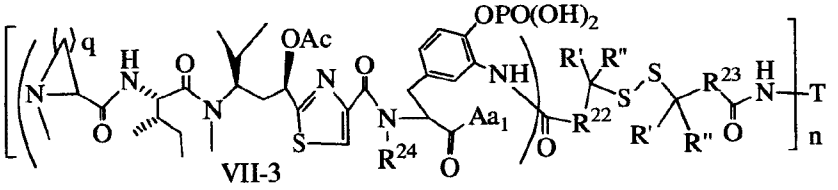
Illustrative examples of compounds of formula VII have the following structures:



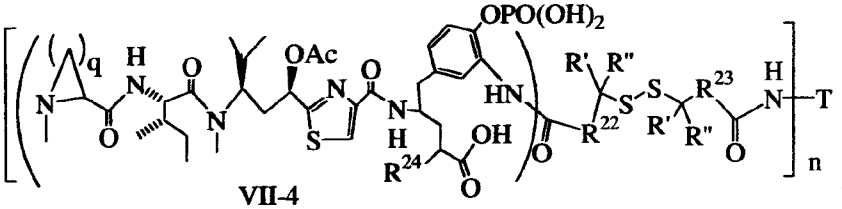
VII-1 VIIa



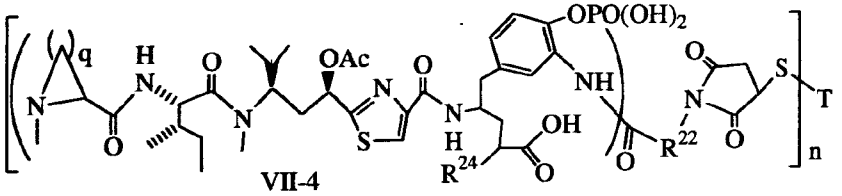
VII-2 VIIb



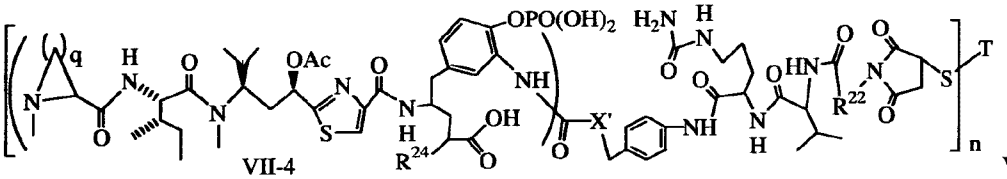
VII-3 VIIc



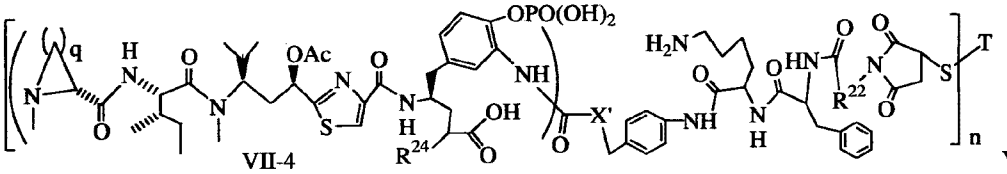
VII-4 VIId



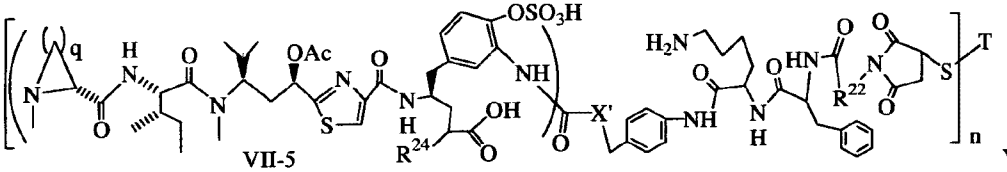
VII-4 VIIe



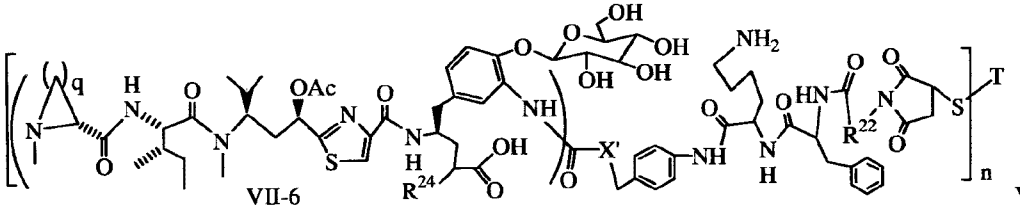
VII-4 VIIf



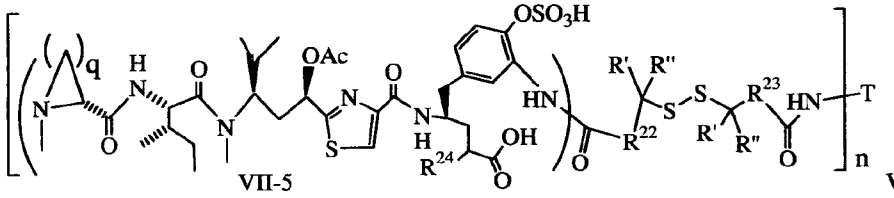
VII-4 VIIg



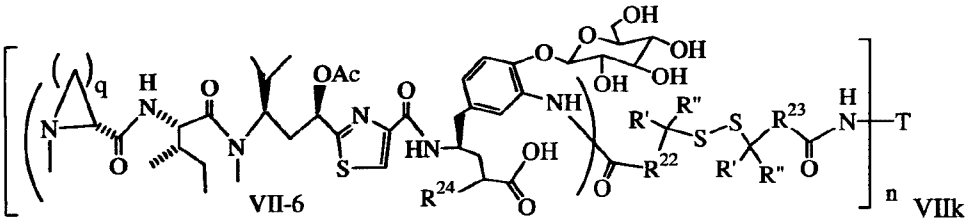
VII-5 VIIh

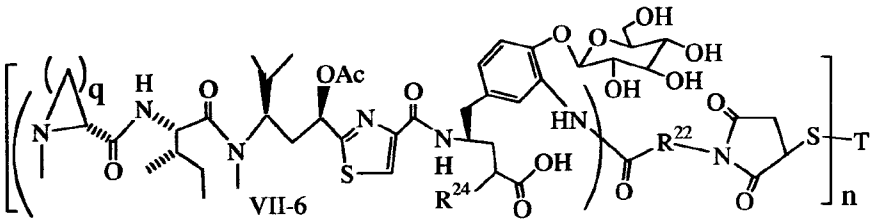


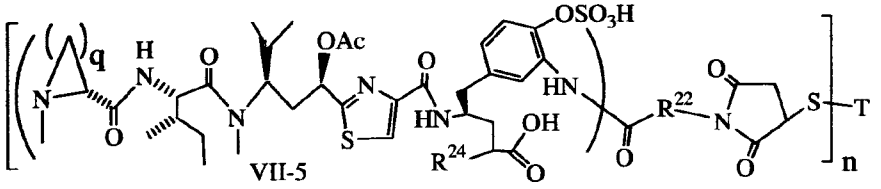
VII-6 VIIi

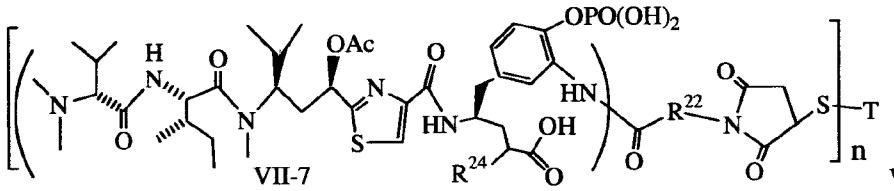


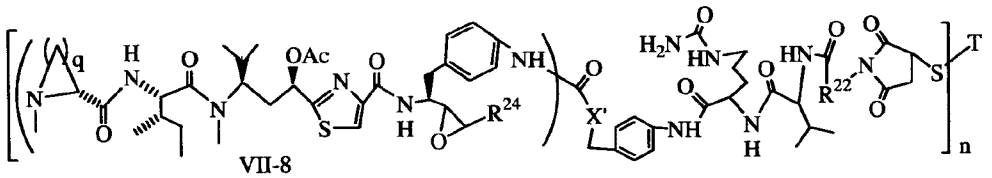
VII-5 VIIj

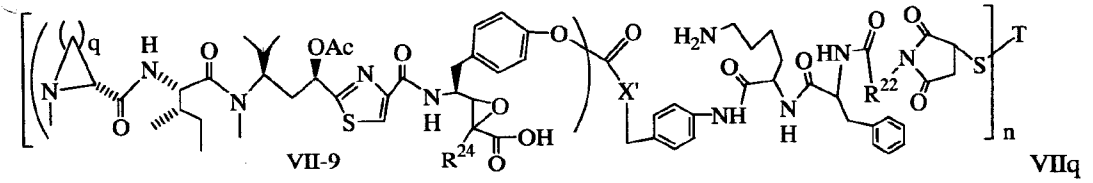

VII-6
VIIk

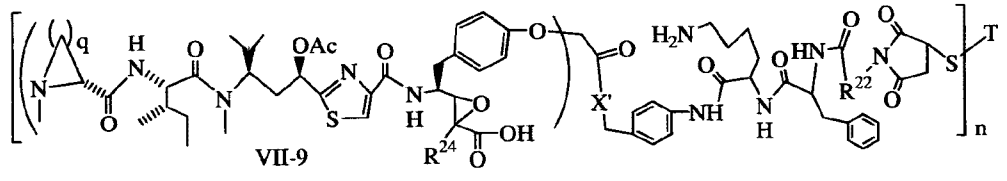

VII-6
VIIl

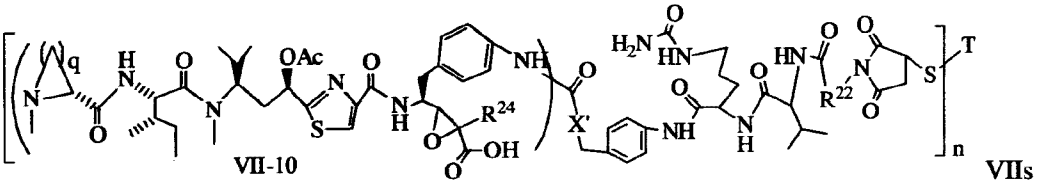

VII-5
VIIm


VII-7
VIIn

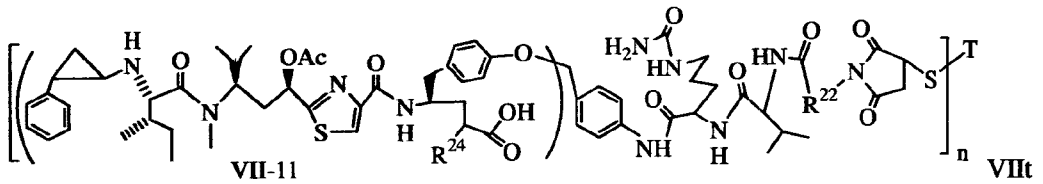

VII-8
VIIo


VII-9
VIIq


VII-9
VIIr



VII-10 VIIs



VII-11 VIIt

Wherein Ar, Aa, n, q, X', R^{22}, R^{23}, R' and R'' are defined the same as for formula IIa ~ IIr; R^{24} is H or CH_3.

In another embodiment, the synthetic routes to produce the antimitotic agents and their conjugation to a cell-surface receptor binding molecules of the present invention are exampled, but not limited to, shown in figures 1-32.

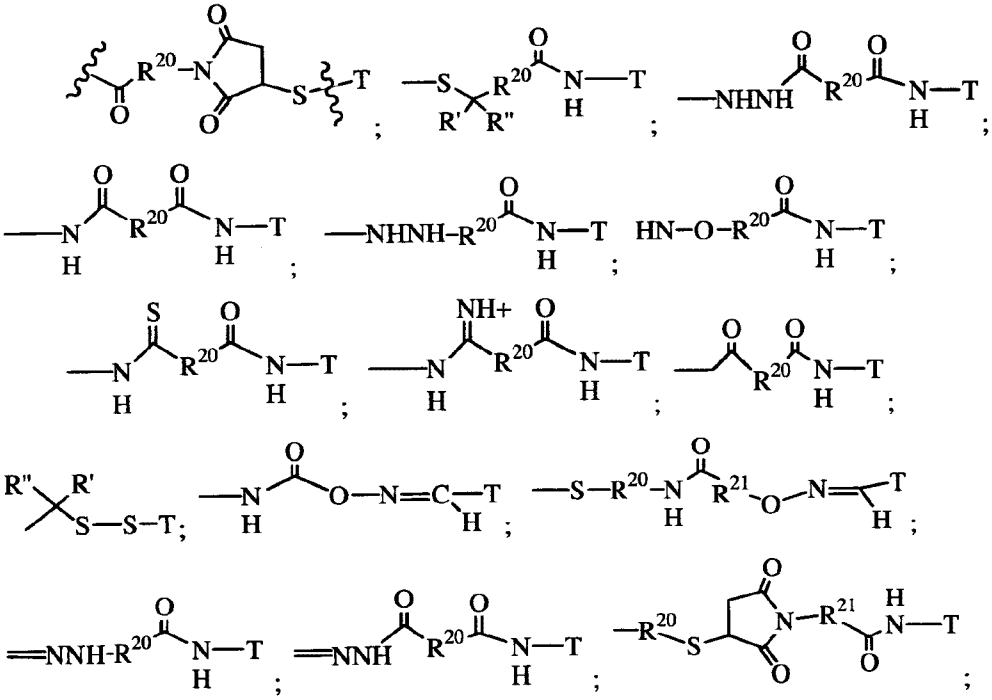
In another embodiment, the releasable linker (L) used for the conjugation of the present invention is a chain of atoms selected from C, N, O, S, Si, and P that covalently connects the cell-surface binding ligand (T) to the potent antimitotic agents. The linker may have a wide variety of lengths, such as in the range from about 2 to about 100 atoms. The atoms used in forming the linker may be combined in all chemically relevant ways, such as forming alkylene, alkenylene, and alkynylene, ethers, polyoxyalkylene, esters, amines, imines, polyamines, hydrazines, hydrazones, amides, ureas, semicarbazides, carbazides, alkoxyamines, alkoxylamines, urethanes, amino acids, acyloxylamines, hydroxamic acids, and many others. In addition, it is to be understood that the atoms forming the releasable linker (L) may be either saturated or unsaturated, or may be radicals, or may be cyclized upon each other to form divalent cyclic structures, including cyclo alkanes, cyclic ethers, cyclic amines, arylenes, heteroarylenes, and the like in the linker.

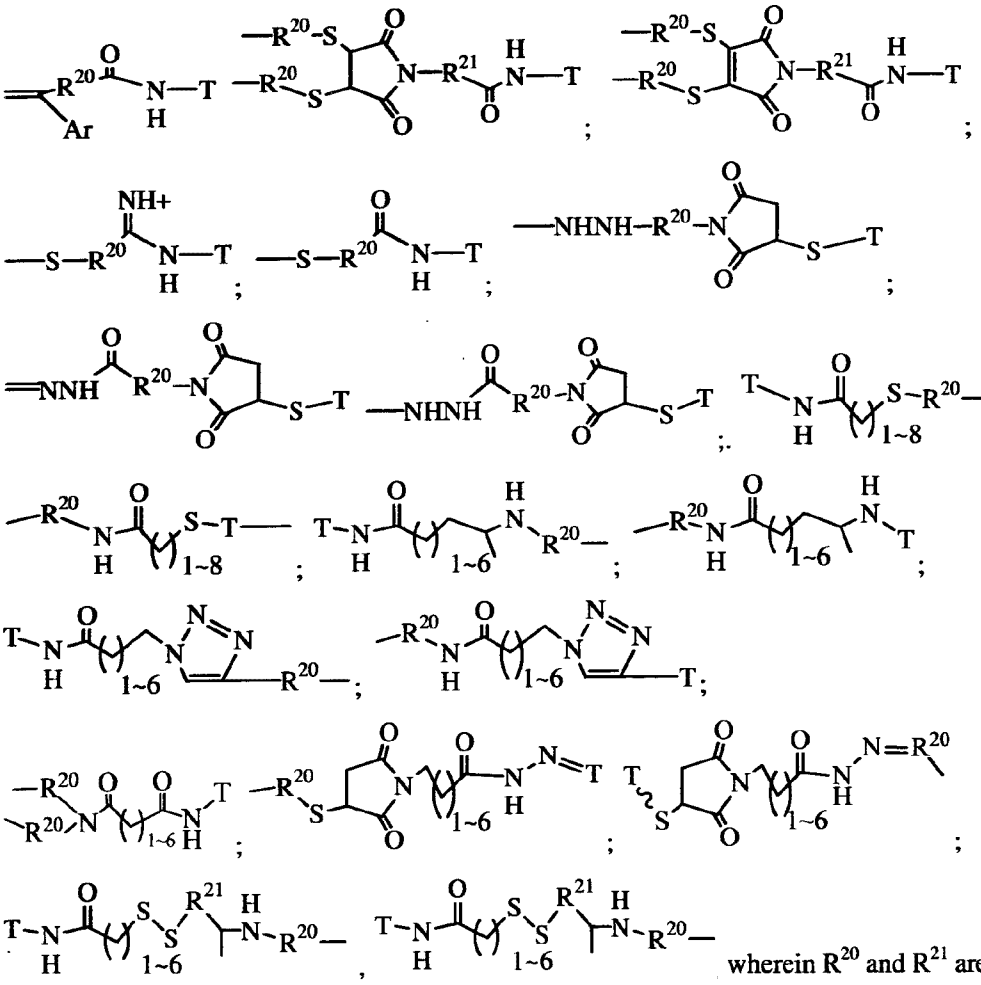
The term releasable linker refers to a linker that includes at least one bond that can be broken under physiological conditions, such as a pH-labile, acid-labile, base-labile, oxidatively labile, metabolically labile, biochemically labile, or enzyme-labile bond. It is appreciated that such physiological conditions resulting in bond breaking do not necessarily include a biological or metabolic process, and instead may include a standard chemical reaction, such as a hydrolysis or substitution reaction, for example, an endosome having a lower pH than cytosolic pH, and/or disulfide bond exchange reaction with a intracellular thiol, such as a millimolar range of abundant of glutathione inside the malignant cells.

The releasable linker L of conjugates may have the formula: --Ww—(Aa)r--Vv-- wherein: --W-- is a Stretcher unit; w is 0 or 1; each --Aa-- is independently an Amino Acid unit; r is independently an integer ranging from 0 to 12; --V-- is a Spacer unit; and v is 0, 1 or 2.

The Stretcher unit (--W--), when present, links a targeted binding molecular unit (T) to an amino acid unit (--Aa--), or links V when an Aa is not present. The Stretcher unit W may independently contain a self-immolative spacer, peptidyl units, a hydrazone bond, disulfide or thioether bonds. In this regard a binding molecular (T) has a functional group that can form a bond with a functional group of a Stretcher. Useful functional groups that can be present on a binding molecular, either naturally or via chemical manipulation include, but are not limited to, sulfhydryl (--SH), amino, hydroxyl, carbonyl, the anomeric hydroxyl group of a carbohydrate, and carboxyl. Preferred functional groups are sulfhydryl, carboxy and amino. Sulfhydryl groups can be generated by reduction of an intramolecular disulfide bond of a binding ligand, such as of an antibody. Alternatively, sulfhydryl groups can be generated by reaction of an amino group of a lysine moiety of a binding molecular using 2-iminothiolane (Traut's reagent) or thiolactone or another sulfhydryl generating reagent, such as modifies T with a disulfide bond linker, or a thiol ester following by reduction or hydrolysis respectively.

Illustrative examples of W linked to T have the structures:

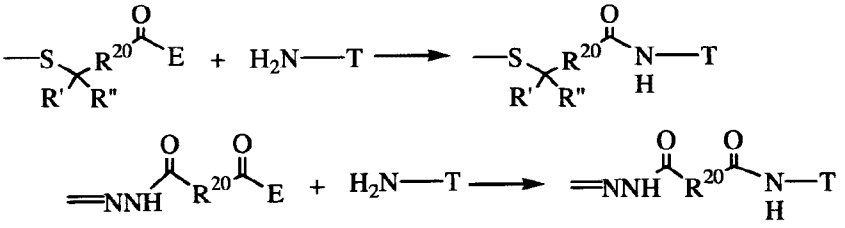




wherein R^{20} and R^{21} are selected from –C_1~C_9 alkylene-, -- C_1~C_7 carbocyclo-, -O-(C_1~C_8 alkyl)-, -arylene-, -- C_1~C_9 alkylene-arylene-, -arylene, -C_1~C_9 alkylene-, -C_1~C_9 alkylene-(C_1~C_8 carbocyclo)-, -(C_3~C_7 carbocyclo)- C_1~C_9 alkylene-, -C_3~C_8 heterocyclo-, -C_1~C_{10} alkylene-(C_3~C_8 heterocyclo)-, -(C_3~C_8 heterocyclo)- C_1~C_9 alkylene-, -$(CH_2CH_2O)_k$-, -$(CH(CH_3)CH_2O)_k$-, and -($CH_2CH_2O)_k$-CH_2-; k is an integer ranging from 1-20.; R' and R'' are independently H or CH_3.

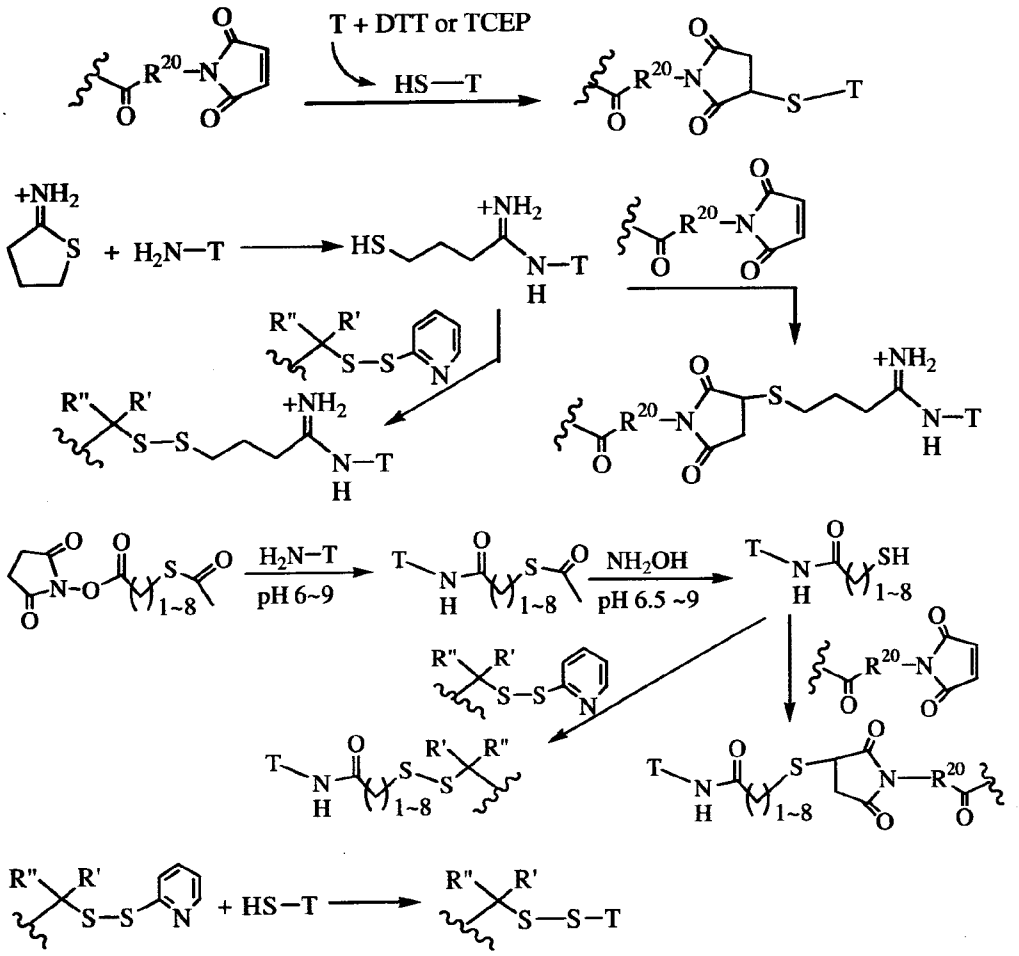
In another embodiment, conjugation of W to T covalently as illustrated above can be via various chemical reactions.

Examples of the formation of amide linkages:



Wherein the Stretcher unit contains a reactive site of E, which can form an amide bond with a primary or secondary amino group of a binding molecule. Example of the reactive E, includes, but is not limited to, such as hydroxysuccinimidyl esters (NHS, Sulfo-NHS, etc), 4-nitrophenyl esters, pentafluorophenyl esters, tetrafluorophenyl (includes sulfo-tetrafluorophenyl) esters, anhydrides, acid chlorides, sulfonyl chlorides, isocyanates and isothiocyanates.

Examples of thiol ether or disulfide bond linkages:

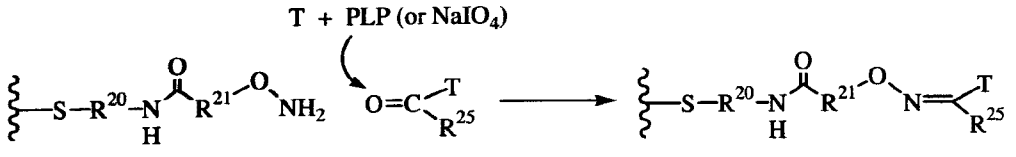


Wherein the Stretcher unit contains a sulfhydryl reactive site, which can form a thiol ether or disulfide bond with a thiol group which is generated by reduction of an intramolecular disulfide bond of the binding ligand T, or generated by a chemical modification on the binding ligand T.

In yet another aspect of the invention, the reactive group of the Stretcher contains a reactive site that is reactive to an aldehyde (--CHO) or a ketone (-C(=O)R) group that can be chemically modified on a binding molecular T. For example, a carbohydrate on a binding molecular T can be mildly oxidized using a reagent such as sodium periodate to generate an

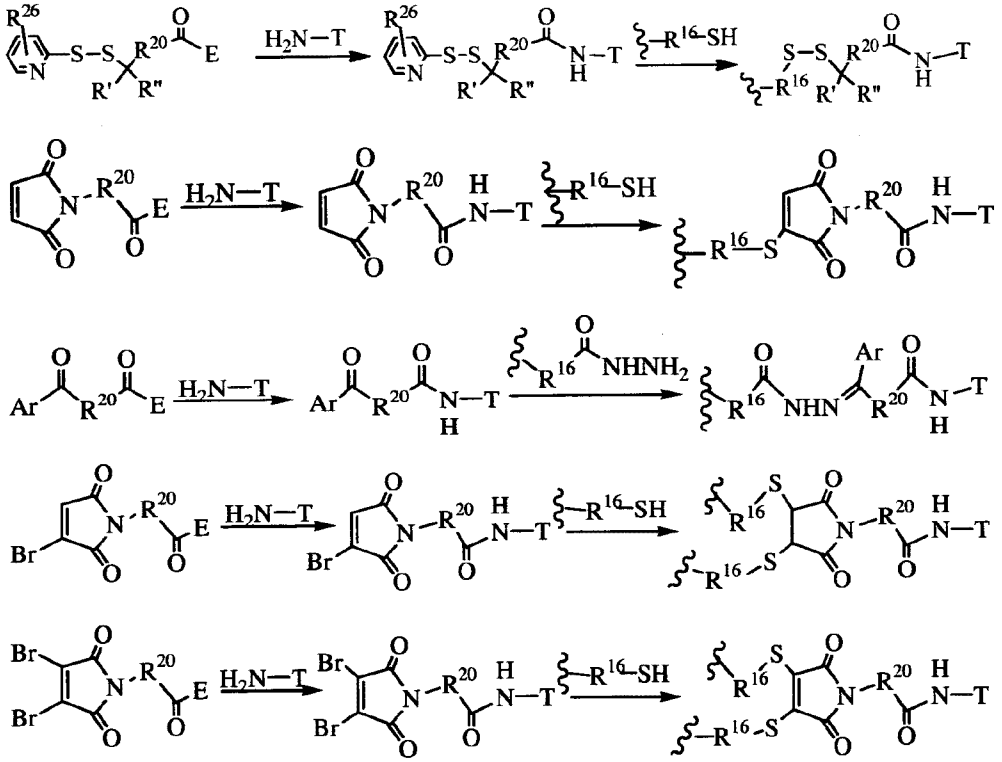
aldehyde or a ketone (-C(=O)R) group; or an amine on an amino acid at the N-termini of antibodies (or proteins or peptides) can react with pyridoxal 5'-phosphate (PLP) in a buffer solution to introduce ketone groups. The resulting (--C=O) unit can be condensed with a Stretcher that contains a functionality such as a hydrazide, an oxime, a primary or secondary amine, a hydrazine, a thiosemicarbazone, a hydrazine carboxylate, and an arylhydrazide.

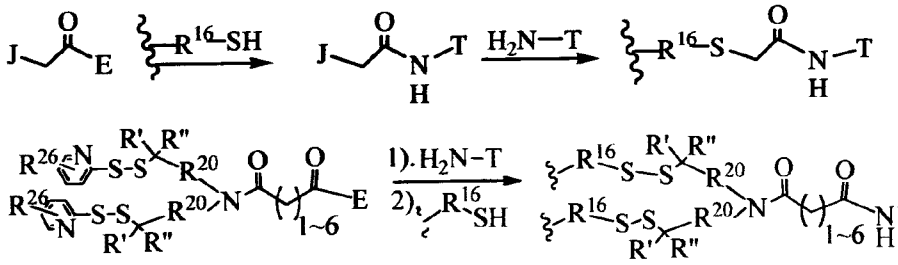
Examples of the conjugation of the hydrazone, or the oxime or imine linkages:

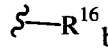


wherein R^{20} and R^{21} are described above, R^{25} is an organic substituent of an amino acid.

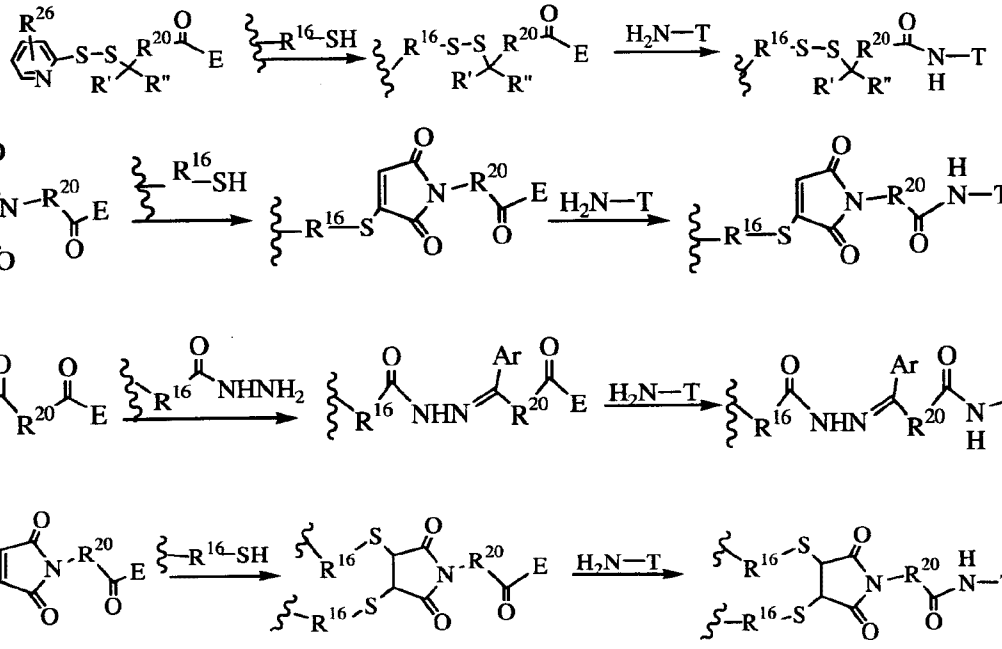
In another aspect of the invention, the Stretchers (which may contain a spacer V and/or an amino acid) can be linked to the binding molecules (T), followed by conjugation of a potent antimitotic agent to the binding molecule-stretcher moiety in an aqueous buffered solution. Examples of these two-step conjugations (a cytotoxic drug linked to R^{16} is omitted here) are:

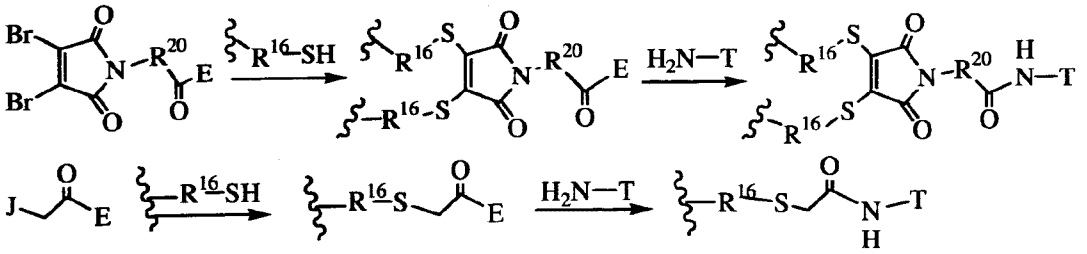




wherein E includes, but is not limited to, such as hydroxysuccinimidyl esters (NHS, Sulfo-NHS, etc), 4-nitrophenyl esters, pentafluorophenyl esters, tetrafluorophenyl (includes sulfo-tetrafluorophenyl) esters, anhydrides, acid chlorides, sulfonyl chlorides, isocyanates and isothiocyanates. R' and R'' are independently H or CH_3; R^{20}, R^{16} and Ar are defined in various embodiment throughout this inventions; R^{26} is H, or F, or NO_2 independently; J is F, Cl, Br, I, tosylate (TsO) or mesylate (MsO) independently and wherein  bears at least one antimitotic agent/drug as 

In another aspect of the invention, the Stretchers can be linked to a potent antimitotic agent first, followed by conjugation of the binding molecules (T) in an aqueous pH 3 ~ 10 (preferably pH 5 ~ 8.5) buffered solution containing up to 50% of organic cosolvents. Examples of these kinds of two-step conjugations:





wherein E includes, but is not limited to, such as hydroxysuccinimidyl esters (NHS, Sulfo-NHS, etc), 4-nitrophenyl esters, pentafluorophenyl esters, tetrafluorophenyl (includes sulfo-tetrafluorophenyl) esters, anhydrides, acid chlorides, sulfonyl chlorides, isocyanates and isothiocyanates. R' and R'' are independently H or CH_3; R^{16}, R^{20} and Ar are defined in various embodiment throughout this inventions; R^{26} is H, or F, or NO_2 independently; J is F, Cl, Br, I, tosylate (TsO) or mesylate (MsO) independently and wherein $\sim R_{16}$ bears at least one antimitotic agent/drug

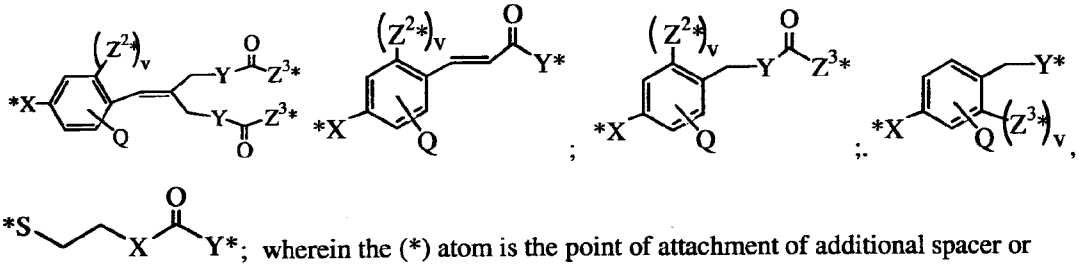
The Amino Acid unit (--Aa--), when present, links the Stretcher unit to the Spacer unit if the Spacer unit is present, links the Stretcher unit to the antimitotic agent unit if the Spacer unit is absent, and links the binding molecule (T) unit to the antimitotic agent unit if the Stretcher unit and Spacer unit are absent. --(Aa)r-- is a natural or unnatural amino acid, dipeptide, tripeptide, tetrapeptide, pentapeptide, hexapeptide, heptapeptide, octapeptide, nonapeptide, decapeptide, undecapeptide or dodecapeptide unit, and r is an integer ranging from 0 to 12. The term amino acid as used herein refers generally to aminoalkylcarboxylate, where the alkyl radical is optionally substituted, such as with alkyl, acyl, hydroxy alkyl, sulfhydrylalkyl, aminoalkyl, carboxyalkyl, and the like, The structures of the natural and unnatural amino acids and peptides are described in the book: G. C. Barrett and D. T. Elmore, "Amino Acid and Peptide", Cambridge University Press, 2004. In addition, amino acid refers to beta, gamma, and longer amino acids with intra chain containing methyl, benzyl, hydroxymethyl, thiomethyl, carboxyl, carboxylmethyl, guanidinopropyl, and the like. More preferably the amino acid is selected from arginine, asparagine, aspartic acid, citrulline, cysteine, glycine, glutamic acid, leucine, lysine, glutamic acid, glutamine, serine, ornithine, phenylalanine, threonine, tyrosine, valine and the like.

The Amino Acid unit used in this invention can be enzymatically cleaved by one or more enzymes, including a tumor-associated protease, to liberate the antimitotic agent, which in one embodiment is protonated in vivo upon release to provide an antimitotic agent.

The Spacer unit (--V--), when present, links an Amino Acid unit to the antimitotic agent when an Amino Acid unit is present. Alternately, the Spacer unit links the Stretcher unit to antimitotic agent when the Amino Acid unit is absent. The Spacer unit also links antimitotic

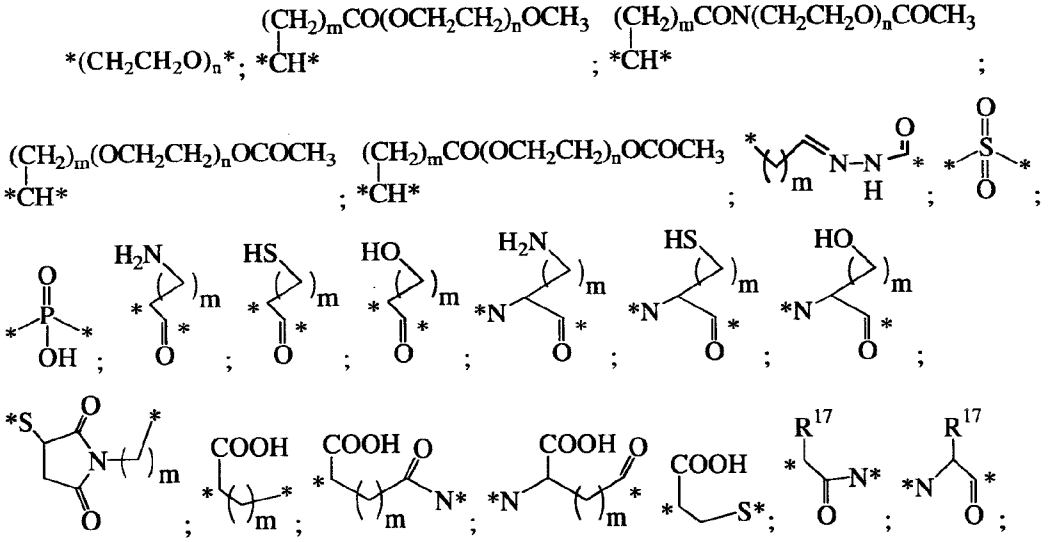
agent to the binding molecule (T) when both the Amino Acid unit and Stretcher unit are absent. The spacer linkers may contain function groups that substantially increase the water solubility, biological transport, preferential renal clearance, uptake, absorption, biodistribution, and/or bioavailability of the conjugate are described herein. Spacer units are of two general types: self-immolative and non self-immolative. A non self-immolative Spacer unit is one in which part or all of the Spacer unit remains bound to antimitotic agent after cleavage, particularly enzymatic, of an Amino Acid unit from the antimitotic agent-Linker- binding molecule conjugate or the antimitotic agent-Linker Compound.

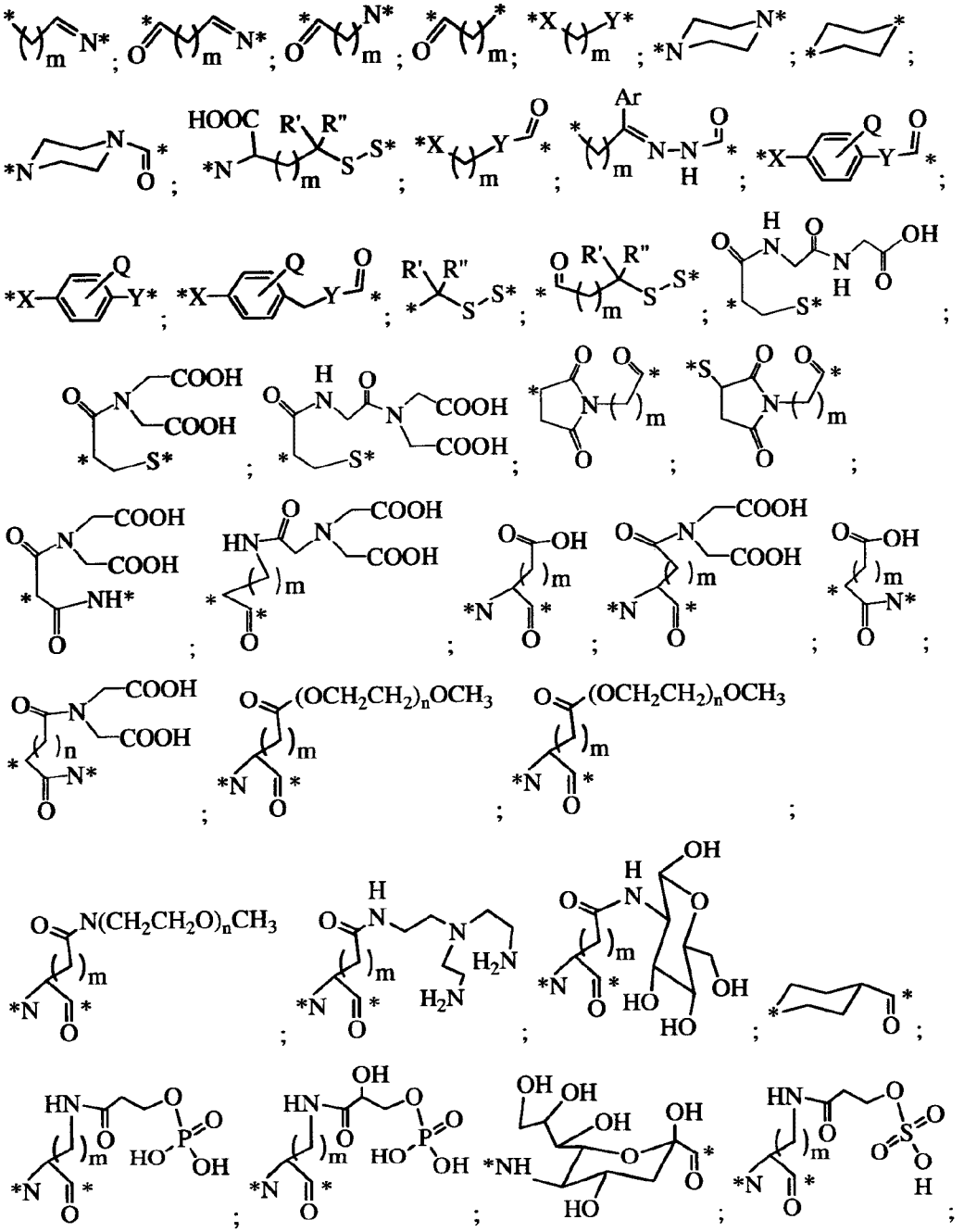
Examples of the self-immolative spacer linkers:

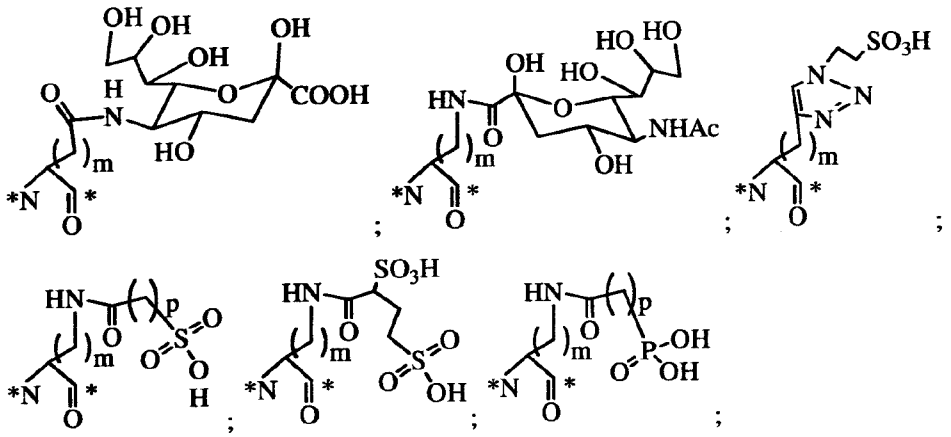


; wherein the (*) atom is the point of attachment of additional spacer or releasable linker units, the antimitotic agent, and/or the binding molecule (T); X, Y and $Z^3$are independently NH, O, or S; Z^2 is H, NH, O or S independently. v is 0 or 1; Q is independently H, OH, C_1~C_6 alkyl, $(OCH_2CH_2)_n$ F, Cl, Br, I, OR^{17}, or SR^{17}, $NR^{17}R^{18}$, N=NR^{17}, N=R^{17}, $NR^{17}R^{18}$, NO_2, $SOR^{17}R^{18}$, SO_2R^{17}, SO_3R^{17}, OSO_3R^{17}, $PR^{17}R^{18}$, $POR^{17}R^{18}$, $PO_2R^{17}R^{18}$, $OPO(OR^{17})(OR^{18})$, $OC(O)PO(OR^{17}(OR^{18})$ or $OCH_2PO(OR^{17}(OR^{18})$ wherein R^{17}, R^{18} are independently H, C_1~C_8 of alkyl; C_2~C_8 of alkenyl, alkynyl, heteroalkyl; C_3~C_8 of aryl, heterocyclic, carbocyclic, cycloalkyl, heterocycloalkyl, heteroaralkyl, alkylcarbonyl; or pharmaceutical cation salts.

Examples of the non-self-immolative spacer linkers:







Where the (*) atom is the point of attachment of additional spacer or releasable linkers, the antimitotic agents, and/or the binding molecules; m is 1~10; n is 1~20.

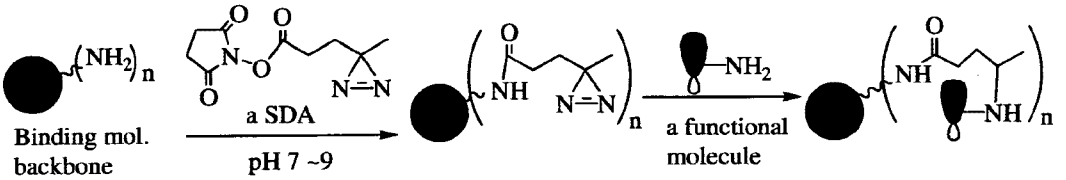
The binding molecule (T) may be of any kind presently known, or that become known, molecule that binds to, complexes with or reacts with a moiety of a cell population sought to be therapeutically or otherwise biologically modified. The binding molecule unit acts to deliver the antimitotic agents to the particular target cell population with which the binding molecule (T) reacts.

The cell binding agents include, but are not limited to, large molecular weight proteins such as, for example, full-length antibodies (polyclonal and monoclonal antibodies); single chain antibodies; fragments of antibodies such as Fab, Fab', $F(ab')_2$, F_v, [Parham, J. Immunol. 131, 2895-2902 (1983)], fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies, CDR's, and epitope-binding fragments of any of the above which immuno-specifically bind to cancer cell antigens, viral antigens or microbial antigens; interferons (such as type I, II, III); peptides; lymphokines such as IL-2, IL-3, IL-4, IL-6, GM-CSF, interferon-gamma (IFN-γ); hormones such as insulin, TRH (thyrotropin releasing hormones), MSH (melanocyte-stimulating hormone), steroid hormones, such as androgens and estrogens, melanocyte-stimulating hormone (MSH); growth factors and colony-stimulating factors such as epidermal growth factors (EGF), granulocyte-macrophage colony-stimulating factor (GM-CSF), transforming growth factors (TGF), such as TGFα, TGFβ, insulin and insulin like growth factors (IGF-I, IGF-II) G-CSF, M-CSF and GM-CSF [Burgess, Immunology Today, 5, 155-158 (1984)]; vaccinia growth factors (VGF); fibroblast growth factors (FGFs); smaller molecular weight proteins, poly-peptide, peptides and peptide hormones, such as bombesin, gastrin, gastrin-releasing peptide; platelet-derived growth factors; interleukin and cytokines, such as interleukin-2 (IL-2), interleukin-6 (IL-6), leukemia inhibitory factors, granulocyte-macrophage colony-stimulating factor (GM-CSF); vitamins, such as folate; apoproteins and glycoproteins, such as

transferrin {O'Keefe et al, 260 J. Biol. Chem. 932-937 (1985)}; sugar-binding proteins or lipoproteins, such as lectins; cell nutrient-transport molecules; and small molecular inhibitors, such as prostate-specific membrane antigen (PSMA) inhibitors and small molecular tyrosine kinase inhibitors (TKI), non-peptides or any other cell binding molecule or substance, such as bioactive polymers (Dhar, et al, Proc. Natl. Acad. Sci. 2008, 105, 17356-61); dendrimers (Lee, et al, Nat. Biotechnol. 2005, 23, 1517-26; Almutairi, et al; Proc. Natl. Acad. Sci. 2009, 106, 685-90); nanoparticles (Liong, et al, ACS Nano, 2008, 19, 1309-12; Medarova, et al, Nat. Med. 2007, 13, 372-7; Javier, et al, Bioconjugate Chem. 2008, 19, 1309-12); liposomes (Medinai, et al, Curr. Phar. Des. 2004, 10, 2981-9); viral capsides (Flenniken, et al, Viruses Nanotechnol. 2009, 327, 71-93). In general monoclonal antibodies are preferred as a cell-surface binding agent if an appropriate one is available.

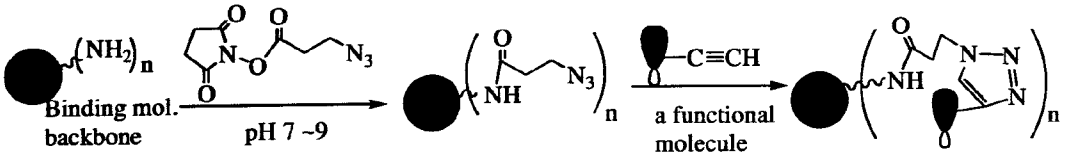
Prior to conjugating with the antimitotic agents of this invention, the binding molecules can be modified through attachment of a more specific peptide, a protein, or a drug, or the other functional molecules with a heterobifunctional cross linker such as with linkers of Amine-to-Nonselective (succinimidyl (NHS)-diazirine (SDA), NHS ester /Azide), Amine-to-Sulfhydryl (NHS ester/maleimide, NHS ester/ pyridyldithiol, NHS esters/ haloacetyl), Sulfhydryl-to-Carbohydrate (Maleimide/Hydrazide, Pyridyldithiol /Hydrazide), Hydroxyl-to-Sulfhydryl (Isocyanate / Maleimide), Amine-to-DNA (NHS ester/ Psoralen), Amine-to-Carboxyl (Carbodiimide).

In the SDA linkage modification, the NHS ester of a SDA linker reacts with primary an amine group of a binding molecule backbone in pH 6~9 buffer to form a stable amide bond upon release of NHS. Then photoactivation of the diarzirine with long-wave UV light (330-370nm) creates a reactive carbene intermediate that can react with an amine group of a more specific peptide or a protein or the other functional molecule. The order of these two steps can be different as this: an amine group of a functional molecule reacts with a SDA linker first following by photoactive reaction of a binding molecule with long-wave UV light (330-370nm). The SDA crosslinkers can be cleavable (with a disulfide bond inside such as SDAD linker).

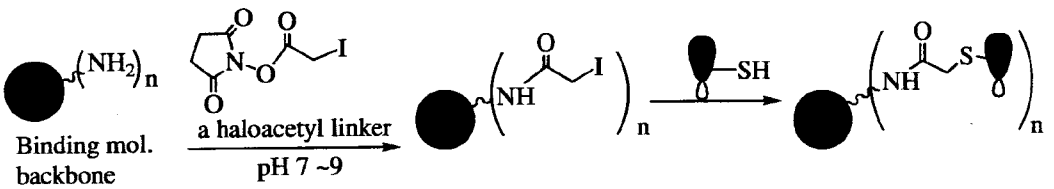


In the NHS ester /Azide linkage modification, the NHS ester of the linker reacts with primary an amine group of a binding molecule backbone in pH 6~9 buffer to form a stable amide. Then an alkynyl group on a more specific peptide or a protein or the other functional molecule

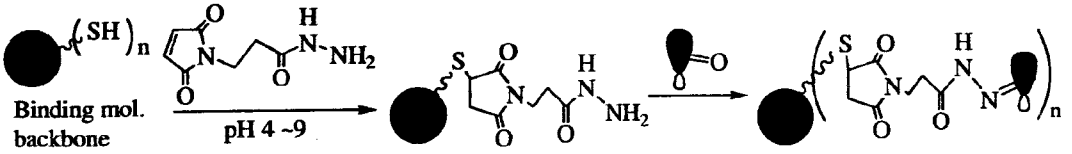
reacts to the azide on the other side of the linker via Azide-Alkyne Huisgen Cycloaddition to form a 1,2,3-triazole linkage (click chemistry). Also, the NHS ester of the linker reacts with primary an amine group of a functional molecule in pH 6~9 buffer to form a stable amide. Then an alkynyl group being linked on a binding molecule reacts to the azide on the other side of the linker via Azide-Alkyne Huisgen Cycloaddition to form a 1,2,3-triazole linkage.



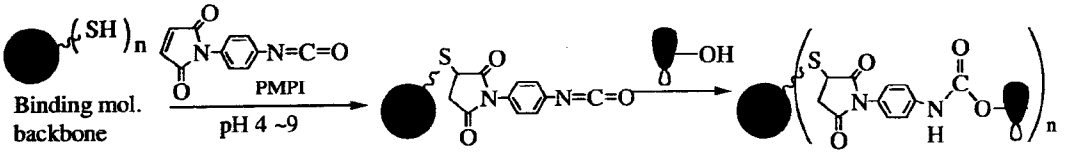
In the Amine-to-Sulfhydryl linkage modification, the NHS ester of the linker reacts with a primary amine group of a binding molecule backbone in pH 6~9 buffer to form a stable amide bond. Then a sulfhydryl on a more specific peptide or a protein or the other functional molecule reacts to the maleimide, or pyridyldithiol, or haloacetyl on the other side of the Amine-to-sulfhydryl linker at pH 4.5 ~ 8.5 to form a thioether or a disulfide bond. The conjugation with the Amine-to-Sulfhydryl linker can be in different orders. For instance, an amine group of a functional molecule can be reacted with the linker to form an amide bond first, following by reaction with a sulfhydryl on a binding molecule. Also a sulfhydryl group of a functional molecule can be reacted with the linker to form a thioether or a disulfide bond at pH 4.5 ~ 7 first, following by reaction with an amine group on a binding molecule at pH 6 ~ 9 to form an amide bond.



In the Sulfhydryl-to-Carbohydrate linkage modification, the sulfhydryl group of a binding molecule can be reacted with the maleimide or the pyridyldithiol on the linker to form a thioether or a disulfide bond at pH 4.5 ~ 8 first, Then a carbonyl (aldehyde/ketone) group on a functional molecule reacts with the hydrazide to form an hydrazone bond. Also the sulfhydryl group on a functional molecule can react with the linker to form a thioether or a disulfide bond at pH 4.5 ~ 8 first, following by reaction with a carbohydrate, or an oxidized carbohydrate, or an carbonyl (aldehyde/ketone) group on a binding molecule form an hydrazone bond.



In the Hydroxyl-to-Sulfhydryl linkage modification, the sulfhydryl group of a binding molecule can be reacted with the maleimide or the pyridyldithiol on the linker to form a thioether or a disulfide bond at pH 6 ~ 8 first, Then a hydroxy group on a functional molecule reacts with the isocyanate on the linker to form a carbamate bond at pH 8 ~9. Also the sulfhydryl group on a functional molecule can react with the linker to form a thioether or a disulfide bond at pH 6~ 8 first, following by reaction with a hydroxy on a binding molecule form a carbamate bond at pH 8 ~9.



In yet another aspect of the invention, the production of antibodies used in the present invention involves *in vivo* or *in vitro* procedures or combinations thereof. Methods for producing polyclonal anti-receptor peptide antibodies are well-known in the art, such as in U.S. Pat. No. 4,493,795 (to Nestor et al). A monoclonal antibody is typically made by fusing myeloma cells with the spleen cells from a mouse that has been immunized with the desired antigen (Köhler, G.; Milstein, C. (1975). *Nature* **256**: 495-497). The detailed procedures are described in "Antibodies--A Laboratory Manual", Harlow and Lane, eds., Cold Spring Harbor Laboratory Press, New York (1988). Particularly monoclonal antibodies are produced by immunizing mice, rats, hamsters or any other mammal with the antigen of interest such as the intact target cell, antigens isolated from the target cell, whole virus, attenuated whole virus, and viral proteins. Splenocytes are typically fused with myeloma cells using polyethylene glycol (PEG) 6000. Fused hybrids are selected by their sensitivity to HAT (hypoxanthine-aminopterin-thymine). Hybridomas producing a monoclonal antibody useful in practicing this invention are identified by their ability to immunoreact specified receptors or inhibit receptor activity on target cells.

A monoclonal antibody used in the present invention can be produced by initiating a monoclonal hybridoma culture comprising a nutrient medium containing a hybridoma that secretes antibody molecules of the appropriate antigen specificity. The culture is maintained under conditions and for a time period sufficient for the hybridoma to secrete the antibody molecules into the medium. The antibody-containing medium is then collected. The antibody

molecules can then be further isolated by well-known techniques, such as using protein-A affinity chromatography; anion, cation, hydrophobic, or size exclusive chromatographies (particularly by affinity for the specific antigen after Protein A, and sizing column chromatography); centrifugation, differential solubility, or by any other standard technique for the purification of proteins.

Media useful for the preparation of these compositions are both well-known in the art and commercially available and include synthetic culture media. An exemplary synthetic medium is Dulbecco's minimal essential medium (DMEM; Dulbecco et al., Virol. 8:396 (1959)) supplemented with 4.5 gm/l glucose, 20 mm glutamine, 20% fetal calf serum and with an anti-foaming agent, such as polyoxyethylene-polyoxypropylene block copolymer.

In addition, antibody-producing cell lines can also be created by techniques other than fusion, such as direct transformation of B lymphocytes with oncogenic DNA, or transfection with an oncovirus, such as Epstein-Barr virus (EBV, also called human herpesvirus 4 (HHV-4)) or Kaposi's sarcoma-associated herpesvirus (KSHV). See, U.S. Pat. Nos. 4,341,761; 4,399,121; 4,427,783; 4,444,887; 4,451,570; 4,466,917; 4,472,500; 4,491,632; 4,493,890. A monoclonal antibody may also be produced via an anti-receptor peptide or peptides containing the carboxyl terminal as described well-known in the art. See Niman et al., Proc. Natl. Acad. Sci. USA, 80: 4949-4953 (1983); Geysen et al., Proc. Natl. Acad. Sci. USA, 82: 178-182 (1985); Lei et al. Biochemistry 34(20): 6675-6688, (1995). Typically, the anti-receptor peptide or a peptide analog is used either alone or conjugated to an immunogenic carrier, as the immunogen for producing anti-receptor peptide monoclonal antibodies.

There are also a number of other well-known techniques for making monoclonal antibodies as binding molecules in this invention. Particularly useful are methods of making fully human antibodies. One method is phage display technology which can be used to select a range of human antibodies binding specifically to the antigen using methods of affinity enrichment. Phage display has been thoroughly described in the literature and the construction and screening of phage display libraries are well known in the art, see, e.g., Dente et al, Gene. 148(1):7-13 (1994); Little et al, Biotechnol Adv. 12(3):539-55 (1994); Clackson et al., Nature 352:264-628 (1991); Huse et al., Science 246:1275-1281 (1989).

Monoclonal antibodies derived by hybridoma technique from another species than human, such as mouse, can be humanized to avoid human anti-mouse antibodies when infused into humans. Among the more common methods of humanization of antibodies are complementarity-determining region grafting and resurfacing. These methods have been extensively described, see e.g. U.S. Pat. 5,859,205 and 6,797,492; Liu et al, Immunol Rev. 222:9-27 (2008); Almagro et al, Front Biosci. 1;13:1619-33 (2008); Lazar et al, Mol Immunol. 44(8):1986-98 (2007); Li et al,

Proc. Natl. Acad. Sci. U S A. 103(10):3557-62 (2006). Fully human antibodies can also be prepared by immunizing transgenic mice, rabbits, monkeys or other mammals, carrying large portions of the human immunoglobulin heavy and light chains, with an immunogen. Examples of such mice are: the Xenomouse. (Abgenix, Inc.), the HuMAb-Mouse (Medarex/BMS), the VelociMouse (Regeneron), see also U.S. Pat. No. 6,596,541, 6,207,418, 6,150,584, 6,111,166, 6,075,181, 5,922,545, 5,661,016, 5,545,806, 5,436,149 and 5,569,825. In human therapy, murine variable regions and human constant regions can also be fused to construct called "chimeric antibodies" that are considerably less immunogenic in man than murine mAbs (Kipriyanov et al, Mol Biotechnol. 26:39-60 (2004); Houdebine, Curr Opin Biotechnol. 13:625-9 (2002). In addition, site-directed mutagenesis in the variable region of an antibody can result in an antibody with higher affinity and specificity for its antigen (Brannigan et al, Nat Rev Mol Cell Biol. 3:964-70, (2002)); Adams et al, J Immunol Methods. 231:249-60 (1999)) and exchanging constant regions of a mAb can improve its ability to mediate effector functions of binding and cytotoxicity.

Antibodies immunospecific for a malignant cell antigen can also be obtained commercially or produced by any method known to one of skill in the art such as, e.g., chemical synthesis or recombinant expression techniques. The nucleotide sequence encoding antibodies immunospecific for a malignant cell antigen can be obtained commercially, e.g., from the GenBank database or a database like it, the literature publications, or by routine cloning and sequencing.

Apart from an antibody, a peptide or protein that bind/block/target or in some other way interact with the epitopes or corresponding receptors on a targeted cell can be used as a binding molecule. These peptides or proteins could be any random peptide or proteins that have an affinity for the epitopes or corresponding receptors and they don't necessarily have to be of the immunoglobulin family. These peptides can be isolated by similar techniques as for phage display antibodies (Szardenings, J Recept Signal Transduct Res. 2003; 23(4):307-49). The use of peptides from such random peptide libraries can be similar to antibodies and antibody fragments. The binding molecules of peptides or proteins may be conjugated on or linked to a large molecules or materials, such as, but is not limited, an albumin, a polymer, a liposome, a nano particle, dendrimers, as long as such attachment permits the peptide or protein to retain its antigen binding specificity.

Examples of antibodies used for conjugation of antimitotic agents in this prevention for treating cancer, autoimmune disease, and infectious disease include, but are not limited to, 3F8 (anti-GD2), Abagovomab (anti CA-125), Abciximab (anti CD41 (integrin alpha-IIb), Adalimumab (anti-TNF-α), Adecatumumab (anti-EpCAM, CD326), Afelimomab (anti-TNF-α);

Afutuzumab (anti-CD20), Alacizumab pegol (anti-VEGFR2), ALD518 (anti-IL-6), Alemtuzumab (Campath, MabCampath, anti- CD52), Altumomab (anti-CEA), Anatumomab (anti-TAG-72), Anrukinzumab (IMA-638, anti-IL-13), Apolizumab (anti-HLA-DR), Arcitumomab (anti-CEA), Aselizumab (anti-L-selectin (CD62L), Atlizumab (tocilizumab, Actemra, RoActemra, anti-IL-6 receptor), Atorolimumab (anti-Rhesus factor), Bapineuzumab (anti-beta amyloid), Basiliximab (Simulect, antiCD25 (α chain of IL-2 receptor), Bavituximab (anti-phosphatidylserine), Bectumomab (LymphoScan, anti-CD22), Belimumab (Benlysta, LymphoStat-B, anti-BAFF), Benralizumab (anti-CD125), Bertilimumab (anti-CCL11 (eotaxin-1)), Besilesomab (Scintimun, anti-CEA-related antigen), Bevacizumab (Avastin, anti-VEGF-A), Biciromab (FibriScint, anti-fibrin II beta chain), Bivatuzumab (anti-CD44 v6), Blinatumomab (BiTE, anti-CD19), Brentuximab (cAC10, anti-CD30 TNFRSF8), Briakinumab (anti-IL-12, IL-23) Canakinumab (Ilaris, anti-IL-1), Cantuzumab (C242, anti-CanAg), Capromab, Catumaxomab (Removab, anti-EpCAM, anti-CD3), CC49 (anti-TAG-72), Cedelizumab (anti-CD4), Certolizumab pegol (Cimzia anti-TNF-α), Cetuximab (Erbitux, IMC-C225, anti-EGFR), Citatuzumab bogatox (anti-EpCAM), Cixutumumab (anti-IGF-1), Clenoliximab (anti-CD4), Clivatuzumab (anti-MUC1), Conatumumab (anti-TRAIL-R2), CR6261 (anti-Influenza A hemagglutinin), Dacetuzumab (anti-CD40), Daclizumab (Zenapax, anti-CD25 (α chain of IL-2 receptor)), Daratumumab (anti-CD38 (cyclic ADP ribose hydrolase), Denosumab (Prolia, anti-RANKL), Detumomab (anti-B-lymphoma cell), Dorlimomab, Dorlixizumab, Ecromeximab (anti-GD3 ganglioside), Eculizumab (Soliris, anti-C5), Edobacomab (anti-endotoxin), Edrecolomab (Panorex, MAb17-1A, anti-EpCAM), Efalizumab (Raptiva, anti-LFA-1 (CD11a), Efungumab (Mycograb, anti-Hsp90), Elotuzumab (anti-SLAMF7), Elsilimomab (anti-IL-6), Enlimomab pegol (anti-ICAM-1 (CD54)), Epitumomab (anti-episialin), Epratuzumab (anti-CD22), Erlizumab (anti-ITGB2 (CD18)), Ertumaxomab (Rexomun, anti-HER2/neu, CD3), Etaracizumab (Abegrin, anti-integrin $\alpha_v\beta_3$), Exbivirumab (anti-hepatitis B surface antigen), Fanolesomab (NeutroSpec, anti-CD15), Faralimomab (anti-interferon receptor), Farletuzumab (anti-folate receptor 1), Felvizumab (anti-respiratory syncytial virus), Fezakinumab (anti-IL-22), Figitumumab (anti-IGF-1 receptor), Fontolizumab (anti-IFN-γ), Foravirumab (anti-rabies virus glycoprotein), Fresolimumab (anti-TGF-β), Galiximab (anti-CD80), Gantenerumab (anti- beta amyloid), Gavilimomab (anti-CD147 (basigin)), Gemtuzumab (anti-CD33), Girentuximab (anti-carbonic anhydrase 9), Glembatumumab (CR011, anti-GPNMB), Golimumab (Simponi, anti-TNF-α), Gomiliximab (anti-CD23 (IgE receptor)), Ibalizumab (anti-CD4), Ibritumomab (anti-CD20), Igovomab (Indimacis-125, anti-CA-125), Imciromab (Myoscint, anti-cardiac myosin), Infliximab (Remicade, anti-TNF-α), Intetumumab (anti-CD51), Inolimomab (anti-CD25 (α chain of IL-2 receptor)), Inotuzumab (anti-CD22), Ipilimumab (anti-CD152), Iratumumab (anti- CD30

(TNFRSF8)), Keliximab (anti-CD4), Labetuzumab (CEA-Cide, anti-CEA), Lebrikizumab (anti-IL-13), Lemalesomab (anti-NCA-90 (granulocyte antigen)), Lerdelimumab (anti-TGF beta 2), Lexatumumab (anti-TRAIL-R2), Libivirumab (anti-hepatitis B surface antigen), Lintuzumab (anti-CD33), Lucatumumab (anti-CD40), Lumiliximab (anti- CD23 (IgE receptor), Mapatumumab (anti-TRAIL-R1), Maslimomab (anti- T-cell receptor), Matuzumab (anti-EGFR), Mepolizumab (Bosatria, anti-IL-5), Metelimumab (anti-TGF beta 1), Milatuzumab (anti-CD74), Minretumomab (anti-TAG-72), Mitumomab (BEC-2, anti-GD3 ganglioside), Morolimumab (anti-Rhesus factor), Motavizumab (Numax, anti-respiratory syncytial virus), Muromonab-CD3 (Orthoclone OKT3, anti-CD3), Nacolomab (anti-C242), Naptumomab (anti-5T4), Natalizumab (Tysabri, anti-integrin α_4), Nebacumab (anti-endotoxin), Necitumumab (anti-EGFR), Nerelimomab (anti-TNF-α), Nimotuzumab (Theracim, Theraloc, anti-EGFR), Nofetumomab, Ocrelizumab (anti-CD20), Odulimomab (Afolimomab, anti-LFA-1 (CD11a)), Ofatumumab (Arzerra, anti-CD20), Olaratumab (anti-PDGF-R α), Omalizumab (Xolair, anti-IgE Fc region), Oportuzumab (anti-EpCAM), Oregovomab (OvaRex, anti-CA-125), Otelixizumab (anti-CD3), Pagibaximab (anti-lipoteichoic acid), Palivizumab (Synagis, Abbosynagis, anti-respiratory syncytial virus), Panitumumab (Vectibix, ABX-EGF, anti-EGFR), Panobacumab (anti-*Pseudomonas aeruginosa*), Pascolizumab (anti-IL-4), Pemtumomab (Theragyn, anti-MUC1), Pertuzumab (Omnitarg, 2C4, anti-HER2/neu), Pexelizumab (anti-C5), Pintumomab (anti-adenocarcinoma antigen), Priliximab (anti-CD4), Pritumumab (anti-vimentin), PRO 140 (anti-CCR5), Racotumomab (1E10, anti-(N-glycolylneuraminic acid (NeuGc, NGNA)-gangliosides GM3)), Rafivirumab (anti-rabies virus glycoprotein), Ramucirumab (anti-VEGFR2), Ranibizumab (Lucentis, anti-VEGF-A), Raxibacumab (anti-anthrax toxin, protective antigen), Regavirumab (anti-cytomegalovirus glycoprotein B), Reslizumab (anti-IL-5), Rilotumumab (anti-HGF), Rituximab (MabThera, Rituxanmab, anti-CD20), Robatumumab (anti-IGF-1 receptor), Rontalizumab (anti-IFN-α), Rovelizumab (LeukArrest, anti-CD11, CD18), Ruplizumab (Antova, anti-CD154 (CD40L)), Satumomab (anti-TAG-72), Sevirumab (anti-cytomegalovirus), Sibrotuzumab (anti-FAP), Sifalimumab (anti-IFN-α), Siltuximab (anti-IL-6), Siplizumab (anti-CD2), (Smart) MI95 (anti-CD33), Solanezumab (anti-beta amyloid), Sonepcizumab (anti-sphingosine-1-phosphate), Sontuzumab (anti-episialin), Stamulumab (anti-myostatin), Sulesomab (LeukoScan, (anti-NCA-90 (granulocyte antigen), Tacatuzumab (anti-alpha-fetoprotein), Tadocizumab (anti-integrin $\alpha_{IIb}\beta_3$), Talizumab (anti-IgE), Tanezumab (anti-NGF), Taplitumomab (anti-CD19), Tefibazumab (Aurexis, (anti-clumping factor A), Telimomab, Tenatumomab (anti-tenascin C), Teneliximab (anti-CD40), Teplizumab (anti-CD3), TGN1412 (anti-CD28), Ticilimumab (Tremelimumab, (anti-CTLA-4), Tigatuzumab (anti-TRAIL-R2), TNX-650 (anti-IL-13), Tocilizumab (Atlizumab, Actemra, RoActemra, (anti-IL-6 receptor),

Toralizumab (anti-CD154 (CD40L)), Tositumomab (anti-CD20), Trastuzumab (Herceptin, (anti-HER2/neu), Tremelimumab (anti-CTLA-4), Tucotuzumab celmoleukin (anti-EpCAM), Tuvirumab (anti-hepatitis B virus), Urtoxazumab (anti- *Escherichia coli*), Ustekinumab (Stelara, anti-IL-12, IL-23), Vapaliximab (anti-AOC3 (VAP-1)), Vedolizumab, (anti-integrin $\alpha_4\beta_7$), Veltuzumab (anti-CD20), Vepalimomab (anti-AOC3 (VAP-1), Visilizumab (Nuvion, anti-CD3), Vitaxin (anti-vascular integrin avb3), Volociximab (anti-integrin $\alpha_5\beta_1$), Votumumab (HumaSPECT, anti-tumor antigen CTAA16.88), Zalutumumab (HuMax-EGFr, (anti-EGFR), Zanolimumab (HuMax-CD4, anti-CD4), Ziralimumab (anti-CD147 (basigin)), Zolimomab (anti-CD5), Etanercept (Enbrel®), Alefacept (Amevive®), Abatacept (Orencia®), Rilonacept (Arcalyst), 14F7 [anti-IRP-2 (Iron Regulatory Protein 2)], 14G2a (anti-GD2 ganglioside, from Nat. Cancer Inst. for melanoma and solid tumors), J591 (anti-PSMA, Weill Cornell Medical School for prostate cancers), 225.28S [anti-HMW-MAA (High molecular weight-melanoma-associated antigen), Sorin Radiofarmaci S.R.L. (Milan, Italy) for melanoma], COL-1 (anti-CEACAM3, CGM1, from Nat. Cancer Inst. USA for colorectal and gastric cancers), CYT-356 (Oncoltad®, for prostate cancers), HNK20 (OraVax Inc. for respiratory syncytial virus), ImmuRAIT (from Immunomedics for NHL), Lym-1 (anti-HLA-DR10, Peregrine Pharm. for Cancers), MAK-195F [anti-TNF (tumor necrosis factor; TNFA, TNF-alpha; TNFSF2), from Abbott / Knoll for Sepsis toxic shock], MEDI-500 [T10B9, anti-CD3, TR$\alpha\beta$ (T cell receptor alpha/beta), complex, from MedImmune Inc for Graft-versus-host disease], RING SCAN [anti-TAG 72 (tumour associated glycoprotein 72), from Neoprobe Corp. for Breast, Colon and Rectal cancers], Avicidin (anti-EPCAM (epithelial cell adhesion molecule), anti-TACSTD1 (Tumor-associated calcium signal transducer 1), anti-GA733-2 (gastrointestinal tumor-associated protein 2), anti-EGP-2 (epithelial glycoprotein 2); anti-KSA; KS1/4 antigen; M4S; tumor antigen 17-1A; CD326, from NeoRx Corp. for Colon, Ovarian, Prostate cancers and NHL]; LymphoCide (Immunomedics, NJ), Smart ID10 (Protein Design Labs), Oncolym (Techniclone Inc, CA), Allomune (BioTransplant, CA), anti-VEGF (Genentech, CA); CEAcide (Immunomedics, NJ), IMC-1C11 (ImClone Systems, NJ) and Cetuximab (ImClone, NJ).

Other antibodies as binding ligands include, but are not limited to, are antibodies against the following antigens: Aminopeptidase N (CD13), Annexin A1, B7-H3 (CD276, various cancers), CA125, CA15-3 (carcinomas), CA19-9 (carcinomas), L6 (carcinomas), Lewis Y (carcinomas), Lewis X (carcinomas), alpha fetoprotein (carcinomas), CA242, placental alkaline phosphatase (carcinomas), prostate specific antigen (prostate), prostatic acid phosphatase (prostate), epidermal growth factor (carcinomas), CD2 (Hodgkin's disease, NHL lymphoma, multiple myeloma), CD3 epsilon (T cell lymphoma, lung, breast, gastric, ovarian cancers, autoimmune diseases, malignant ascites), CD19 (B cell malignancies), CD20 (non-Hodgkin's

lymphoma), CD22 (leukemia, lymphoma, multiple myeloma, SLE), CD30, CD33, CD38 (multiple myeloma), CD40 (lymphoma, multiple myeloma, leukemia), CD51 (Metastatic melanoma, sarcoma), CD52, CD56 (small cell lung cancers, ovarian cancer, Merkel cell carcinoma, and the liquid tumor, multiple myeloma), CD66e (cancers), CD70 (metastatic renal cell carcinoma and non-Hodgkin lymphoma), CD74 (multiple myeloma), CD80 (lymphoma), CD98 (cancers), mucin (carcinomas), CD221 (solid tumors), CD227 (breast, ovarian cancers), CD262 (NSCLC and other cancers), CD309 (ovarian cancers), CD326 (solid tumors), CEACAM3 (colorectal, gastric cancers), CEACAM5 (carcinoembryonic antigen; CEA, CD66e) (breast, colorectal and lung cancers), DLL4 (Δ-like-4), EGFR (Epidermal Growth Factor Receptor, various cancers), CTLA4 (melanoma), CXCR4 (CD184, Heme-oncology, solid tumors), Endoglin (CD105, solid tumors), EPCAM (epithelial cell adhesion molecule, bladder, head, neck, colon, NHL prostate, and ovarian cancers), ERBB2 (Epidermal Growth Factor Receptor 2; lung, breast, prostate cancers), FCGR1 (autoimmune diseases), FOLR (folate receptor, ovarian cancers), GD2 ganglioside (cancers), G-28 (a cell surface antigen glyvolipid, melanoma), GD3 idiotype (cancers), Heat shock proteins (cancers), HER1 (lung, stomach cancers), HER2 (breast, lung and ovarian cancers), HLA-DR10 (NHL), HLA-DRB (NHL, B cell leukemia), human chorionic gonadotropin (carcinoma), IGF1R (insulin-like growth factor 1 receptor, solid tumors, blood cancers), IL-2 receptor (interleukin 2 receptor,T-cell leukemia and lymphomas), IL-6R (interleukin 6 receptor, multiple myeloma, RA, Castleman's disease, IL6 dependent tumors), Integrins (αvβ3, α5β1, α6β4, αIIβ3, α5β5, αvβ5, for various cancers), MAGE-1 (carcinomas), MAGE-2 (carcinomas), MAGE-3 (carcinomas), MAGE 4 (carcinomas), anti-transferrin receptor (carcinomas), p97 (melanoma), MS4A1 (membrane-spanning 4-domains subfamily A member 1, Non-Hodgkin's B cell lymphoma, leukemia), MUC1 or MUC1-KLH (breast, ovarian, cervix, bronchus and gastrointestinal cancer), MUC16 (CA125) (Ovarian cancers), CEA (colorectal), gp100 (melanoma), MART1 (melanoma), MPG (melanoma), MS4A1 (membrane-spanning 4-domains subfamily A, small cell lung cancers, NHL), Nucleolin, Neu oncogene product (carcinomas), P21 (carcinomas), Paratope of anti-(N-glycolylneuraminic acid, Breast, Melanoma cancers), PLAP-like testicular alkaline phosphatase (ovarian, testicular cancers), PSMA (prostate tumors), PSA (prostate), ROBO4, TAG 72 (tumour associated glycoprotein 72, AML, gastric, colorectal, ovarian cancers), T cell transmembrane protein (cancers), Tie (CD202b), TNFRSF10B (tumor necrosis factor receptor superfamily member 10B, cancers), TNFRSF13B (tumor necrosis factor receptor superfamily member 13B, multiple myeloma, NHL, other cancers, RA and SLE), TPBG (trophoblast glycoprotein, Renal cell carcinoma), TRAIL-R1 (Tumor necrosis apoprosis Inducing ligand Receptor 1,lymphoma, NHL, colorectal, lung cancers), VCAM-1 (CD106, Melanoma), VEGF, VEGF-A, VEGF-2 (CD309)

(various cancers). Some other tumor associated antigens recognized by antibodies have been reviewed (Gerber, et al, mAbs 1:3, 247-253 (2009); Novellino et al, Cancer Immunol Immunother. 54(3),187-207 (2005). Franke, et al, Cancer Biother Radiopharm. 2000, 15, 459-76). Many other antigens are: many other Cluster of Differentiations (CD4, CD5, CD6, CD7, CD8, CD9, CD10, CD11a, CD11b, CD11c, CD12w, CD14, CD15, CD16, CDw17, CD18, CD21, CD23, CD24, CD25, CD26, CD27, CD28, CD29, CD31, CD32, CD34, CD35, CD36, CD37, CD41, CD42, CD43, CD44, CD45, CD46, CD47, CD48, CD49b, CD49c, CD53, CD54, CD55, CD58, CD59, CD61, CD62E, CD62L, CD62P, CD63, CD68, CD69, CD71, CD72, CD79, CD79a, CD79b, CD81, CD82, CD83, CD86, CD87, CD88, CD89, CD90, CD91, CD95, CD96, CD100, CD103, CD105, CD106, CD109, CD117, CD120, CD127, CD133, CD134, CD135, CD138, CD141, CD142, CD143, CD144, CD147, CD151, CD152, CD154, CD156, CD158, CD163, CD166, .CD168, CD184, CDw186, CD195, CD202 (a, b), CD209, CD235a, CD271, CD303, CD304), Apo2, ASLG659, BMPR1B (bone morphogenetic protein receptor), CRIPTO, Annexin A1, Nucleolin, Endoglin (CD105), ROBO4, Amino-peptidase N, Δ-like-4 (DLL4), VEGFR-2 (CD309), CXCR4 9CD184), Tie2, B7-H3, WT1, MUC1, LMP2, HPV E6 E7, EGFRvIII, HER-2/neu, Idiotype, MAGE A3, p53 nonmutant, NY-ESO-1, GD2, CEA, MelanA/MART1, Napi3b (NAPI-3B, NPTIIb, SLC34A2, solute carrier family 34, member 2, type II sodium –dependent phosphate transporter 3b), Ras mutant, gp100, p53 mutant, Proteinase3 (PR1), bcr-abl, Tetratocarcinoma-derived growth factors), EphA receptors, EphB receptors, EGFr, EGFRvIII, ETBR (Endothelin), HER2/neu, HER3, HLA-DOB (MHC class II molecule Ia antigen), integrins, IRTA2, MPF (MPF, MSLN, SMR, megakaryocyte potentiating factor, mesothelin), cripto, Sema 5b (FLJ10372, KIAA1445, Mm42015, SEMA5B, 5EMAG, semaphoring 5 bHlog, sdema domain, seven thrombospondin repeats, cytoplasmic domain), PSCA, STEAP1 (six transmembrane epithelial antigen of prostate), and STEAP2 (HGNC 8639, IPCA-1, PCANP1, STAMP1, STEAP2, STMP, prostate)Tyrosinase, Survivin, hTERT, Sarcoma translocation breakpoints, EphA2, PAP, ML-IAP, AFP, EpCAM, ERG (TMPRSS2 ETS fusion gene), NA17, PAX3, ALK, Androgen receptor, Cyclin B1, Polysialic acid, MYCN, RhoC, TRP-2, GD3, Fucosyl GM1, Mesothelin, PSCA, MAGE A1, sLe(a), CYP1B1, PLAC1, GM3, BORIS, Tn, GloboH, ETV6-AML, NY-BR-1, RGS5, SART3, STn, Carbonic anhydrase IX, PAX5, OY-TES1, Sperm protein 17, LCK, HMWMAA, AKAP-4, SSX2, XAGE 1, B7H3, Legumain, Tie 2, Page4, VEGFR2, MAD-CT-1, FAP, PDGFR-β, MAD-CT-2, Fos-related antigen 1.

In another specific embodiment, the antimitotic agent- binding molecule conjugates of the invention are used in accordance with the compositions and methods of the invention for the treatment of cancers. The cancers include, but are not limited, Adrenocortical Carcinoma, Anal

Cancer, Bladder Cancer, Brain Tumor (Adult, Brain Stem Glioma, Childhood, Cerebellar Astrocytoma, Cerebral Astrocytoma, Ependymoma, Medulloblastoma, Supratentorial Primitive Neuroectodermal and Pineal Tumors, Visual Pathway and Hypothalamic Glioma), Breast Cancer, Carcinoid Tumor, Gastrointestinal, Carcinoma of Unknown Primary, Cervical Cancer, Colon Cancer, Endometrial Cancer, Esophageal Cancer, Extrahepatic Bile Duct Cancer, Ewings Family of Tumors (PNET), Extracranial Germ Cell Tumor, Eye Cancer, Intraocular Melanoma, Gallbladder Cancer, Gastric Cancer (Stomach), Germ Cell Tumor, Extragonadal, Gestational Trophoblastic Tumor, Head and Neck Cancer, Hypopharyngeal Cancer, Islet Cell Carcinoma, Kidney Cancer (renal cell cancer), Laryngeal Cancer, Leukemia (Acute Lymphoblastic, Acute Myeloid, Chronic Lymphocytic, Chronic Myelogenous, Hairy Cell), Lip and Oral Cavity Cancer, Liver Cancer, Lung Cancer (Non-Small Cell, Small Cell, Lymphoma (AIDS-Related, Central Nervous System, Cutaneous T-Cell, Hodgkin's Disease, Non-Hodgkin's Disease, Malignant Mesothelioma, Melanoma, Merkel Cell Carcinoma, Metasatic Squamous Neck Cancer with Occult Primary, Multiple Myeloma, and Other Plasma Cell Neoplasms, Mycosis Fungoides, Myelodysplastic Syndrome, Myeloproliferative Disorders, Nasopharyngeal Cancer, Neuroblastoma, Oral Cancer, Oropharyngeal Cancer, Osteosarcoma, Ovarian Cancer (Epithelial, Germ Cell Tumor, Low Malignant Potential Tumor), Pancreatic Cancer (Exocrine, Islet Cell Carcinoma), Paranasal Sinus and Nasal Cavity Cancer, Parathyroid Cancer, Penile Cancer, Pheochromocytoma Cancer, Pituitary Cancer, Plasma Cell Neoplasm, Prostate Cancer Rhabdomyosarcoma, Rectal Cancer, Renal Cell Cancer (kidney cancer), Renal Pelvis and Ureter (Transitional Cell), Salivary Gland Cancer, Sezary Syndrome, Skin Cancer, Skin Cancer (Cutaneous T-Cell Lymphoma, Kaposi's Sarcoma, Melanoma), Small Intestine Cancer, Soft Tissue Sarcoma, Stomach Cancer, Testicular Cancer, Thymoma (Malignant), Thyroid Cancer, Urethral Cancer, Uterine Cancer (Sarcoma), Unusual Cancer of Childhood, Vaginal Cancer, Vulvar Cancer, Wilms' Tumor

In another specific embodiment, the antimitotic agent- binding molecule conjugates of the invention are used in accordance with the compositions and methods of the invention for the treatment or prevention of an autoimmune disease. The autoimmune diseases include, but are not limited, Achlorhydra Autoimmune Active Chronic Hepatitis, Acute Disseminated Encephalomyelitis, Acute hemorrhagic leukoencephalitis, Addison's Disease, Agammaglobulinemia, Alopecia areata, Amyotrophic Lateral Sclerosis, Ankylosing Spondylitis, Anti-GBM/TBM Nephritis, Antiphospholipid syndrome, Antisynthetase syndrome, Arthritis, Atopic allergy, Atopic Dermatitis, Autoimmune Aplastic Anemia, Autoimmune cardiomyopathy, Autoimmune hemolytic anemia, Autoimmune hepatitis, Autoimmune inner ear disease, Autoimmune lymphoproliferative syndrome, Autoimmune peripheral neuropathy, Autoimmune

pancreatitis, Autoimmune polyendocrine syndrome Types I, II, & III, Autoimmune progesterone dermatitis, Autoimmune thrombocytopenic purpura, Autoimmune uveitis, Balo disease/Balo concentric sclerosis, Bechets Syndrome, Berger's disease, Bickerstaff's encephalitis, Blau syndrome, Bullous Pemphigoid, Castleman's disease, Chagas disease, Chronic Fatigue Immune Dysfunction Syndrome, Chronic inflammatory demyelinating polyneuropathy, Chronic recurrent multifocal ostomyelitis, Chronic lyme disease, Chronic obstructive pulmonary disease, Churg-Strauss syndrome, Cicatricial Pemphigoid, Coeliac Disease, Cogan syndrome, Cold agglutinin disease, Complement component 2 deficiency, Cranial arteritis, CREST syndrome, Crohns Disease (a type of idiopathic inflammatory bowel diseases), Cushing's Syndrome, Cutaneous leukocytoclastic angiitis, Dego's disease, Dercum's disease, Dermatitis herpetiformis, Dermatomyositis, Diabetes mellitus type 1, Diffuse cutaneous systemic sclerosis, Dressler's syndrome, Discoid lupus erythematosus, Eczema, Endometriosis, Enthesitis-related arthritis, Eosinophilic fasciitis, Epidermolysis bullosa acquisita, Erythema nodosum, Essential mixed cryoglobulinemia, Evan's syndrome, Fibrodysplasia ossificans progressiva, Fibromyalgia, Fibromyositis, Fibrosing aveolitis, Gastritis, Gastrointestinal pemphigoid, Giant cell arteritis, Glomerulonephritis, Goodpasture's syndrome, Graves' disease, Guillain-Barré syndrome, Hashimoto's encephalitis, Hashimoto's thyroiditis, Haemolytic anaemia, Henoch-Schonlein purpura, Herpes gestationis, Hidradenitis suppurativa, Hughes syndrome (See Antiphospholipid syndrome), Hypogammaglobulinemia, Idiopathic Inflammatory Demyelinating Diseases, Idiopathic pulmonary fibrosis, Idiopathic thrombocytopenic purpura (See Autoimmune thrombocytopenic purpura), IgA nephropathy (Also Berger's disease), Inclusion body myositis, Inflammatory demyelinating polyneuopathy, Interstitial cystitis, Irritable Bowel Syndrome , Juvenile idiopathic arthritis, Juvenile rheumatoid arthritis, Kawasaki's Disease, Lambert-Eaton myasthenic syndrome, Leukocytoclastic vasculitis, Lichen planus, Lichen sclerosus, Linear IgA disease (LAD), Lou Gehrig's Disease (Also Amyotrophic lateral sclerosis), Lupoid hepatitis, Lupus erythematosus, Majeed syndrome, Ménière's disease, Microscopic polyangiitis, Miller-Fisher syndrome, Mixed Connective Tissue Disease, Morphea, Mucha-Habermann disease, Muckle–Wells syndrome, Multiple Myeloma, Multiple Sclerosis, Myasthenia gravis, Myositis, Narcolepsy, Neuromyelitis optica (Devic's Disease), Neuromyotonia, Occular cicatricial pemphigoid, Opsoclonus myoclonus syndrome, Ord thyroiditis, Palindromic rheumatism, PANDAS (Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcus), Paraneoplastic cerebellar degeneration, Paroxysmal nocturnal hemoglobinuria, Parry Romberg syndrome, Parsonnage-Turner syndrome, Pars planitis, Pemphigus, Pemphigus vulgaris, Pernicious anaemia, Perivenous encephalomyelitis, POEMS syndrome, Polyarteritis nodosa, Polymyalgia rheumatica, Polymyositis, Primary biliary cirrhosis, Primary sclerosing cholangitis,

Progressive inflammatory neuropathy, Psoriasis, Psoriatic Arthritis, Pyoderma gangrenosum, Pure red cell aplasia, Rasmussen's encephalitis, Raynaud phenomenon, Relapsing polychondritis, Reiter's syndrome, Restless leg syndrome, Retroperitoneal fibrosis, Rheumatoid arthritis, Rheumatoid fever, Sarcoidosis, Schizophrenia, Schmidt syndrome, Schnitzler syndrome, Scleritis, Scleroderma, Sjögren's syndrome, Spondyloarthropathy, Sticky blood syndrome, Still's Disease, Stiff person syndrome, Subacute bacterial endocarditis, Susac's syndrome, Sweet syndrome, Sydenham Chorea, Sympathetic ophthalmia, Takayasu's arteritis, Temporal arteritis (giant cell arteritis), Tolosa-Hunt syndrome, Transverse Myelitis, Ulcerative Colitis (a type of idiopathic inflammatory bowel diseases), Undifferentiated connective tissue disease, Undifferentiated spondyloarthropathy, Vasculitis, Vitiligo, Wegener's granulomatosis, Wilson's syndrome, Wiskott-Aldrich syndrome

In another specific embodiment, a binding molecule used for the conjugate for the treatment or prevention of an autoimmune disease includes, but are not limited to, anti-elastin antibody; Abys against epithelial cells antibody; Anti-Basement Membrane Collagen Type IV Protein antibody; Anti-Nuclear Antibody; Anti ds DNA; Anti ss DNA, Anti Cardiolipin Antibody IgM, IgG; anti-celiac antibody; Anti Phospholipid Antibody IgK, IgG; Anti SM Antibody; Anti Mitochondrial Antibody; Thyroid Antibody; Microsomal Antibody, T-cells antibody; Thyroglobulin Antibody, Anti SCL-70; Anti-Jo; Anti-U.sub.1RNP; Anti-La/SSB; Anti SSA; Anti SSB; Anti Perital Cells Antibody; Anti Histones; Anti RNP; C-ANCA; P-ANCA; Anti centromere; Anti-Fibrillarin, and Anti GBM Antibody, Anti-ganglioside antibody; Anti-Desmogein 3 antibody; Anti-p62 antibody; Anti-sp100 antibody; Anti-Mitochondrial(M2) antibody; Rheumatoid factor antibody; Anti-MCV antibody; Anti-topoisomerase antibody; Anti-neutrophil cytoplasmic(cANCA) antibody.

In certain preferred embodiments, the binding molecule for the conjugate in the present invention, can bind to either a receptor or a receptor complex expressed on an activated lymphocyte which is associated with an autoimmune disease. The receptor or receptor complex can comprise an immunoglobulin gene superfamily member (e.g. CD2, CD3, CD4, CD8, CD19, CD22, CD28, CD79, CD90, CD152/CTLA-4, PD-1, or ICOS), a TNF receptor superfamily member (e.g. CD27, CD40, CD95/Fas, CD134/OX40, CD137/4-1BB, INF-R1, TNFR-2, RANK, TACI, BCMA, osteoprotegerin, Apo2/TRAIL-R1, TRAIL-R2, TRAIL-R3, TRAIL-R4, and APO-3), an integrin, a cytokine receptor, a chemokine receptor, a major histocompatibility protein, a lectin (C-type, S-type, or I-type), or a complement control protein.

In another specific embodiment, useful binding ligands that are immunospecific for a viral or a microbial antigen are humanized or human monoclonal antibodies. As used herein, the term "viral antigen" includes, but is not limited to, any viral peptide, polypeptide protein (e.g. HIV

gp120, HIV nef, RSV F glycoprotein, influenza virus neuraminidase, influenza virus hemagglutinin, HTLV tax, herpes simplex virus glycoprotein (e.g. gB, gC, gD, and gE) and hepatitis B surface antigen) that is capable of eliciting an immune response. As used herein, the term "microbial antigen" includes, but is not limited to, any microbial peptide, polypeptide, protein, saccharide, polysaccharide, or lipid molecule (e.g., a bacteria, fungi, pathogenic protozoa, or yeast polypeptide including, e.g., LPS and capsular polysaccharide 5/8) that is capable of eliciting an immune response. Examples of antibodies available l for the viral or microbial infection include, but are not limited to, Palivizumab which is a humanized anti-respiratory syncytial virus monoclonal antibody for the treatment of RSV infection; PRO542 which is a CD4 fusion antibody for the treatment of HIV infection; Ostavir which is a human antibody for the treatment of hepatitis B virus; PROTVIR which is a humanized IgG.sub.1 antibody for the treatment of cytomegalovirus; and anti-LPS antibodies.

The binding molecules –antimitotic agent conjugates of this invention can be used in the treatment of infectious diseases. These infectious diseases include, but are not limited to, Acinetobacter infections, Actinomycosis, African sleeping sickness (African trypanosomiasis), AIDS (Acquired immune deficiency syndrome), Amebiasis, Anaplasmosis, Anthrax, Arcano-bacterium haemolyticum infection, Argentine hemorrhagic fever, Ascariasis, Aspergillosis, Astrovirus infection, Babesiosis, Bacillus cereus infection, Bacterial pneumonia, Bacterial vaginosis, Bacteroides infection, Balantidiasis, Baylisascaris infection, BK virus infection, Black piedra, Blastocystis hominis infection, Blastomycosis, Bolivian hemorrhagic fever, Borrelia infection, Botulism (and Infant botulism), Brazilian hemorrhagic fever, Brucellosis, Burk-holderia infection, Buruli ulcer, Calicivirus infection (Norovirus and Sapovirus), Campylo-bacteriosis, Candidiasis (Moniliasis; Thrush), Cat-scratch disease, Cellulitis, Chagas Disease (American trypanosomiasis), Chancroid, Chickenpox, Chlamydia, Chlamydophila pneumoniae infection, Cholera, Chromoblastomycosis, Clonorchiasis, Clostridium difficile infection, Coccidioidomycosis, Colorado tick fever, Common cold (Acute viral rhinopharyngitis; Acute coryza), Creutzfeldt-Jakob disease, Crimean-Congo hemorrhagic fever, Cryptococcosis, Cryptosporidiosis, Cutaneous larva migrans, Cyclosporiasis, Cysticercosis, Cytomegalovirus infection, Dengue fever, Dientamoebiasis, Diphtheria, Diphyllobothriasis, Dracunculiasis, Ebola hemorrhagic fever, Echinococcosis, Ehrlichiosis, Enterobiasis (Pinworm infection), Entero-coccus infection, Enterovirus infection, Epidemic typhus, Erythema infectiosum (Fifth disease), Exanthem subitum, Fasciolopsiasis, Fasciolosis, Fatal familial insomnia, Filariasis, Food poisoning by Clostridium perfringens, Free-living amebic infection, Fusobacterium infection, Gas gangrene (Clostridial myonecrosis), Geotrichosis, Gerstmann-Sträussler-Scheinker syndrome, Giardiasis, Glanders, Gnathostomiasis, Gonorrhea, Granuloma inguinale

(Donovanosis), Group A streptococcal infection, Group B streptococcal infection, Haemophilus influenzae infection, Hand, foot and mouth disease (HFMD), Hantavirus Pulmonary Syndrome, Helicobacter pylori infection, Hemolytic-uremic syndrome, Hemorrhagic fever with renal syndrome, Hepatitis A, Hepatitis B, Hepatitis C, Hepatitis D, Hepatitis E, Herpes simplex, Histoplasmosis, Hookworm infection, Human bocavirus infection, Human ewingii ehrlichiosis, Human granulocytic anaplasmosis, Human metapneumovirus infection, Human monocytic ehrlichiosis, Human papillomavirus infection, Human parainfluenza virus infection, Hymenolepiasis, Epstein-Barr Virus Infectious Mononucleosis (Mono), Influenza, Isosporiasis, Kawasaki disease, Keratitis, Kingella kingae infection, Kuru, Lassa fever, Legionellosis (Legionnaires' disease), Legionellosis (Pontiac fever), Leishmaniasis, Leprosy, Leptospirosis, Listeriosis, Lyme disease (Lyme borreliosis), Lymphatic filariasis (Elephantiasis), Lymphocytic choriomeningitis, Malaria, Marburg hemorrhagic fever, Measles, Melioidosis (Whitmore's disease), Meningitis, Meningococcal disease, Metagonimiasis, Microsporidiosis, Molluscum contagiosum, Mumps, Murine typhus (Endemic typhus), Mycoplasma pneumonia, Mycetoma, Myiasis, Neonatal conjunctivitis (Ophthalmia neonatorum), (New) Variant Creutzfeldt-Jakob disease (vCJD, nvCJD), Nocardiosis, Onchocerciasis (River blindness), Paracoccidioidomycosis (South American blastomycosis), Paragonimiasis, Pasteurellosis, Pediculosis capitis (Head lice), Pediculosis corporis (Body lice), Pediculosis pubis (Pubic lice, Crab lice), Pelvic inflammatory disease, Pertussis (Whooping cough), Plague, Pneumococcal infection, Pneumocystis pneumonia, Pneumonia, Poliomyelitis, Prevotella infection, Primary amoebic meningoencephalitis, Progressive multifocal leukoencephalopathy, Psittacosis, Q fever, Rabies, Rat-bite fever, Respiratory syncytial virus infection, Rhinosporidiosis, Rhinovirus infection, Rickettsial infection, Rickettsialpox, Rift Valley fever, Rocky mountain spotted fever, Rotavirus infection, Rubella, Salmonellosis, SARS (Severe Acute Respiratory Syndrome), Scabies, Schistosomiasis, Sepsis, Shigellosis (Bacillary dysentery), Shingles (Herpes zoster), Smallpox (Variola), Sporotrichosis, Staphylococcal food poisoning, Staphylococcal infection, Strongyloidiasis, Syphilis, Taeniasis, Tetanus (Lockjaw), Tinea barbae (Barber's itch), Tinea capitis (Ringworm of the Scalp), Tinea corporis (Ringworm of the Body), Tinea cruris (Jock itch), Tinea manuum , Tinea nigra, Tinea pedis (Athlete's foot), Tinea unguium (Onychomy-cosis), Tinea versicolor (Pityriasis versicolor), Toxocariasis (Ocular Larva Migrans), Toxocariasis (Visceral Larva Migrans), Toxoplasmosis, Trichinellosis, Trichomoniasis, Trichuriasis (Whipworm infection), Tuberculosis, Tularemia, Ureaplasma urealyticum infection, Venezuelan equine encephalitis, Venezuelan hemorrhagic fever, Viral pneumonia, West Nile Fever, White piedra (Tinea blanca), Yersinia pseudotuberculosis infection, Yersiniosis, Yellow fever, Zygomycosis.

The binding molecules, preferable antibodies described in this patent that are against pathogenic strains include, but are not limit, Acinetobacter baumannii, Actinomyces israelii, Actinomyces gerencseriae and Propionibacterium propionicus, Trypanosoma brucei, HIV (Human immunodeficiency virus), Entamoeba histolytica, Anaplasma genus, Bacillus anthracis, Arcanobacterium haemolyticum, Junin virus, Ascaris lumbricoides, Aspergillus genus, Astroviridae family, Babesia genus, Bacillus cereus, multiple bacteria, Bacteroides genus, Balantidium coli, Baylisascaris genus, BK virus, Piedraia hortae, Blastocystis hominis, Blastomyces dermatitides, Machupo virus, Borrelia genus, Clostridium botulinum, Sabia, Brucella genus, usually Burkholderia cepacia and other Burkholderia species, Mycobacterium ulcerans, Caliciviridae family, Campylobacter genus, usually Candida albicans and other Candida species, Bartonella henselae, Group A Streptococcus and Staphylococcus, Trypanosoma cruzi, Haemophilus ducreyi, Varicella zoster virus (VZV), Chlamydia trachomatis, Chlamydophila pneumoniae, Vibrio cholerae, Fonsecaea pedrosoi, Clonorchis sinensis, Clostridium difficile, Coccidioides immitis and Coccidioides posadasii, Colorado tick fever virus, rhinoviruses, coronaviruses, CJD prion, Crimean-Congo hemorrhagic fever virus, Cryptococcus neoformans, Cryptosporidium genus, Ancylostoma braziliense; multiple parasites, Cyclospora cayetanensis, Taenia solium, Cytomegalovirus, Dengue viruses (DEN-1, DEN-2, DEN-3 and DEN-4) – Flaviviruses, Dientamoeba fragilis, Corynebacterium diphtheriae, Diphyllobothrium, Dracunculus medinensis, Ebolavirus, Echinococcus genus, Ehrlichia genus, Enterobius vermicularis, Enterococcus genus, Enterovirus genus, Rickettsia prowazekii, Parvovirus B19, Human herpesvirus 6 and Human herpesvirus 7, Fasciolopsis buski, Fasciola hepatica and Fasciola gigantica, FFI prion, Filarioidea superfamily, Clostridium perfringens, Fusobacterium genus, Clostridium perfringens; other Clostridium species, Geotrichum candidum, GSS prion, Giardia intestinalis, Burkholderia mallei, Gnathostoma spinigerum and Gnathostoma hispidum, Neisseria gonorrhoeae, Klebsiella granulomatis, Streptococcus pyogenes, Streptococcus agalactiae, Haemophilus influenzae, Enteroviruses, mainly Coxsackie A virus and Enterovirus 71, Sin Nombre virus, Helicobacter pylori, Escherichia coli O157:H7, Bunyaviridae family, Hepatitis A Virus, Hepatitis B Virus, Hepatitis C Virus, Hepatitis D Virus, Hepatitis E Virus, Herpes simplex virus 1, Herpes simplex virus 2, Histoplasma capsulatum, Ancylostoma duodenale and Necator americanus, Hemophilus influenzae, Human bocavirus, Ehrlichia ewingii, Anaplasma phagocytophilum, Human metapneumovirus, Ehrlichia chaffeensis, Human papillomavirus, Human parainfluenza viruses, Hymenolepis nana and Hymenolepis diminuta, Epstein-Barr Virus, Orthomyxoviridae family, Isospora belli, Kingella kingae, Klebsiella pneumoniae, Klebsiella ozaenas, Klebsiella rhinoscleromotis, Kuru prion, Lassa virus, Legionella pneumophila, Legionella pneumophila, Leishmania genus,

Mycobacterium leprae and Mycobacterium lepromatosis, Leptospira genus, Listeria monocytogenes, Borrelia burgdorferi and other Borrelia species, Wuchereria bancrofti and Brugia malayi, Lymphocytic choriomeningitis virus (LCMV), Plasmodium genus, Marburg virus, Measles virus, Burkholderia pseudomallei, Neisseria meningitides, Metagonimus yokagawai, Microsporidia phylum, Molluscum contagiosum virus (MCV), Mumps virus, Rickettsia typhi, Mycoplasma pneumoniae, numerous species of bacteria (Actinomycetoma) and fungi (Eumycetoma), parasitic dipterous fly larvae, Chlamydia trachomatis and Neisseria gonorrhoeae, vCJD prion, Nocardia asteroides and other Nocardia species, Onchocerca volvulus, Paracoccidioides brasiliensis, Paragonimus westermani and other Paragonimus species, Pasteurella genus, Pediculus humanus capitis, Pediculus humanus corporis, Phthirus pubis, Bordetella pertussis, Yersinia pestis, Streptococcus pneumoniae, Pneumocystis jirovecii, Poliovirus, Prevotella genus, Naegleria fowleri, JC virus, Chlamydophila psittaci, Coxiella burnetii, Rabies virus, Streptobacillus moniliformis and Spirillum minus, Respiratory syncytial virus, Rhinosporidium seeberi, Rhinovirus, Rickettsia genus, Rickettsia akari, Rift Valley fever virus, Rickettsia rickettsii, Rotavirus, Rubella virus, Salmonella genus, SARS coronavirus, Sarcoptes scabiei, Schistosoma genus, Shigella genus, Varicella zoster virus, Variola major or Variola minor, Sporothrix schenckii, Staphylococcus genus, Staphylococcus genus, Staphylococcus aureus, Streptococcus pyogenes, Strongyloides stercoralis, Treponema pallidum, Taenia genus, Clostridium tetani, Trichophyton genus, Trichophyton tonsurans, Trichophyton genus, Epidermophyton floccosum, Trichophyton rubrum, and Trichophyton mentagrophytes, Trichophyton rubrum, Hortaea werneckii, Trichophyton genus, Malassezia genus, Toxocara canis or Toxocara cati, Toxoplasma gondii, Trichinella spiralis, Trichomonas vaginalis, Trichuris trichiura, Mycobacterium tuberculosis, Francisella tularensis, Ureaplasma urealyticum, Venezuelan equine encephalitis virus, Vibrio colerae, Guanarito virus, West Nile virus, Trichosporon beigelii, Yersinia pseudotuberculosis, Yersinia enterocolitica, Yellow fever virus, Mucorales order (Mucormycosis) and Entomophthorales order (Entomophthoramycosis), Pseudomonas aeruginosa, Campylobacter (Vibrio) fetus, Aeromonas hydrophila, Edwardsiella tarda, Yersinia pestis, Shigella dysenteriae, Shigella flexneri, Shigella sonnei, Salmonella typhimurium, Treponema pertenue, Treponema carateneum, Borrelia vincentii, Borrelia burgdorferi, Leptospira icterohemorrhagiae, Pneumocystis carinii, Brucella abortus, Brucella suis, Brucella melitensis, Mycoplasma spp., Rickettsia prowazeki, Rickettsia tsutsugumushi, Clamydia spp.; pathogenic fungi (Aspergillus fumigatus, Candida albicans, Histoplasma capsulatum); protozoa (Entomoeba histolytica, Trichomonas tenas, Trichomonas hominis, Tryoanosoma gambiense, Trypanosoma rhodesiense, Leishmania donovani, Leishmania tropica, Leishmania braziliensis, Pneumocystis pneumonia, Plasmodium vivax, Plasmodium falciparum,

Plasmodium malaria); or Helminiths (Schistosoma japonicum, Schistosoma mansoni, Schistosoma haematobium, and hookworms).

Other antibodies as a binding ligand in this invention for treatment of viral disease include, but are not limited to, antibodies against antigens of pathogenic viruses, including as examples and not by limitation: Poxyiridae, Herpesviridae, Adenoviridae, Papovaviridae, Enteroviridae, Picornaviridae, Parvoviridae, Reoviridae, Retroviridae, influenza viruses, parainfluenza viruses, mumps, measles, respiratory syncytial virus, rubella, Arboviridae, Rhabdoviridae, Arenaviridae, Non-A/Non-B Hepatitis virus, Rhinoviridae, Coronaviridae, Rotoviridae, Oncovirus [such as, HBV (Hepatocellular carcinoma), HPV (Cervical cancer, Anal cancer), Kaposi's sarcoma-associated herpesvirus (Kaposi's sarcoma), Epstein-Barr virus (Nasopharyngeal carcinoma, Burkitt's lymphoma, Primary central nervous system lymphoma), MCPyV (Merkel cell cancer), SV40 (*Simian virus 40)*, HCV (Hepatocellular carcinoma), HTLV-I (Adult T-cell leukemia/lymphoma)], Immune disorders caused virus: [such as Human Immunodeficiency Virus (AIDS)]; Central nervous system virus: [such as, JCV (Progressive multifocal leukoencephalopathy), MeV (Subacute sclerosing panencephalitis), LCV (Lymphocytic choriomeningitis), Arbovirus encephalitis, Orthomyxoviridae (probable) (Encephalitis lethargica), RV (Rabies), Chandipura virus, Herpesviral meningitis, Ramsay Hunt syndrome type II; Poliovirus (Poliomyelitis, Post-polio syndrome), HTLV-I (Tropical spastic paraparesis)]; Cytomegalovirus (Cytomegalovirus retinitis, HSV (Herpetic keratitis)); Cardiovascular virus [such as CBV (Pericarditis, Myocarditis)]; Respiratory system/acute viral nasopharyngitis/viral pneumonia: [Epstein-Barr virus (EBV infection/Infectious mononucleosis), Cytomegalovirus; SARS coronavirus (Severe acute respiratory syndrome) Orthomyxoviridae: Influenzavirus A/B/C (Influenza/Avian influenza), Paramyxovirus: Human parainfluenza viruses (Parainfluenza), RSV (Human respiratory syncytial virus), hMPV]; Digestive system virus [MuV (Mumps), Cytomegalovirus (Cytomegalovirus esophagitis); Adenovirus (Adenovirus infection); Rotavirus, Norovirus, Astrovirus, Coronavirus; HBV (Hepatitis B virus), CBV, HAV (Hepatitis A virus), HCV (Hepatitis C virus), HDV (Hepatitis D virus), HEV (Hepatitis E virus), HGV (Hepatitis G virus)]; Urogenital virus [such as, BK virus, MuV (Mumps)].

According to a further object, the present invention also concerns pharmaceutical compositions comprising the conjugate of the invention together with a pharmaceutically acceptable carrier for treatment of cancer and autoimmune disorders. The method for treatment of cancer and autoimmune disorders can be practiced *in vitro*, *in vivo*, or *ex vivo*. Examples of *in vitro* uses include treatments of cell cultures in order to kill all cells except for desired variants that do not express the target antigen; or to kill variants that express undesired antigen. Examples of *ex vivo* uses include treatments of hematopoietic stem cells (HSC) prior to the

performance of the transplantation (HSCT) into the same patient in order to kill diseased or malignant cells. For instance, clinical *ex vivo* treatment to remove tumour cells or lymphoid cells from bone marrow prior to autologous transplantation in cancer treatment or in treatment of autoimmune disease, or to remove T cells and other lymphoid cells from allogeneic bone marrow or tissue prior to transplant in order to prevent graft-versus-host disease, can be carried out as follows. Bone marrow is harvested from the patient or other individual and then incubated in medium containing serum to which is added the conjugate of the invention, concentrations range from about 1 pM to 0.1 mM, for about 30 minutes to about 48 hours at about 37 °C. The exact conditions of concentration and time of incubation (=dose) are readily determined by the skilled clinicians. After incubation the bone marrow cells are washed with medium containing serum and returned to the patient by i.v. infusion according to known methods. In circumstances where the patient receives other treatment such as a course of ablative chemotherapy or total-body irradiation between the time of harvest of the marrow and reinfusion of the treated cells, the treated marrow cells are stored frozen in liquid nitrogen using standard medical equipment.

For clinical *in vivo* use, the conjugate of the invention will be supplied as solutions or as a lyophilized solid that can be redisolved in sterile water for injection. Examples of suitable protocols of conjugate administration are as follows. Conjugates are given weekly for 8 weeks as an i.v. bolus. Bolus doses are given in 50 to 500 ml of normal saline to which human serum albumin (e.g. 0.5 to 1 mL of a concentrated solution of human serum albumin, 100 mg/mL) can be added. Dosages will be about 50 μg to 20 mg/kg of body weight per week, i.v. (range of 10 μg to 200 mg/kg per injection). 8 weeks after treatment, the patient may receive a second course of treatment. Specific clinical protocols with regard to route of administration, excipients, diluents, dosages, times, etc., can be determined by the skilled clinicians.

Examples of medical conditions that can be treated according to the *in vivo* or *ex vivo* methods of killing selected cell populations include malignancy of any types of cancer, autoimmune diseases, graft rejections, and infections (viral, bacterial or parasite).

The amount of a conjugate which is required to achieve the desired biological effect, will vary depending upon a number of factors, including the chemical characteristics, the potency, and the bioavailability of the conjugates, the type of disease, the species to which the patient belongs, the diseased state of the patient, the route of administration, all factors which dictate the required dose amounts, delivery and regimen to be administered.

In general terms, the conjugates of this invention may be provided in an aqueous physiological buffer solution containing 0.1 to 10% w/v conjugates for parenteral administration. Typical dose ranges are from 1 μg/kg to 0.1 g/kg of body weight per day; a preferred dose range

is from 0.01 mg/kg to 20 mg/kg of body weight per day or an equivalent dose in a human child. The preferred dosage of drug to be administered is likely to depend on such variables as the type and extent of progression of the disease or disorder, the overall health status of the particular patient, the relative biological efficacy of the compound selected, the formulation of the compound, the route of administration (intravenous, intramuscular, or other), the pharmacokinetic properties of the compound by the chosen delivery route, and the speed (bolus or continuous infusion) and schedule of administrations (number of repetitions in a given period of time).

The conjugates of the present invention are also capable of being administered in unit dose forms, wherein the term "unit dose" means a single dose which is capable of being administered to a patient, and which can be readily handled and packaged, remaining as a physically and chemically stable unit dose comprising either the active conjugate itself, or as a pharmaceutically acceptable composition, as described hereinafter. As such, typical total daily dose ranges are from 0.01 to 100 mg/kg of body weight. By way of general guidance, unit doses for humans range from 1 mg to 3000 mg per day. Preferably the unit dose range is from 1 to 500 mg administered one to four times a day, and even more preferably from 10 mg to 500 mg, once a day. Conjugates provided herein can be formulated into pharmaceutical compositions by admixture with one or more pharmaceutically acceptable excipients. Such unit dose compositions may be prepared for use by oral administration, particularly in the form of tablets, simple capsules or soft gel capsules; or intranasally, particularly in the form of powders, nasal drops, or aerosols; or dermally, for example, topically in ointments, creams, lotions, gels or sprays, or via trans-dermal patches. The compositions may conveniently be administered in unit dosage form and may be prepared by any of the methods well known in the pharmaceutical art, for example, as described in *Remington: The Science and Practice of Pharmacy*, 21th ed.; Lippincott Williams & Wilkins: Philadelphia, PA, 2005.

Preferred formulations include pharmaceutical compositions in which a compound of the present invention is formulated for oral or parenteral administration. For oral administration, tablets, pills, powders, capsules, troches and the like can contain one or more of any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, or gum tragacanth; a diluent such as starch or lactose; a disintegrant such as starch and cellulose derivatives; a lubricant such as magnesium stearate; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, or methyl salicylate. Capsules can be in the form of a hard capsule or soft capsule, which are generally made from gelatin blends optionally blended with plasticizers, as well as a starch capsule. In addition, dosage unit forms can contain various other materials that modify the physical form of the dosage unit, for

example, coatings of sugar, shellac, or enteric agents. Other oral dosage forms syrup or elixir may contain sweetening agents, preservatives, dyes, colorings, and flavorings. In addition, the active compounds may be incorporated into fast dissolve, modified-release or sustained-release preparations and formulations, and wherein such sustained-release formulations are preferably bi-modal. Preferred tablets contain lactose, cornstarch, magnesium silicate, croscarmellose sodium, povidone, magnesium stearate, or talc in any combination.

Liquid preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. The liquid compositions may also include binders, buffers, preservatives, chelating agents, sweetening, flavoring and coloring agents, and the like. Non-aqueous solvents include alcohols, propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and organic esters such as ethyl oleate. Aqueous carriers include mixtures of alcohols and water, buffered media, and saline. In particular, biocompatible, biodegradable lactide polymer, lactide/glycolide copolymer, or polyoxyethylene-polyoxypropylene copolymers may be useful excipients to control the release of the active compounds. Intravenous vehicles can include fluid and nutrient replenishers, electrolyte replenishers, such as those based on Ringer's dextrose, and the like. Other potentially useful parenteral delivery systems for these active compounds include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes.

Alternative modes of administration include formulations for inhalation, which include such means as dry powder, aerosol, or drops. They may be aqueous solutions containing, for example, polyoxyethylene-9-lauryl ether, glycocholate and deoxycholate, or oily solutions for administration in the form of nasal drops, or as a gel to be applied intranasally. Formulations for buccal administration include, for example, lozenges or pastilles and may also include a flavored base, such as sucrose or acacia, and other excipients such as glycocholate. Formulations suitable for rectal administration are preferably presented as unit-dose suppositories, with a solid based carrier, such as cocoa butter, and may include a salicylate. Formulations for topical application to the skin preferably take the form of an ointment, cream, lotion, paste, gel, spray, aerosol, or oil. Carriers which can be used include petroleum jelly, lanolin, polyethylene glycols, alcohols, or their combinations. Formulations suitable for transdermal administration can be presented as discrete patches and can be lipophilic emulsions or buffered, aqueous solutions, dissolved and/or dispersed in a polymer or an adhesive.

In a specific embodiment, a conjugate of the invention is administered concurrently with the other known or will be known therapeutic agents such as the chemotherapeutic agent, the radiation therapy, immunotherapy agents, autoimmune disorder agents, anti-infectious agents or the other antibody-drug conjugates, resulting in a *synergistic effect.* In another specific

embodiment, the *synergistic* drugs or radiation therapy are administered prior or subsequent to administration of a conjugate, in one aspect at least an hour, 12 hours, a day, a week, a month, in further aspects several months, prior or subsequent to administration of a conjugate of the invention.

In other embodiments, the synergistic drugs include, but not limited to:

1). Chemotherapeutic agents: a). Alkylating agents: such as [Nitrogen mustards: (chlorambucil, cyclophosphamide, ifosfamide, mechlorethamine, melphalan, trofosfamide); Nitrosoureas: (carmustine, lomustine); Alkylsulphonates: (busulfan, treosulfan); Triazenes: (dacarbazine); Platinum containing compounds: (carboplatin, cisplatin, oxaliplatin)]; b). Plant Alkaloids: such as [Vinca alkaloids: (vincristine, vinblastine, vindesine, vinorelbine); Taxoids: (paclitaxel, docetaxol)]; c). DNA Topoisomerase Inhibitors: such as [Epipodophyllins: (9-aminocamptothecin, camptothecin, crisnatol, etoposide, etoposide phosphate, irinotecan, teniposide, topotecan,); Mitomycins: (mitomycin C)]; d). Anti-metabolites: such as {[Anti-folate: DHFR inhibitors: (methotrexate, trimetrexate); IMP dehydrogenase Inhibitors: (mycophenolic acid, tiazofurin, ribavirin, EICAR); Ribonucleotide reductase Inhibitors: (hydroxyurea, deferoxamine)]; [Pyrimidine analogs: Uracil analogs: (5-Fluorouracil, doxifluridine, floxuridine, ratitrexed(Tomudex)); Cytosine analogs: (cytarabine, cytosine arabinoside, fludarabine); Purine analogs: (azathioprine, mercaptopurine, thioguanine)]}; e). Hormonal therapies: such as {Receptor antagonists: [Anti-estrogen: (megestrol, raloxifene, tamoxifen); LHRH agonists: (goscrclin, leuprolide acetate); Anti-androgens: (bicalutamide, flutamide)]; Retinoids/Deltoids: [Vitamin D3 analogs: (CB 1093, EB 1089 KH 1060, cholecalciferol, ergocalciferol); Photodynamic therapies: (verteporfin, phthalocyanine, photosensitizer Pc4, demethoxy-hypocrellin A); Cytokines: (Interferon-alpha, Interferon-gamma, tumor necrosis factor (TNFs), human proteins containing a TNF domain)]}; f). Kinase inhibitors, such as BIBW 2992 (anti-EGFR/Erb2), imatinib, gefitinib, pegaptanib, sorafenib, dasatinib, sunitinib, erlotinib, nilotinib, lapatinib, axitinib, pazopanib. vandetanib, E7080 (anti-VEGFR2), mubritinib, ponatinib (AP24534), bafetinib (INNO-406), bosutinib (SKI-606), cabozantinib, vismodegib, iniparib, ruxolitinib, CYT387, axitinib, tivozanib, sorafenib, bevacizumab, cetuximab, Trastuzumab, Ranibizumab, Panitumumab, ispinesib; g). Others: such as gemcitabine, epoxomicins (e. g. carfilzomib), bortezomib, thalidomide, lenalidomide, pomalidomide, tosedostat, zybrestat, PLX4032, STA-9090, Stimuvax, allovectin-7, Xegeva, Provenge, Yervoy, Isoprenylation inhibitors (such as Lovastatin), Dopaminergic neurotoxins (such as 1-methyl-4-phenylpyridinium ion), Cell cycle inhibitors (such as staurosporine), Actinomycins (such as Actinomycin D, dactinomycin), Bleomycins (such as bleomycin A2, bleomycin B2, peplomycin), Anthracyclines (such as daunorubicin, doxorubicin (adriamycin), idarubicin, epirubicin,

pirarubicin, zorubicin, mtoxantrone, MDR inhibitors (such as verapamil), Ca^{2+}ATPase inhibitors (such as thapsigargin), Histone deacetylase inhibitors (Vorinostat, Romidepsin, Panobinostat, Valproic acid, Mocetinostat (MGCD0103), Belinostat, PCI-24781, Entinostat, SB939, Resminostat, Givinostat, AR-42, CUDC-101, sulforaphane, Trichostatin A) ; Thapsigargin, Celecoxib, glitazones, epigallocatechin gallate, Disulfiram, Salinosporamide A.

2). An anti-autoimmune disease agent includes, but is not limited to, cyclosporine, cyclosporine A, aminocaproic acid, azathioprine, bromocriptine, chlorambucil, chloroquine, cyclophosphamide, corticosteroids (e.g. amcinonide, betamethasone, budesonide, hydrocortisone, flunisolide, fluticasone propionate, fluocortolone danazol, dexamethasone, Triamcinolone acetonide, beclometasone dipropionate), DHEA, enanercept, hydroxychloroquine, infliximab, meloxicam, methotrexate, mofetil, mycophenylate, prednisone, sirolimus, tacrolimus.

3). An anti-infectious disease agent includes, but is not limited to, a). Aminoglycosides: amikacin, astromicin, gentamicin (netilmicin, sisomicin, isepamicin), hygromycin B, kanamycin (amikacin, arbekacin, bekanamycin, dibekacin, tobramycin), neomycin (framycetin, paromomycin, ribostamycin), netilmicin, spectinomycin, streptomycin, tobramycin, verdamicin; b). Amphenicols: azidamfenicol, chloramphenicol, florfenicol, thiamphenicol; c). Ansamycins: geldanamycin, herbimycin; d). Carbapenems: biapenem, doripenem, ertapenem, imipenem/ cilastatin, meropenem, panipenem; e). Cephems: carbacephem (loracarbef), cefacetrile, cefaclor, cefradine, cefadroxil, cefalonium, cefaloridine, cefalotin or cefalothin, cefalexin, cefaloglycin, cefamandole, cefapirin, cefatrizine, cefazaflur, cefazedone, cefazolin, cefbuperazone, cefcapene, cefdaloxime, cefepime, cefminox, cefoxitin, cefprozil, cefroxadine, ceftezole, cefuroxime, cefixime, cefdinir, cefditoren, cefepime, cefetamet, cefmenoxime, cefodizime, cefonicid, cefoperazone, ceforanide, cefotaxime, cefotiam, cefozopran, cephalexin, cefpimizole, cefpiramide, cefpirome, cefpodoxime, cefprozil, cefquinome, cefsulodin, ceftazidime, cefteram, ceftibuten, ceftiolene, ceftizoxime, ceftobiprole, ceftriaxone, cefuroxime, cefuzonam, *cephamycin* (cefoxitin, cefotetan, cefmetazole), oxacephem (flomoxef, latamoxef); f). Glycopeptides: bleomycin, vancomycin (oritavancin, telavancin), teicoplanin (dalbavancin), ramoplanin, cubicin; g). Glycylcyclines: e. g. tigecycline; g). β-Lactamase inhibitors: penam (sulbactam, tazobactam), clavam (clavulanic acid); i). Lincosamides: clindamycin, lincomycin; j). Lipopeptides: daptomycin, A54145, calcium-dependent antibiotics (CDA); k). Macrolides: azithromycin, cethromycin, clarithromycin, dirithromycin, erythromycin, flurithromycin, josamycin, ketolide (telithromycin, cethromycin), midecamycin, miocamycin, oleandomycin, rifamycins (rifampicin, rifampin, rifabutin, rifapentine), rokitamycin, roxithromycin, spectinomycin, spiramycin, tacrolimus (FK506), troleandomycin, telithromycin; l). Monobactams: aztreonam, tigemonam; m). Oxazolidinones: linezolid; n). Penicillins:

amoxicillin, ampicillin (pivampicillin, hetacillin, bacampicillin, metampicillin, talampicillin), azidocillin, azlocillin, benzylpenicillin, benzathine benzylpenicillin, benzathine phenoxymethylpenicillin, clometocillin, procaine benzylpenicillin, carbenicillin (carindacillin), cloxacillin, dicloxacillin, epicillin, flucloxacillin, mecillinam (pivmecillinam), mezlocillin, meticillin, nafcillin, oxacillin, penamecillin, penicillin, pheneticillin, phenoxymethylpenicillin, piperacillin, propicillin, sulbenicillin, temocillin, ticarcillin; o). Polypeptides: bacitracin, colistin, polymyxin B; p). Quinolones: alatrofloxacin, balofloxacin, ciprofloxacin, clinafloxacin, danofloxacin, difloxacin, enoxacin, enrofloxacin, floxin, garenoxacin, gatifloxacin, gemifloxacin, grepafloxacin, kano trovafloxacin, levofloxacin, lomefloxacin, marbofloxacin, moxifloxacin, nadifloxacin, norfloxacin, orbifloxacin, ofloxacin, pefloxacin, trovafloxacin, grepafloxacin, sitafloxacin, sparfloxacin, temafloxacin, tosufloxacin, trovafloxacin; q). Streptogramins: pristinamycin, quinupristin/dalfopristin); r). Sulfonamides: mafenide, prontosil, sulfacetamide, sulfamethizole, sulfanilimide, sulfasalazine, sulfisoxazole, trimethoprim, trimethoprim-sulfamethoxazole (co-trimoxazole); s). Steroid antibacterials: e.g. fusidic acid; t). Tetracyclines: doxycycline, chlortetracycline, clomocycline, demeclocycline, lymecycline, meclocycline, metacycline, minocycline, oxytetracycline, penimepicycline, rolitetracycline, tetracycline, glycylcyclines (e.g. tigecycline); u). Other types of antibiotics: annonacin, arsphenamine, bactoprenol inhibitors (Bacitracin), DADAL/AR inhibitors (cycloserine), dictyostatin, discodermolide, eleutherobin, epothilone, ethambutol, etoposide, faropenem, fusidic acid, furazolidone, isoniazid, laulimalide, metronidazole, mupirocin, mycolactone, NAM synthesis inhibitors (e. g. fosfomycin), nitrofurantoin, paclitaxel, platensimycin, pyrazinamide, quinupristin/dalfopristin, rifampicin (rifampin), tazobactam tinidazole, uvaricin;

4). Anti-viral drugs: a). Entry/fusion inhibitors: aplaviroc, maraviroc, vicriviroc, *gp41* (enfuvirtide), PRO 140, CD4 (ibalizumab); b). Integrase inhibitors: raltegravir, elvitegravir, globoidnan A; c). Maturation inhibitors: bevirimat, vivecon; d). Neuraminidase inhibitors: oseltamivir, zanamivir, peramivir; e). Nucleosides &_nucleotides: abacavir, aciclovir, adefovir, amdoxovir, apricitabine, brivudine, cidofovir, clevudine, dexelvucitabine, didanosine (ddI), elvucitabine, emtricitabine (FTC), entecavir, famciclovir, fluorouracil (5-FU), 3'-fluoro-substituted 2', 3'-dideoxynucleoside analogues (e.g. 3'-fluoro-2',3'-dideoxythymidine (FLT) and 3'-fluoro-2',3'-dideoxyguanosine (FLG), fomivirsen, ganciclovir, idoxuridine, lamivudine (3TC), l-nucleosides (e.g. β-l-thymidine and β-l-2'-deoxycytidine), penciclovir, racivir, ribavirin, stampidine, stavudine (d4T), taribavirin (viramidine), telbivudine, tenofovir, trifluridine valaciclovir, valganciclovir, zalcitabine (ddC), zidovudine (AZT); f). Non-nucleosides: amantadine, ateviridine, capravirine, diarylpyrimidines (etravirine, rilpivirine), delavirdine, docosanol, emivirine, efavirenz, foscarnet (phosphonoformic acid), imiquimod, interferon alfa,

loviride, lodenosine, methisazone, nevirapine, NOV-205, peginterferon alfa, podophyllotoxin, rifampicin, rimantadine, resiquimod (R-848), tromantadine; g). Protease inhibitors: amprenavir, atazanavir, boceprevir, darunavir, fosamprenavir, indinavir, lopinavir, nelfinavir, pleconaril, ritonavir, saquinavir, telaprevir (VX-950), tipranavir; h). Other types of anti-virus drugs: abzyme, arbidol, calanolide a, ceragenin, cyanovirin-n, diarylpyrimidines, epigallocatechin gallate (EGCG), foscarnet, griffithsin, taribavirin (viramidine), hydroxyurea, KP-1461, miltefosine, pleconaril, portmanteau inhibitors, ribavirin, seliciclib.

5). Other immunotheraphy drugs: e.g. imiquimod, interferons (e.g. α, β), granulocyte colony-stimulating factors, cytokines, Interleukins (IL-1 ~ IL-35), antibodies (e. g. trastuzumab, pertuzumab, bevacizumab, cetuximab, panitumumab, infliximab, adalimumab, basiliximab, daclizumab, omalizumab), Protein-bound drugs (e.g., Abraxane), an antibody conjugated with drugs selected from calicheamicin derivative, of maytansine derivatives (DM1 and DM4), CC-1065 and duocarmycin minor groove binders, potent taxol derivatives, doxorubicin, auristatin antimitotic drugs (e. g. Trastuzumab-DM1, Inotuzumab ozogamicin, Brentuximab vedotin, Glembatumumab vedotin, lorvotuzumab mertansine, AN-152 LMB2, TP-38, VB4-845, Cantuzumab mertansine, AVE9633, SAR3419, CAT-8015 (anti-CD22), IMGN388, IMGN529, IMGN853, milatuzumab-doxorubicin, SGN-75 (anti-CD70), Anti-CD22-MCC-DM1).

According to a still further object, the present invention is also concerned with the process of preparation of the conjugate of the invention. The conjugate and process of the present invention may be prepared in a number of ways well known to those skilled in the art. The antimitotic agents used in the conjugate can be synthesized, for example, by application or adaptation of the methods described below, or variations thereon as appreciated by the skilled artisan. The appropriate modifications and substitutions will be readily apparent and well known or readily obtainable from the scientific literature to those skilled in the art. In particular, such methods can be found in R.C. Larock, *Comprehensive Organic Transformations*, 2nd Edition, Wiley-VCH Publishers, 1999.

In the reactions described hereinafter, it may be necessary to protect reactive functional groups, for example hydroxy, amino, imino, thio or carboxy groups, where these are desired in the final product, to avoid their unwanted participation in the reactions. Conventional protecting groups may be used in accordance with standard practice, for examples see P. G. Wuts and T.W. Greene, Greene's Protective Groups in Organic Synthesis, Wiley-Interscience; 4th edition (2006). Some reactions may be carried out in the presence of a base, or an acid or in a suitable solvent. There is no particular restriction on the nature of the base, acid and solvent to be used in this reaction, and any base, acid or solvent conventionally used in reactions of this type may equally be used here, provided that it has no adverse effect on other parts of the molecule. The reactions can take place over a wide

range of temperatures. In general, we find it convenient to carry out the reaction at a temperature of from -80°C to 150°C (more preferably from about room temperature to 100°C). The time required for the reaction may also vary widely, depending on many factors, notably the reaction temperature and the nature of the reagents. However, provided that the reaction is effected under the preferred conditions outlined above, a period of from 3 hours to 20 hours will usually suffice.

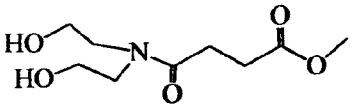
The work-up of the reaction can be carried out by conventional means. For example, the reaction products may be recovered by distilling off the solvent from the reaction mixture or, if necessary after distilling off the solvent from the reaction mixture, pouring the residue into water followed by extraction with a water-immiscible organic solvent and distilling off the solvent from the extract. Additionally, the product can, if desired, be further purified by various well known techniques, such as recrystallization, reprecipitation or the various chromatography techniques, notably column chromatography or preparative thin layer chromatography. The synthesis of the antimitotic agents and their conjugates of this invention are illustrated in the figures 1 ~28.

The comjugates of binding molecules with potent antimitotic agents are further illustrated but not restricted by the description in the following examples.

6. EXPERIMENTAL MATERIALS:

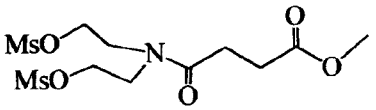
Mass spectra were obtained using a Bruker Esquire 3000 system. NMR spectra were recorded on a Bruker AVANCE300 spectrometer. Chemical shifts are reported in ppm relative to TMS as an internal standard. Ultraviolet spectra were recorded on a Hitachi U1200 spectrophotometer. HPLC was performed using an Agilent 1100 HPLC system equipped with a fraction collector and a variable wavelength detector. Thin layer chromatography was performed on Analtech GF silica gel TLC plates. Aminal acids and their derivatives as well as preloaded resins were either from Merck Chemicals International Co, or Synthetech Co., or Peptides International Inc or Chembridge International Co. or Sigma –Aldrich Co. Some of the linkers, Linkers of NHS ester /Maleimide (AMAS, BMPS, GMBS, MBS, SMCC, EMCS or Sulfo-EMCS, SMPB, SMPH, LC-SMCC, Sulfo-KMUS, SM(PEG)4, SM(PEG)6, SM(PEG)8, SM(PEG)12, SM(PEG)24); NHS ester /Pyridyldithiol (SPDP, LC-SPDP or Sulfo-LC-SPDP, SMPT, Sulfo-LC-SMPT); NHS esters /Haloacetyl (SIA, SBAP, SIAB or Sulfo-SIAB); NHS ester /Diazirine (SDA or Sulfo-SDA, LC-SDA or Sulfo-LC-SDA, SDAD or Sulfo-SDAD); Maleimide /Hydrazide (BMPH, EMCH, MPBH, KMUH); Pyridyldithiol /Hydrazide (PDPH); Isocyanate /Maleimide (PMPI) were purchased from Thermo Fisher Scientific Co. SPDB, SPP linkers were made according to the references (Cumber, A. et al, *Bioconjugate Chem.*, **1992**, *3*, 397–401). Human anti-CD22 antibody was from Santa Cruz Biotechnology, Inc. and Trastuzumab was from Genentech. All other chemicals or anhydrous solvents were from Sigma-Aldrich International.

Example 1. **Methyl 4-(bis(2-hydroxyethyl)amino)-4-oxobutanoate (3)**



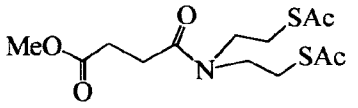
Dimethyl succinate (20.0 g, 136.9 mmol) and dihydroxyethylamine (7.20 g, 68.7 mmol) in the mixture of anhydrous toluene (500 ml) and pyridine (50 ml) were refluxed at 150 °C for 28 h. The mixture was concentrated and purified on SiO_2 column eluted with EtOAc/DCM (5% ~ 25% EtOAc) to afford the title compound (12.5 g, 83% yield). ESI MS m/z+ for $C_9H_{17}NaNO_5$ (M + Na) cald 242.2, found 242.4.

Example 2. **Methyl 4-(bis(2-((methylsulfonyl)oxy)ethyl)amino)-4-oxobutanoate (4)**



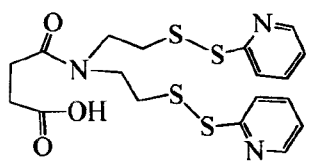
Methyl 4-(bis(2-hydroxyethyl)amino)-4-oxobutanoate (12.0 g, 49.56 mmol) in anhydrous pyridine (350 ml) was added methanesulfonyl chloride (20.0 g, 175.4 mmol). After stirred overnight the mixture was concentrated, diluted with EtOAc (350 ml), washed with cold 1 M NaH_2PO_4 (2 x 300ml), dried over $MgSO_4$, filtered and evaporated to afford crude product (~18.8 g, 101% yield). The crude product was used for next step without further purification. ESI MS m/z+ for $C_{11}H_{21}NaNO_9S_2$ (M + Na) cald 398.2, found 398.4.

Example 3. **Methyl 4-(bis(2-(acetylthio)ethyl)amino)-4-oxobutanoate (5)**



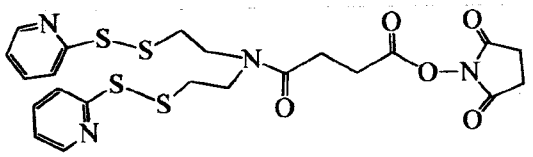
Methyl 4-(bis(2-((methylsulfonyl)oxy)ethyl)amino)-4-oxobutanoate (fresh made, 90% pure, 8.5 g, ~20 mmol) in DMA (350 ml) at 0 °C was added thioacetic acid (10 ml, 134 mmol), followed by addtion of Et_3N (30 ml, 215 mmol). The mixture was then stirred at room temperature overnight, concentrated, diluted with EtOAc (350 ml), washed with $NaHCO_3$ (sat, 300 ml), NaCl sat solution (300 ml) and 1 M NaH_2PO_4 (300 ml). The organic layer was dried over Na_2SO_4, filtered, evaporated and purified on SiO_2 column eluted with EtOAc/hexane (10% ~ 25% EtOAc) to afford the title compound (5.1 g, 76% yield). ESI MS m/z+ for $C_{13}H_{21}NaNO_5S_2$ (M + Na) cald 358.1, found 358.2.

Example 4. **4-(Bis(2-(pyridin-2-yldisulfanyl)ethyl)amino)-4-oxobutanoic acid (6)**



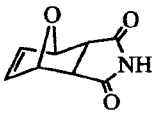
Methyl 4-(bis(2-(acetylthio)ethyl)amino)-4-oxobutanoate (5.0 g, 14.9 mmol) in THF (150 ml) was added NaOH (5.0 g, 125 mmol) in water (100 ml). The mixture was stirred at RT for 35 min, neutralized with H_3PO_4 to pH 7. Then 1,2-di(pyridin-2-yl)disulfane (Aldrithiol-2, 26.0 g, 118 mmol) in THF (100 ml) was added and the mixture was stirred for 4h, concentrated and purified on SiO_2 column eluted with MeOH/DCM/HOAc (1:20/1%) to afford the title product (5.8 g, 85.6% yield). ESI MS m/z+ for $C_{18}H_{21}NaN_3O_3S_4$ (M + Na) cald 478.0, found 478.2.

Example 5. **2,5-dioxopyrrolidin-1-yl 4-(bis(2-(pyridin-2-yldisulfanyl)ethyl)amino)-4-oxobutanoate (7)**



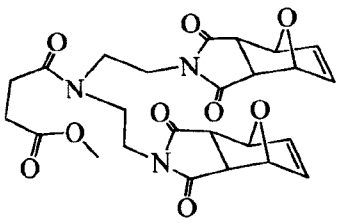
4-(Bis(2-(pyridin-2-yldisulfanyl)ethyl)amino)-4-oxobutanoic acid (5.2 g, 11.5 mmol) in DMA (100 ml) was added NHS (1.6 g, 13.9 mmol) and EDC (5.0 g, 26.1 mmol). The mixture was stirred overnight, evaporated and purified on SiO_2 column eluted with EtOAc/DCM (5% to 15% EtOAc) to afford the title product (5.8 g, 85.6% yield). ESI MS m/z+ for $C_{22}H_{24}NaN_4O_5S_4$ (M + Na) cald 575.1, found 575.2.

Example 6. **3,6-endoxo-Δ-tetrahydrophthalimide (12)**



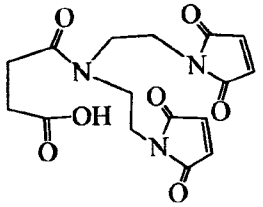
Maleimide (10.0 g, 103.0 mmol) in toluene (200 ml) was added furan (10.0 ml, 137.4 mmol). The mixture was heated inside a 1 L of autoclave bomb at 100 °C for 8 h. The bomb was cooled to room temperature, and the inside solid was rinsed with methanol, concentrated and crystallized in ethyl acetate/hexane to afford 16.7 g (99%) of the title compound. 1H NMR ($CDCl_3$): 11.12 (s, 1H) (NH), 6.68~6.64 (m, 2H), 5.18~5.13 (m, 2H), 2.97 ~2.92 (m, 2H). MS m/z+ for $C_8H_7NaNO_3$ (M + Na) cald 188.04, found 188.04.

Example 7. **Methyl 4-((2-((3aR,4R,7S,7aS)-1,3-dioxo-3a,4,7,7a-tetrahydro-1H-4,7-epoxyisoindol-2(3H)-yl)ethyl)(2-((4R,7S,7aS)-1,3-dioxo-3a,4,7,7a-tetrahydro-1H-4,7-epoxyisoindol-2(3H)-yl)ethyl)amino)-4-oxobutanoate (13)**



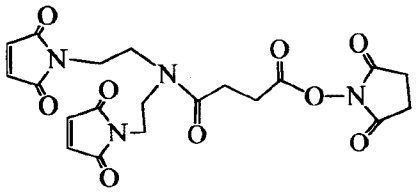
Methyl 4-(bis(2-((methylsulfonyl)oxy)ethyl)amino)-4-oxobutanoate (**4**, fresh made, 90% pure, 8.5 g, ~20 mmol) in DMA (350 ml) was added 3,6-endoxo-Δ-tetrahydrophthalimide (10.2 g, 61.8 mmol), sodium carbonate (8.0 g, 75.5 mmol) and sodium iodide (0.3 g, 2.0 mmol). The mixture was then stirred at room temperature overnight, concentrated, diluted with EtOAc (350 ml), washed with $NaHCO_3$ (sat, 300 ml), NaCl sat solution (300 ml) and 1 M NaH_2PO_4 (300 ml). The organic layer was dried over Na_2SO_4, filtered, evaporated and purified on SiO_2 column eluted with EtOAc/hexane (10% ~ 30% EtOAc) to afford the title compound (7.9 g, 77% yield). ESI MS m/z+ for $C_{25}H_{27}NaN_3O_9$ (M + Na) cald 536.2, found 536.4.

Example 8. **4-(bis(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)amino)-4-oxobutanoic acid (14)**



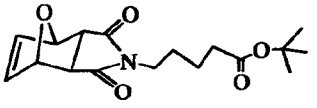
Compound **13** (3.0 g, 5.8 mmol) and trimethylstannanol (4.8 g, 26.4 mmol) in 1,2-dichloroethane (150 ml) was refluxed at 80 °C for 8 h. It was cooled to room temperature and the residue was passed a short silica gel column and eluted with dichloromethane/methanol to remove the extra trimethyltin hydroxide. Then the pooled fractions were combined, concentrated and diluted with DMA and toluene, refluxed at 120 °C overnight and and purified on SiO_2 column eluted with MeOH/DCM (5% ~ 10% MeOH) to afford the title compound (1.62 g, 76% yield). ESI MS m/z+ for $C_{16}H_{17}NaN_3O_9$ (M + Na) cald 386.1, found 386.2.

Example 9. **2,5-Dioxopyrrolidin-1-yl 4-(bis(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)amino)-4-oxobutanoate**



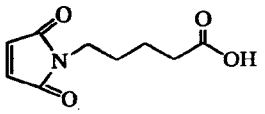
Compound (**14**)) (1.60 g, 4.4 mmol) in DMA (100 ml) was added NHS (0.76 g, 6.61 mmol) and EDC (1.70 g, 8.90 mmol). The mixture was stirred overnight, evaporated and purified on SiO_2 column eluted with EtOAc/DCM (5% to 15% EtOAc) to afford the title product (1.72 g, 85.0% yield). ESI MS m/z+ for $C_{20}H_{20}NaN_4O_9$ (M + Na) cald 483.1, found 483.2.

Example 10. **t-Butyl 5-(3',6'-endoxo-Δ-tetrahydrophthalimido) pentanoate.**



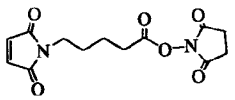
t-Butyl 5-hydroxy pentanote (10.0 g, 57.4 mmol) in pyridine (60 ml) was added mesyl chloride (8.0 ml, 103.3 mmol) and the mixture was stirred for 6 h, evaporated, diluted with EtOAc, washed with cold 1M NaH_2PO_4, pH 6, dried over $MgSO_4$, filtered and evaporated to dryness. To the mixture of compound **12** (9.90 g, 60.0 mmol) and Na_2CO_3 (8.5 g, 80.1 mmol) in DMF (80 ml) was added the dryness mesylate compound. The mixture was stirred overnight, evaporated, diluted with EtOAc, washed with saline water and 1M NaH_2PO_4, pH 6, dried over $MgSO_4$, evaporated and purified on SiO_2 column eluted with $EtOAc/CH_2Cl_2$ (1:12) to afford the title compound (14.01 g, 76%). MS m/z+ for $C_{17}H_{23}NaNO_5$ (M + Na) cald 344.16, found 344.16.

Example 11. **5-maleimido-pentanoic acid (21b).**



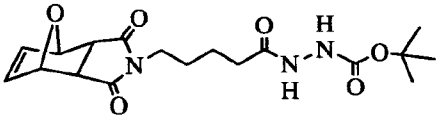
Compound **17** (5.0 g, 15.57 mmol) in 1,4-dioxanne (40 ml) was added HCl (10 ml, 36%) at 4 °C and the mixture was stirred for 30 min, evaporated to dryness to form 5-(3',6'-endoxo-Δ-tetrahydrophthalimido) pentanoic acid (4.08 g, 99%). The dried compound in mixture of DMF/toluene (1:1, 40 ml) was refluxed for 6h, evaporated and crystallized from EtOH/ether/hexane to afford the title compound (2.76 g, 90%). MS m/z+ for $C_9H_{12}NO_4$ (M + H) cald 198.07, found 198.07.

Example 12. **N-succinimidyl 5-maleimido-pentanoate (23b) (DMPS linker)**



5-maleimido-pentanoic acid **21b** (2.0 g, 10.1 mmol) in CH_2Cl_2 (20 ml) was added N-hydroxysuccimide (1.50 g, 13.0 mmol) and EDC (7.0 g, 36.4 mmol) and the mixture was stirred overnight, evaporated and purified on SiO_2 column ($EtOAc/CH_2Cl_2$, 1:10) to afford the title compound **23b** (2.43 g, 82%). MS m/z+ for $C_{13}H_{14}NaN_2O_6$ (M + Na) cald 317.09, found 317.09.

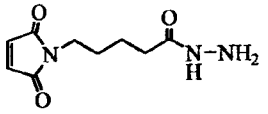
Example 13. **t-Butyl 5-(3',6'-endoxo-Δ-tetrahydrophthalimido)pentanoyl hydrazine-carboxylate (25a-a).**



5-(3',6'-endoxo-Δ-tetrahydrophthalimido) pentanoic acid (1.0 g, 3.77) in DMF (30 ml) was added tert-butyl carbazate (0.60 g, 4.53 mmol) and EDC (2.0 g, 10.4 mmol). The mixture was

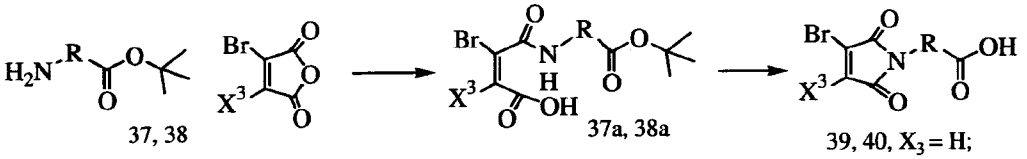
stirred overnight, evaporated and purified on SiO2 column (EtOAc/CH_2Cl_2, 1:10) to afford the title compound (1.18 g, 83%). MS m/z+ for $C_{18}H_{25}NaN_3O_6$ (M + Na) cald 402.17, found 402.18.

Example 14. **5-maleimido-pentanoic acid hydrazide (25a-b)**



Compound **25a-a** (1.18 g, 3.11 mmol) was dissolved in the mixture of DMF/toluene (1:1, 20 ml), refluxed for 6h and envaporated. Then the mixture dissolved in 1,4-dioxanne (20 ml) was added HCl (5 ml, 36%) at 4 °C and the mixture was stirred for 30 min, evaporated to dryness and crystallized from EtOH/ether/hexane to afford the title compound (577 mg, 88%). MS m/z+ for $C_9H_{14}N_3O_3$ (M + H) cald 212.10, found 212.10.

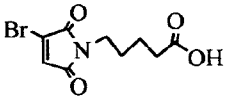
Example 15. **General procedure for 3'-bromo-maleimdo compounds 39 and 40, and 3',4'-dibromo-maleimdo compounds 43 and 44.**



R = C_1 ~ C_8 alkyl or $C_2H_4(OC_2H_4)_n$, n= 1~20; X^3 = H or Br

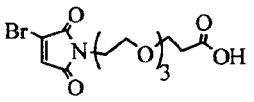
Amino compound 37 or 38 (~ 6 g) in DMF (60 ml) was added bromomaleic anhydride (1 eq) or 2,3-dibromomaleic anhydride (1 eq) and the mixture was stirred overnight, evaporated via oil pump to dryness to afford the crude enoic acids. To the crude enoic acids were added HOAc (~50 ml) and Ac_2O (2 ~ 4 g) and the reaction mixture was fluxed at 120 °C for 6 ~ 12 h, concentrated and purified on SiO_2 column eluted with EtOAc/CH_2Cl_2 (1:10 ~ 1:1) to afford (61% ~ 87% yield) of the 3'-bromo-maleimdo compounds **39** and **40**, and 3',4'-dibromo-maleimdo compounds **43** and **44** respectively.

5-(3-bromo-2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)pentanoic acid



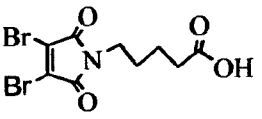
MS m/z+ for $C_9H_{11}BrNO_4$ (M + H) cald 275.98, found 275,98.

3-(2-(2-(2-(3-bromo-2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)ethoxy)ethoxy)-propanoic acid.



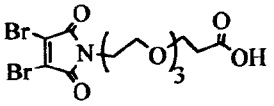
MS m/z+ for $C_{13}H_{19}BrNO_7$ (M + H) cald 380.03, found 380.03.

5-(3,4-dibromo-2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)pentanoic acid



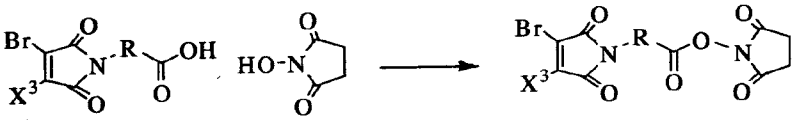
MS m/z+ for $C_9H_{10}Br_2NO_4$ (M + H) cald 353.89, found 353.89.

3-(2-(2-(2-(3,4-dibromo-2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)ethoxy)ethoxy)-propanoic acid



MS m/z+ for $C_{13}H_{18}Br_2NO_7$ (M + H) cald 457.94, found 457.94.

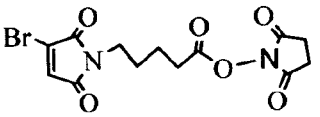
Example 16. **General procedure for NHS ester of 3'-bromo-maleimdo compounds 41 and 42, and NHS ester of 3',4'-dibromo-maleimdo compounds 45 and 46.**



R = C_1 ~ C_8 alkyl or $C_2H_4(OC_2H_4)_n$, n= 1~20; X^3 = H or Br 41, 42, X_3 = H; 45, 46, X_3 = Br

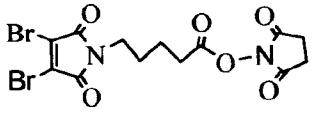
To the solution of 3'-bromo-maleimdo compounds 39 and 40 (1 eq), or 3',4'-dibromo-maleimdo compounds 43 and 44 in DMA (~0.15 M) were added *N*-hydroxysuccinimide (1.1 eq) and EDC (2~4 eq) and the mixture was stirred overnight, concentrated and purified on SiO_2 column eluted with EtOAc/CH_2Cl_2 (1:20 ~ 1:5) to afford (70% ~ 93% yield) of the 3'-bromo-maleimdo linkers **41** and **42**, and 3',4'-dibromo-maleimdo linkers **45** and **46** respectively.

2,5-dioxopyrrolidin-1-yl 5-(3-bromo-2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)pentanoate.



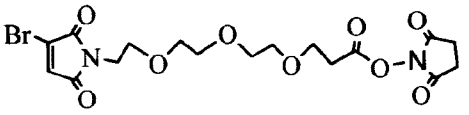
MS m/z+ for $C_{13}H_{13}BrN_2NaO_7$ (M + Na) cald 395.00, found 395.00.

2,5-dioxopyrrolidin-1-yl 5-(3,4-dibromo-2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)pentanoate



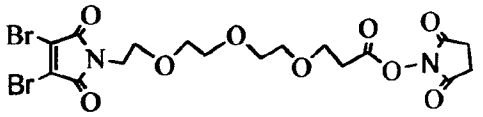
MS m/z+ for $C_{13}H_{12}Br_2N_2NaO_6$ (M + Na) cald 472.91, found 472.91.

2,5-dioxopyrrolidin-1-yl 3-(2-(2-(2-(3-bromo-2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)ethoxy)ethoxy)propanoate



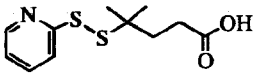
MS m/z+ for $C_{17}H_{21}BrN_2NaO_9$ (M + Na) cald 499.04, found 499.04.

2,5-dioxopyrrolidin-1-yl 3-(2-(2-(2-(3,4-dibromo-2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)ethoxy)ethoxy)propanoate



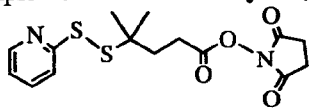
MS m/z+ for $C_{17}H_{20}Br_2N_2NaO_9$ (M + Na) cald 576.95, found 576.95.

Example 17. **4-(2-Pyridyldithio)-4-methylpentanoic acid.**



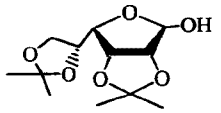
4-Mercapto-4-methylpentanoic Acid (Goff, D. et al, *Bioconjugate Chem.* **1990**, *1*, 381–386) (4.67 g, 31.5 mmol) in methanol (15 ml) was added the solution of 2,2'-Dithiodipyridine (30.0 g, 136.2 mmol) in the mixture of methanol (80 ml) and 100 mM sodium phosphate buffer, pH 7.5 (70 ml). After stirred for 6 h, the mixture was concentrated, extracted with EtOAc/Hexane (1:1). The aqueous solution was adjusted to pH 3 and extracted with EtOAc (3 x 100 ml). The organic layers were combined, dried over Na_2SO_4, filtered, evaporated and purified on SiO2 column (MeOH/CH2Cl2/HOAc, 1:15:0.01) to afford the title compound (7.05 g, 87%). MS m/z+ for $C_{11}H_{16}NO_2S_2$ (M + H) cald 258.05, found 258.05.

Example 18. **N-Succinimidyl 4-(2-pyridyldithio) -4-methylpentanoate (243) (SMDP linker)**



4-(2-pyridyldithio) -4-methylpentanoic acid (2.0 g, 7.78 mmol) in CH_2Cl_2 (20 ml) was added N-hydroxysuccimide (1.10 g, 9.56 mmol) and EDC (4.0 g, 20.8 mmol) and the mixture was stirred overnight, evaporated and purified on SiO_2 column ($EtOAc/CH_2Cl_2$, 1:10) to afford the title compound (2.48 g, 90%). MS m/z+ for $C_{15}H_{18}NaN_2O_4S_2$ (M + Na) cald 377.07, found 377.08.

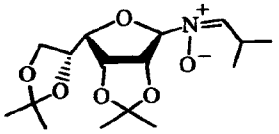
Example 19. **(3aR,4R,6S,6aR)-6-((R)-2,2-dimethyl-1,3-dioxolan-4-yl)-2,2-dimethyltetrahydro-furo[3,4-d][1,3]dioxol-4-ol (62).**



To a stirred slurry of D-gulonic-lactone (20.01 g, 112.37 mmol) and anhydrous $CuSO_4$ (25.0 g, 157.22 mmol) in dry acetone (450 mL) was added conc. H_2SO_4 (1.6 mL), and the mixture was stirred for 24 h at room temperature. The pH of the solution was adjusted to 7 with $Ca(OH)_2$, and the resulting slurry was filtered and evaporated *in vacuo* to afford a diacetonide

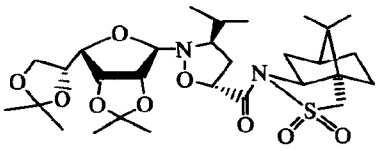
(2,3:5,6-O-diisopropylidene-D-gulono-1,4-lactone) as a light-yellow syrup which was used in the next step without further purification. To a stirred solution of diacetonide in THF (300 mL) at -78°C was added slowly 1 M solution of DIBAL-H (180 ml, 180 mM) in toluene. After being stirred for 1 h at -78 °C, the reaction mixture was quenched with water (50 mL) and filtered through Celite. The organic layer was separated, dried (Na_2SO_4,), and concentrated in vacuo. The residue was purified by silica gel chromatography with hexane-ethyl acetate (5:1) to give the title compound (25.27 g, 83% two steps) as colorless syrup. ESI MS m/z+ for $C_{12}H_{20}NaO_6$ (M + Na) cald 283.12, found 283.12.

Example 20. **(3aR,4R,6S,6aR,Z)-6-((R)-2,2-dimethyl-1,3-dioxolan-4-yl)-2,2-dimethyl-N-(2-methylpropylidene)tetrahydrofuro[3,4-d][1,3]dioxol-4-amine oxide (64)**



A mixture of 2,3:5,6-O-diisopropylidene-D-gulofuranose (**62**) (10.0 g, 38.4 mmol) and hydroxylamine hydrochloride (25.01 g, 360.87 mmol) in pyridine (150 mL) was stirred at room temperature for 1 h. The reaction mixture was concentrated *in vacuo,* added water (250 mL) and extracted with dichloromethane. The combined organic extracts were washed with brine, dried ($MgSO_4$), concentrated in vacuo and filtered through short silica gel column eluted with ethyl acetate to give 2,3:5,6-O-diisopropylidene-D-gulose oxime (**63**) (10.34 g, 98%) as a colorless vitreous substance which was used directly without further purification. A mixture of this crude oxime (**63**) (10.30 g, 37.43 mmol), isobutyraldehyde (3.00 g, 41.66 mmol), and $MgSO_4$ (3 g, 25 mmol) was stirred at the room temperature overnight. The mixture was filtered through a pad of Celite. The filtered was concentrated in vacuo and the residue was passed through a short SiO_2 column eluted with ethyl acetate to afford the title compound (11.57 g, 94% yield) as white solid. ESI MS m/z+ for $C_{16}H_{27}NO_6$ cald 329.18., found 329.18.

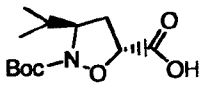
Example 21. **(3R,5R)-2-((3aR,4R,6S,6aR)-6-((R)-2,2-dimethyl-1,3-dioxolan-4-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-3-[(-)-10',2'-camphorsultam]-N-propylisoxazolidine-5-carboxamide (65).**



A mixture of (3aR,4R,6S,6aR,Z)-6-((R)-2,2-dimethyl-1,3-dioxolan-4-yl)-2,2-dimethyl-N-(2-methylpropylidene)tetrahydrofuro[3,4-d][1,3]dioxol-4-amine oxide (6.00 g 18.22 mmol) and (2R)-N-(acryloyl) bornane-10,2-sultams (5.10 g, 18.95 mmol) in CH_2Cl_2 (50 mL) was heated

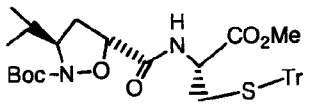
under reflux for 37 h. After concentration, the residue was recrystallized from EtOH (30 mL) to give the title compound (8.72 g, 80% yield) as a colorless solid. Flash chromatography (silica gel, hexane/AcOEt 7:3) of the mother liquor gave further (0.47 g, 4%) of the title compound as a colorless solid. MS ESI: m/z: [M+Na]+, calcd for C29H46N2NaO9S 621.28, Found, 621.28

Example 22. **(3R,5R)-2-(tert-butoxycarbonyl)-3-isopropylisoxazolidine-5-carboxylic acid (67).**



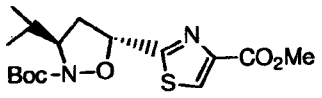
A solution of LiOH (5.0 g, 208.7 mmol) in H_2O (60 mL) at 45°C was added to a solution of (3R,5R)-2-((3aR,4R,6S,6aR)-6-((R)-2,2-dimethyl-1,3-dioxolan-4-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-3-[(-)-10',2'-camphorsultam]-N-propylisoxazolidine-5-carboxamide (30.0 g, 48.2 mmol) in THF (100 mL) and MeOH (60 mL). After stirred for 1 h, the mixture was concentrated, poured into H_2O (150 mL) and the mixture was adjusted to pH 9 with 4M HCl aq. The mixture was extracted with EtOAc, then the aqueous layer was adjusted to pH 3 with 4M HCl, The mixture was extracted with EtOAc, the organic extract was washed with brine, dried over Na_2SO_4, filtered, and then concentrated in vacuo. The residue was triturated with hexane to give (3R,5R)-2-{(3aR,4R,6S,6aR)-6-[(R)-2,2-dimethyl-1,3-dioxolan-4-yl]-2,2-dimethyltetrahydrofuro[3,4-d,1,3]dioxol-4-yl}-3-isopropylisoxazolidine-5-carboxylic acid (66a) (17.1 g 88%) as a colorless solid. This material was used for the next step without further purification. MS ESI: m/z: [M+Na]+, calcd for $C_{19}H_{31}NNaO_8$, 424.19, Found, 424.19. To a solution of this material (8.0 g, 19.95 mmol) in MeCN (80 mL) was added 60% $HClO_4$ aq. (6.0 mL, 35.77 mmol) at 45°C, and the mixture was stirred at RT for 1 h. After concentration, the residue was dissolved in 1,4-dioxane (40 mL), and then a suspension of $NaHCO_3$ (25 g, 297 mmol) in H_2O (32 mL) and Boc_2O (4.80 g, 22.00 mmol) was added at 4 °C. The mixture was stirred at RT for 4 h, concentrated, diluted with H_2O and EtOAc/Hexane (1:1) and separated. The aqueous layer was adjusted to pH 3 with 4 M HCl aq. The mixture was extracted with EtOAc, the organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated in vacuo. The residue was triturated with hexane to give (3R,5R)-2-(tert-butoxycarbonyl)-3-isopropylisoxazolidine-5-carboxylic acid (4.91 g, 95%) as a colorless amorphous solid. MS ESI: m/z: [M+Na]+, calcd for $C_{12}H_{21}NNaO_5$, 282.13, Found, 282.13.

Example 23. **Methyl (R)-2-[(3R,5R)-2-[(t-butyl-yl)methoxycarbonyl]-3-isopropylisoxazolidine-5-carboxamido]-3-(triphenylmethylthio)propionate (68)**



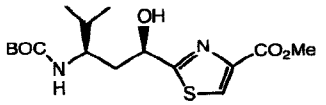
iPr2NEt (0.75 mL, 4.31 mmol) and TBTU (2.50 g, 7.78 mmol) were added to a solution of (3R,5R)-2-(tert-butoxycarbonyl)-3-isopropylisoxazolidine-5-carboxylic acid (1.01 g, 3.89 mmol) and l-(S)-Tr-cysteine methyl ester hydrochloride (1.76 g, 4.27 mmol) in CH_2Cl_2 (15 mL) at 4 °C and the mixture was stirred at RT for overnight. The mixture was poured into $NaHCO_3$ (sat.) solution, extracted with CH_2Cl_2, dried over Na_2SO_4, filtered, concentrated and silica gel chromatography (hexane/AcOEt 1:2) to give the title compound (2.10 g, 87%) as colorless amorphous solid. MS ESI: m/z: [M+Na]+, calcd for $C_{35}H_{42}N_2NaO_6S$, 641.27, Found, 641.26.

Example 24. **(3R,5R)-tert-butyl 3-isopropyl-5-(4-(methoxycarbonyl)thiazol-2-yl)isoxazolidine-2-carboxylate (69)**



Tf_2O (2.0 mL, 12.0 mmol) was added to a solution of $Ph_3P{=}O$ (4.10 g, 14.74 mmol) in CH_2Cl_2 (40.0 mL) and the mixture was stirred at -10 °C for 1 h. A solution of Methyl (R)-2-[(3R,5R)-2-[(t-butyl-yl)methoxycarbonyl]-3-isopropylisoxazolidine-5-carboxamido]-3-(triphenylmethylthio)propionate (**68**) (4.00 g, 6.47 mmol) in CH_2Cl_2 (20 mL) was added to the reaction mixture at -10 °C and the mixture was stirred at RT for 6 h, added to $NaHCO_3$ saturated solution at 4 °C and extracted with CH_2Cl_2. The organic extracts were washed with brine, dried over Na_2SO_4, filtered, concentrated in vacuo and SiO_2 chromatography (hexane/AcOEt 3:2) to give the corresponding thiazolidine derivative as a yellow amorphous solid. MnO_2 (5.80 g, 66.7 mmol) was added to a solution of this material in CH_2Cl_2 (60 mL) and the mixture was stirred at RT for 24 h. The mixture was filtrated via Celite, concentrated in vacuo and SiO_2 chromatography (hexane/AcOEt 3:2) to give the title compound (**69**) (1.75 g, 75%) as a colorless amorphous solid. ESI: m/z: [M+Na]+, calcd for $C_{16}H_{24}N_2NaO_5S$, 379.13, Found, 379.14

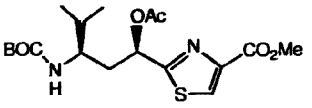
Example 25. **Methyl 2-((1R,3R)-3-(tert-butoxycarbonylamino)-1-hydroxy-4-methylpentyl)-thiazole-4-carboxylate (70).**



$Mo(CO)_6$ (1.10 g, 3.12 mmol) was added to a solution of (3R,5R)-tert-butyl 3-isopropyl-5-(4-(methoxycarbonyl)thiazol-2-yl)isoxazolidine-2-carboxylate (**69**) (1.00 g, 2.81 mmol) in CH_3CN (20 ml) and H_2O (2 ml) and the mixture was stirred at 70 °C for 16 h. After concentration, the residue was diluted with EtOAc (50 mL) and a 10% aq. solution of citric acid (50 ml). $NaIO_4$ was added to the mixture until the aqueous layer became clear, and extracted with EtOAc. The organic extracts were washed with a 10% aq. $Na_2S_2O_3$ and brine, dried over

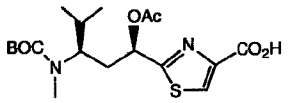
Na_2SO_4, filtered, concentrated and SiO_2 chromatography (hexane/EtOAc 3:2) to give the title compound (906 mg, 90%) as a colorless solid. MS ESI: m/z: [M+Na]+, calcd for $C_{16}H_{26}N_2NaO_5S$, 381.14, Found, 381.14

Example 26. **Methyl 2-((1R,3R)-1-acetoxy-3-(tert-butoxycarbonyl-amino)-4-methylpentyl)-thiazole-4-carboxylate (71)**



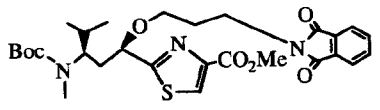
Compound **70** (900 mg, 2.51 mmol) in pyridine (15 ml) was added Ac_2O (0.5 ml, 5.29 mmol) and the mixture was stirred overnight, concentrated, SiO_2 chromatography (hexane/EtOAc 4:1) to give the title compound (950 mg, 95%) as a colorless solid. MS ESI: m/z: [M+Na]+, calcd for $C_{18}H_{28}N_2NaO_6S$, 423.15, Found, 423.16.

Example 27. **2-((1R,3R)-1-acetoxy-3-(tert-butoxycarbonylamino)-4-methylpentyl)thiazole-4-carboxylic acid (72)**



Compound **71** (940 mg, 2.35 mmol) in THF (15 ml) was added NaH (120 mg, 3.0 mmol, 60% in oil) at 4 °C and the mixture was stirred for 2 h, then CH_3I (0.155 ml, 2.49 mmol) was added. After stirred overnight, concentrated, re-dissolved in EtOAc, filtered through short SiO_2 column and evaporated to dryness to give a crude 2-((1R,3R)-1-acetoxy-3-(tert-butoxycarbonyl-(methyl)amino)-4-methylpentyl)thiazole-4-carboxylic acid (73a). To the dryness compound (73a) in 1,2-dichloroethane (20 ml) was added Trimethyltin hydroxide (620 mg, 3.43 mmol). The mixture was stirred at 80 °C overnight, concentrated, re-dissolved MeOH/CH_2Cl_2/HOAc (1:5:0.01, 20 ml), filtered through short SiO_2 column, concentrated and coevaporated with toluene to a dryness. To the dryness compound in pyridine (15 ml) was added Ac_2O (0.4 ml, 4.23 mmol). After stirred overnight, the mixture was concentrated, SiO_2 chromatography (MeOH/CH_2Cl_2/HOAc (1:10:0.01) to give the title compound (735 mg, 78%) as a colorless solid. ESI: m/z: [M+Na]+, calcd for $C_{18}H_{28}N_2NaO_6S$, 423.15, Found, 423.16.

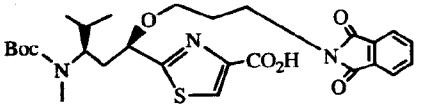
Example 28. **Methyl 2-((1R,3R)-3-(tert-butoxycarbonyl(methyl)amino)-1-(3-(1,3-dioxoisoindolin-2-yl)propoxy)-4-methylpentyl)thiazole-4-carboxylate (86).**



Compound **70** (850 mg, 2.37 mmol) in THF (15 ml) at -20 °C was added NaH (100 mg, 2.5 mmol, 60% in oil). After stirred for 30 min at -20 °C, *N*-(3-Bromopropyl)phthalimide (655 mg,

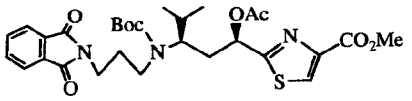
2.4 mmol) was added and the mixture was stirred at -20 °C for 30 min and then warmed up to room temperature in 4 h. The reaction mixture was quenched with methanol (0.5 ml), diluted with CH_2Cl_2 (60 ml), filtered through a short silica gel column, evaporated to dryness to provide crude methyl 2-((1R,3R)-3-(tert-butoxycarbonyl-amino)-1-(3-(1,3-dioxoisoindolin-2-yl)propoxy)-4-methylpentyl)thiazole-4-carboxylate **85** which was used directly without further purification. To the crude compound **85** in THF (25 ml) at room temperature was added NaH (170 mg, 4.25 mmol, 60% in oil). After stirred for 45 min, CH_3I (0.20 ml, 3.21 mmol) was added. The mixture was stirred at room temperature overnight, quenched with NaH_2PO_4 (2.0 M, 2 ml). The mixture was added DMA (5ml), evaporated in vacuo, SiO_2 chromatography with EtOAc/CH_2Cl_2 (1:10 ~ 1:4) to afford the title compound (921 mg, 69%). ESI: m/z: [M+Na]+, calcd for $C_{28}H_{37}N_3NaO_7S$, 582.22, Found, 582.22.

Example 29. **2-((1R,3R)-3-(tert-butoxycarbonyl(methyl)amino)-1-(3-(1,3-dioxoisoindolin-2-yl)propoxy)-4-methylpentyl)thiazole-4-carboxylic acid (87).**



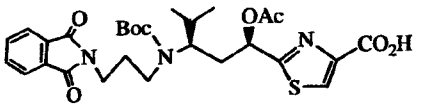
To the dryness compound **86** (910 mg, 1.63 mmol) in 1,2-dichloroethane (20 ml) was added Trimethyltin hydroxide (400 mg, 2.21 mmol). The mixture was stirred at 80 °C overnight, concentrated, purified on SiO_2 column eluted with CH_3OH/CH_2Cl_2/HOAc (1:10:0.01) to afford the title compound (756 mg, 85%). ESI: m/z: [M+Na]+, calcd for $C_{27}H_{36}N_3NaO_7S$, 546.22, Found, 546.22.

Example 30. **Methyl 2-((1R,3R)-1-acetoxy-3-(tert-butoxycarbonyl(3-(1,3-dioxoisoindolin-2-yl)propyl)amino)-4-methylpentyl)thiazole-4-carboxylate (89)**



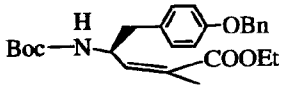
To the compound **71** (800 mg, 2.00 mmol) in THF (30 ml) at room temperature was added NaH (150 mg, 3.75 mmol, 60% in oil). After stirred for 45 min, *N*-(3-Bromopropyl)phthalimide (655 mg, 2.4 mmol) was added. The mixture was stirred at room temperature overnight, quenched with NaH_2PO_4 (2.0 M, 2 ml). The mixture was added DMA (5ml), evaporated and purified on SiO_2 column eluted with EtOAc/CH_2Cl_2 (1:10 ~ 1:4) to afford the title compound (971 mg, 82%). ESI: m/z: [M+Na]+, calcd for $C_{29}H_{37}N_3NaO_8S$, 610.22, Found, 610.22.

Example 31. **2-((1R,3R)-1-acetoxy-3-(tert-butoxycarbonyl(3-(1,3-dioxoisoindolin-2-yl)propyl)amino)-4-methylpentyl)thiazole-4-carboxylic acid (90)**



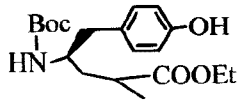
To the dryness compound (**89**) (900 mg, 1.53 mmol) in 1,2-dichloroethane (35 ml) was added trimethyltin hydroxide (400 mg, 2.21 mmol). The mixture was stirred at 80 °C overnight, concentrated. Then the mixture in pyridine (20 ml) was added Ac_2O (3 ml) and stirred overnight, evaporated, purified on SiO_2 column ($MeOH/CH_2Cl_2$/HOAc, 1:10:0.01) to afford the title compound (755 mg, 86%). ESI: m/z: [M+Na]+, calcd for $C_{28}H_{35}N_3NaO_8S$, 596.20, Found, 596.20.

Example 32. **(S)-Ethyl 5-(4-(benzyloxy)phenyl)-4-(tert-butoxy-carbonylamino)-2-methylpent-2-enoate (185)**



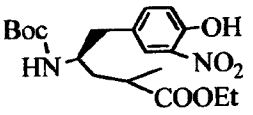
(S)-Methyl 3-(4-(benzyloxy)phenyl)-2-(tert-butoxy-carbonylamino)propanoate **184** (8.00 g, 20.76 mmol) in CH_2Cl_2 (250 ml) at -78 °C was added dropwise DIBAL (40 ml, 40 mmol, 1.0 M) in CH_2Cl_2. After stirred at -78 °C for 2h, the reaction was quenched with addition of MeOH (5 ml). The mixture was warmed to RT, acidified with 1 M HCl to pH 4 and separated. The aqueous layer was extracted with DCM (2 x 150 ml). The organic layers were washed with water, dried over Na_2SO_4, filtered and evaporated to dryness to form cude aldehyde intermediate. Then the crude intermediate aldehyde was dissolved in DCM, the ylide solution prepared from 1-(1-ethoxycarbonyl ethyl)-triphenylphosphonium bromide (18.0 g, 40.64 mmol) and KOtBu (5.00g, 44.64 mmol) in CH_2Cl_2 (80 ml) at RT was added at. After stirred at RT over night, the mixture was extracted concentrated and purified by SiO_2 chromatography (EtOAc/Hexane, 1:8 ~ 1:4) to afford (6.90 g, 76%) of the title compound. ESI: m/z: [M+Na]+, calcd for $C_{26}H_{33}NNaO_5$, 462.22, Found, 462.22.

Example 33. **(4R)-ethyl 4-((tert-butoxycarbonyl)amino)-5-(4-hydroxyphenyl)-2-methylpentanoate (186)**



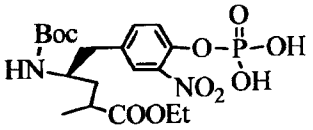
(S)-Ethyl 5-(4-(benzyloxy)phenyl)-4-(tert-butoxy-carbonylamino)-2-methylpent-2-enoate **(185)** (6.70 g, 15.26 mmol) in a hydrogenation bottle was charged methanol (150 ml), Pd/C (0.3 g, 10% Pd). The hydrogenation reaction was conducted at 30 psi for 6 h. The mixture was filtered through Celite, evaporated and crystallized with EtOH/hexane to the title compound (**186**) (4.61 g, 86% yield). ESI: m/z: [M+Na]+, calcd for $C_{19}H_{29}NNaO_5$, 374.20, Found, 374.30.

Example 34. **(4R)-ethyl 4-((tert-butoxycarbonyl)amino)-5-(4-hydroxy-3-nitrophenyl)-2-methylpentanoate (187)**



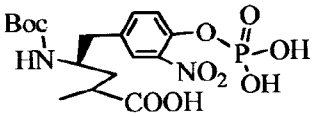
To a solution of compound **186** (4.50 g, 12.81 mmol) in anhydrous CH_2Cl_2 (200 ml) was added Ac_2O (2 ml, 21.16 mmol) and fuming HNO_3 (0.65 ml, 14.07 mmol). The mixture was stirred at RT for 4h, diluted with water (150 ml), separated and the aqueous layer was extracted with EtOAc. The organic layers were combined, dried over Na_2SO_4, evaporated and purified on SiO_2 column (EtOAc/DCM, 1:10) to afford (4.21 g, 83%) of the title compound. ESI: m/z: [M+Na]+, calcd for $C_{19}H_{28}N_2NaO_7$, 419.19, Found, 419.20.

Example 35. **ethyl 4-((tert-butoxycarbonyl)amino)-2-methyl-5-(3-nitro-4-(phosphonooxy)phenyl)pentanoate (188)**



Compound **187** (4.00 g, 10.09 mmol) in a mixture of CH_3CN (70 ml) and DMA (30 ml) was added DIPEA (4.00 ml, 23.00 mmol) at 0 °C. After stirred for 2 min, $POCl_3$ (2.00 ml, 21.45 mmol) was added dropwise at 0 °C. The mixture was stirred at RT for 8 h, and quenched with slowly addition of $NaHCO_3$ (3.5 g, 41.60 mmol) in water (20 ml) at 0 °C. After stirred at 0 °C overnight, the mixture was concentrated and purified on C-18 cartridge (20 x 4 cm) eluted with gradient mixture, 25 ml/min, A: 0.5% HOAc, B: CH_3OH, from 100% A in 10 min, then to 75% A and 25% B in 45 min. The fractions containing the product was pooled and evaporated to afford the title compound (3.89 g, 81% yield). ESI: m/z: $[M-H]^-$, calcd for $C_{19}H_{28}N_2O_{10}P$, 475.16, Found, 475.20.

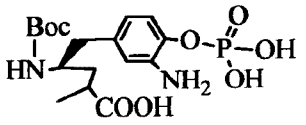
Example 36. **(4R)-4-((tert-butoxycarbonyl)amino)-2-methyl-5-(3-nitro-4-(phosphonooxy)phenyl)pentanoic acid (189)**



To a solution of LiOH (5.0 g, 208.7 mmol) in H_2O (60 mL) was added to a solution of compound (**188**) (3.75 g, 7.87 mmol) in THF (100 mL). After stirred for 4 h at 0 °C, the mixture was was adjusted to pH ~6 with 4M HCl, concentrated, and purified by C-18 chromatography eluted with gradient mixture, 25 ml/min, A: 0.5% HOAc, B: CH_3OH, from 100% A in 10 min, then to 75% A and 25% B in 45 min. The fractions containing the product was pooled and

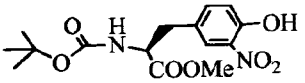
evaporated to afford the title compound (2.82 g, 80% yield). ESI: m/z: $[M-H]^-$, calcd for $C_{17}H_{24}N_2O_{10}P$, 447.12, Found, 447.20.

Example 37. **(4R)-5-(3-amino-4-(phosphonooxy)phenyl)-4-((tert-butoxycarbonyl)amino)-2-methylpentanoic acid (190)**



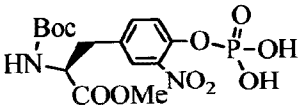
Compound (189) (2.60 g, 5.80 mmol) in a hydrogenation bottle was charged methanol (80 ml), Pd/C (0.2 g, 10% Pd). The hydrogenation reaction was conducted at 35 psi of H_2 for 6 h. The mixture was filtered through Celite, evaporated to afford crude title compound (**190**) (2.18 g, 90% yield), which was used directly without further purification. ESI: m/z: [M+Na]+, calcd for $C_{17}H_{26}N_2O_8P$, 417.15, Found, 417.15.

Example 38. **(S)-methyl 2-((tert-butoxycarbonyl)amino)-3-(4-hydroxy-3-nitrophenyl)propanoate (196)**



To a solution of (S)-methyl 2-((tert-butoxycarbonyl)amino)-3-(4-hydroxyphenyl) propanoate (**195**) (4.5 g, 15.24 mmol) in anhydrous CH_2Cl_2 (240 ml) was added Ac_2O (4 ml, 42.32 mmol) and fuming HNO_3 (0.85 ml, 18.40 mmol). The mixture was stirred at RT for 4h, diluted with water (150 ml), separated and the aqueous layer was extracted with EtOAc. The organic layers were combined, dried over Na_2SO_4, evaporated and purified on SiO_2 column (EtOAc/DCM, 1:10) to afford (4.30 g, 83%) of the title compound. ESI: m/z: [M+Na]+, calcd for $C_{15}H_{20}N_2NaO_7$, 363.13, Found, 363.20.

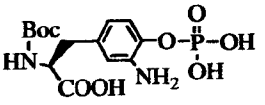
Example 39. **(S)-methyl 2-((tert-butoxycarbonyl)amino)-3-(3-nitro-4-(phosphonooxy)phenyl)propanoate (197)**



To a solution of compound **196** (4.10 g, 12.05 mmol) in a mixture of CH_3CN (90 ml) was added DIPEA (4.00 ml, 23.00 mmol) at 0 °C. After stirred for 2 min, $POCl_3$ (2.00 ml, 21.45 mmol) was added dropwise at 0 °C. The mixture was stirred at RT for 8 h, and quenched with slowly addition of $NaHCO_3$ (3.5 g, 41.60 mmol) in water (20 ml) at 0 °C. After stirred at 0 °C overnight, the mixture was concentrated and purified on C-18 cartridge (20 x 4 cm) eluted with gradient mixture, 25 ml/min, A: 0.5% HOAc, B: CH_3OH, from 100% A in 10 min, then to 75% A and 25% B in 45 min. The fractions containing the product was pooled and evaporated to

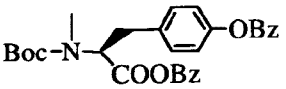
afford (4.20 g, 83%) the title compound. ESI: m/z: $[M-H]^-$, calcd for $C_{15}H_{20}N_2O_{10}P$, 419.08, Found, 419.10.

Example 40. **3-[3-amino-4-(phosphonooxy)phenyl]-(2R)-2-{[(tert-butoxy)carbonyl]amino}-propanoic acid (198)**



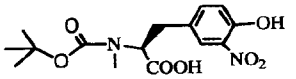
To the dryness compound (**197**) (4.0 g, 9.52 mmol) in the mixture of 1,2-dichloroethane (50 ml) and DMA (60 ml) was added trimethyltin hydroxide (4.00 g, 22.1 mmol). The mixture was stirred at 80 °C for 6h, evaporated, filtered through short SiO_2 column eluted with water/MeCN (1;4). The fractions containing the product were pooled, concentrated to generate (S)-2-((tert-butoxycarbonyl)amino)-3-(3-nitro-4-(phosphonooxy)phenyl)propanoic acid. In a hydrogenation bottle was charged DMA (70 ml), Pd/C (0.3 g, 10% Pd), followed by the addition of the prepared propanoic acid. The hydrogenation reaction was conducted at 30 psi of hydrogen for 6 h. The mixture was filtered through Celite, evaporated, and crystallized to afford (2.86 g, 80% yield) of the title compound (**198**), which was used directly without further purification. ESI: m/z: $[M-H]^-$, calcd for $C_{14}H_{20}N_2O_8P$, 375.10, Found, 375.10.

Example 41. **Benzyl 3-[4-(benzyloxy)phenyl]-(2R)-2-{[(tert-butoxy)carbonyl]-(methyl)-amino}-propanoate (200).**



A solution of benzyl 3-[4-(benzyloxy)phenyl]-(2R)-2-{[(tert-butoxy)carbonyl]-amino}propanoate (4.0 g, 8.67 mmol) in THF (60 ml) was added NaH (430 mg, 10.75 mmol, 60% in oil). After stirred at RT for 1 h, CH_3I (1.82 g, 12.82 mmol) was added, and the mixture was stirred overnight, quenched with CH_3OH (0.5 ml), evaporated and purified on SiO2 column ($EtOAc/CH_2Cl_2$, 1:10) to afford the title compound (3.83 g, 93%). MS ESI: m/z: [M+Na]+, calcd for $C_{29}H_{33}NNaO_5$, 498.24, Found, 498.24.

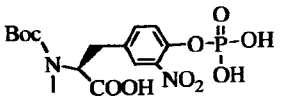
Example 42. **(2R)-2-{[(tert-butoxy)carbonyl](methyl)amino}-3-(4-hydroxy-3-nitrophenyl)propanoic acid (201)**



Compound **200** (3.80 g, 8.00 mmol) in a hydrogenation bottle was charged methanol (80 ml), Pd/C (0.3 g, 10% Pd). The hydrogenation reaction was conducted at 30 psi of hydrogen for 6 h. The mixture was filtered through Celite, evaporated to afford crude (2R)-2-{[(tert-butoxy)carbonyl](methyl)amino}-3-(4-hydroxyphenyl)propanoic acid (**201a**), which was used

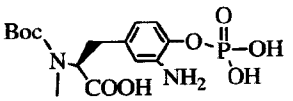
directly without further purification. To the compound **201a** in anhydrous CH_2Cl_2 (240 ml) at -25 °C was added dropwise a mixture of $SnCl_4$ (1.5 ml, 12.75 mmol) and fuming HNO_3 (0.60 ml, 12.98 mmol) in CH_2Cl_2 (40 ml). The mixture was stirred at -25 °C for 75 min, quenched with saturated $NaHCO_3$ to pH 3~4, separated and the aq. layer was extracted with EtOAc. The organic layers were combined, dried over Na_2SO_4, concentrated and purified on SiO_2 column (MeOH/DCM/HOAc 1:8:0.01) to afford (1.98 g, 73%) of the title compound. ESI: m/z: [M+Na]+, calcd for $C_{15}H_{20}N_2NaO_7$, 363.13, Found, 363.13.

Example 43. **(2R)-2-{[(tert-butoxy)carbonyl](methyl)amino}-3-[3-nitro-4-(phosphonooxy)-phenyl]propanoic acid (202).**



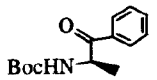
To a solution of compound **201** (1.98 g, 5.82 mmol) in a mixture of CH_3CN (30 ml) and DMA (30 ml) was added DIPEA (2.00 ml, 11.50 mmol) at 0 °C. After stirred for 2 min, $POCl_3$ (1.10 ml, 11.79 mmol) was added dropwise at 0 °C. The mixture was stirred at RT for 8 h, and quenched with slowly addition of $NaHCO_3$ (2.0 g, 23.80 mmol) in water (10 ml) at 0 °C. After stirred at 0 °C overnight, the mixture was concentrated and purified on C-18 cartridge (20 x 4 cm) eluted with gradient mixture, 25 ml/min, A: 0.5% HOAc, B: CH_3OH, from 100% A in 10 min, then to 75% A and 25% B in 45 min. The fractions containing the product was pooled and evaporated to afford (1.96, 80%) the title compound. ESI: m/z: $[M-H]^-$, calcd for $C_{15}H_{20}N_2O_{10}P$, 419.09, Found, 419.09.

Example 44. **3-[3-amino-4-(phosphonooxy)phenyl]-(2R)-2-[(tert-butoxycarbonyl)(methyl)-amino]-propanoic acid (203)**



Compound **202** (1.96 g, 4.67 mmol) in a hydrogenation bottle was charged DMA (60 ml), Pd/C (0.2 g, 10% Pd). The hydrogenation reaction was conducted at 30 psi of hydrogen for 6 h. The mixture was filtered through Celite, evaporated to dryness to afford (1.74 g, 95%) of the title compound (**203**), which was used directly without further purification. ESI: m/z: $[M-H]^-$, calcd for $C_{15}H_{22}N_2O_8P$, 389.12, Found, 389.12.

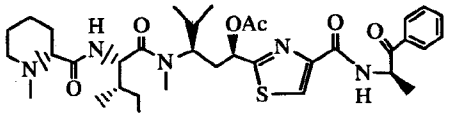
Example 45. **Tert-butyl N-(1-oxo-1-phenylpropan-2-(2R)-yl)carbamate (204)**



(1*S*,2*R*)-(+)-Norephedrine (7.0 g, 46.29 mmol) in the mixture of THF (40 ml) and 1M $NaHCO_3$ (100 ml) at 4 °C was added dropwise Boc_2O (10.15 g, 46.53 mmol) in THF (60 ml) in

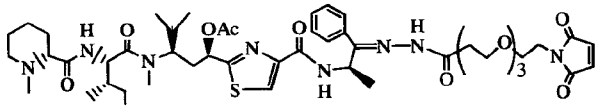
45 min. The mixture then stirred at RT for 6 h, concentrated, extracted with EtOAc, dried over Na_2SO_4, concentrated and filtered through short SiO_2 column eluted with EtOAc/Hexane (1:2), concentrated to afford the crude tert-butyl N-((1S)-hydroxy-1-phenylpropan-2-(2R)-yl)carbamate (**204b**) (10.81, 93%). MS ESI: m/z+: $[M+Na]^+$, calcd for $C_{14}H_{21}NaNO_3$, 274.15, Found, 274.15. The crude compound was used directly without further purification. The compound (**204b**) in CH_2Cl_2 (50 ml) was added Dess-Martin periodinane solution in CH_2Cl_2 (180 ml, 0.3 M). After stirred for 1h, the mixture was added ice cold NaOH (1 M, 100 ml), separated and the organic layers were washed with 1M NaH_2PO_4, pH 6 (100 ml), dried over Na_2SO_4, evaporated and purified on SiO2 column (EtOAc/hexane 1:5) to afford the title compound **204** (9.34 g, 81% in two steps). MS ESI: m/z+: $[M+Na]^+$, calcd for $C_{14}H_{19}NaNO_3$, 272.14, Found, 272.14.

Example 46. **(1R,3R)-3-((2S,3S)-N-(methyl)-3-methyl-2-((R)-1-methylpiperidine-2-carboxamido)-pentamido)-4-methyl-1-(4-((1-oxo-1-phenylpropan-2-yl)carbamoyl)thiazol-2-yl)-pentyl acetate (205).**



Compound **204** (180 mg, 0.722 mmol) in 4 ml of dioxane was added HCl conc. (1.0 ml, 37%) at 4 °C and the mixture was stirred at RT for 30 min, evaporated and coevaporated with toluene to dryness. Then to the dryness solid in DMA (7 ml) were added compound **106** (251 mg, 0.466 mmol), EDC (305 mg, 1.56 mmol) and DIPEA (0.13 ml, 0.747 mmol) and the mixture was stirred for 8h, evaporated and purified on SiO_2 column ($EtOAc/CH_2Cl_2$, 1:4) to afford the title compound **205** (255.3 mg, 82%). ESI: m/z+: $[M+Na]^+$, calcd for $C_{35}H_{51}NaN_5O_6S$, 692.36, Found, 692.36.

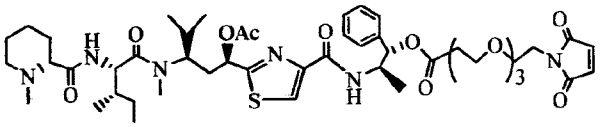
Example 47. **(1R,3R)-3-((2S,3S)-N,3-dimethyl-2-((R)-1-methylpiperidine-2-carboxamido)-pentanamido)-1-(4-((R)-1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-12-oxo-15-phenyl-3,6,9-trioxa-13,14-diazaheptadec-14-en-16-ylcarbamoyl)thiazol-2-yl)-4-methylpentyl acetate (206).**



Compound **205** (75 mg, 0.112 mmol) in methanol (5 ml) was added compound **12** (50 mg in HCl salt, 0.126 mmol) and HOAc (3 ul, 0.052 mmol). The mixture was stirred overnight, neutralized with DIPEA (23 ul, 0.132 mmol), evaporated and purified on SiO_2 cartridge (4g,

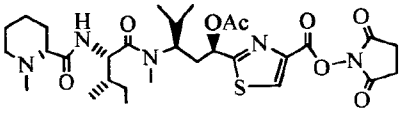
EtOAc/CH_2Cl_2, 1:5 ~1:3) to afford the title compound **206** (79.3 mg, 70%). MS ESI: m/z+: $[M+Na]^+$, calcd for $C_{50}H_{74}NaN_8O_{12}S$, 1033.51, Found, 1033.50.

Example 48. **(1S,2R)-2-(2-((1R,3R)-1-acetoxy-3-((2S,3S)-N,3-dimethyl-2-((R)-1-methylpiperidine-2-carboxamido)pentanamido)-4-methylpentyl)thiazole-4-carboxamido)-1-phenyl-propyl 3-(2-(2-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)ethoxy)ethoxy)-propanoate (211)**



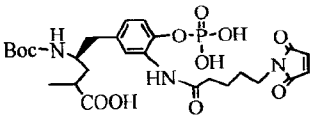
Compound **208a** (95 mg, 0.141 mmol) and 3-(2-(2-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)ethoxy)ethoxy)propanoic acid (55 mg, 0.182 mmol) in CH_2Cl_2 (5 ml) were added DCC (122 mg, 0.591 mmol) and DMAP (25 mg, 0.204 mmol) and the mixture was stirred overnight, evaporated and purified with SiO_2 chromatography (EtOAc/CH_2Cl_2, 1:3) to afford the title compound (95.1 mg, 71%). MS ESI: m/z+: $[M+Na]^+$, calcd for $C_{48}H_{70}NaN_6O_{12}S$, 977.47, Found, 977.47.

Example 49. **2,5-dioxopyrrolidin-1-yl 2-((1R,3R)-1-acetoxy-3-((2S,3S)-N,3-dimethyl-2-((R)-1-methylpiperidine-2-carboxamido)pentanamido)-4-methylpentyl)thiazole-4-carboxylate (234).**



Compound **106** (788.1 mg, 1.464 mmol) in DMF (10 ml) were added NHS (202.0 mg, 1.756 mmol) and EDC (980 mg, 5.104 mmol) and the mixture was stirred overnight, evaporated and purified with SiO_2 chromatography (EtOAc/CH_2Cl_2, 1:3) to afford the title compound (762.8 mg, 82%). MS ESI: m/z+: $[M+Na]^+$, calcd for $C_{30}H_{45}NaN_5O_8S$, 658.30, Found, 658.30.

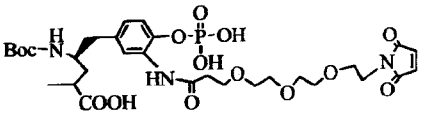
Example 50. **(4R)-4-(tert-butoxycarbonylamino)-5-(3-(5-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)pentanamido)-4-(phosphonooxy)phenyl)-2-methylpentanoic acid (235)**



Compound **190** (825.1 mg, 1.973 mmol) in DMF (7 ml) was added 2,5-dioxopyrrolidin-1-yl 5-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)pentanoate (**23d**) (711 mg, 2.417 mmol) and DIPEA (0.250 ml, 1.438 mmol), and the mixture was stirred overnight, evaporated and purified with C-18 chromatography (4x25 cm, v = 15 ml/min, 100% of 1% HOAc to 75% of 1% HOAc/25%

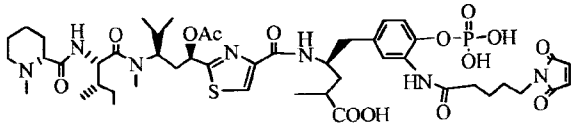
MeOH in 45 min) to afford the title compound **235** (895.7 mg, 76%). MS ESI: m/z-: $[M-H]^-$, calcd for $C_{26}H_{35}N_3O_{11}P$, 596.21, Found, 596.21.

Example 51. **(4R)-4-(tert-butoxycarbonylamino)-5-(3-(3-(2-(2-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)ethoxy)ethoxy)propanamido)-4-(phosphonooxy)phenyl)-2-methylpentanoic acid (236)**



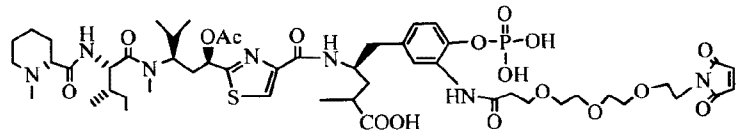
Compound **190** (632.5 mg, 1.512 mmol) in DMF (7 ml) were added 2,5-dioxopyrrolidin-1-yl 3-(2-(2-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)ethoxy)ethoxy)propanoate (**24c**) (727 mg, 1.826 mmol) and DIPEA (0.250 ml, 1.438 mmol), and the mixture was stirred overnight, evaporated and purified with C-18 chromatography (4x25 cm, v = 15 ml/min, 100% of 1% HOAc to 75% of 1% HOAc/25% MeOH in 45 min) to afford the title compound **236** (763.2 mg, 72%). MS ESI: m/z-: $[M-H]^-$, calcd for $C_{30}H_{44}N_3O_{14}P$, 700.25, Found, 700.25.

Example 52. **(4R)-4-(2-((1R,3R)-1-acetoxy-3-((2S,3S)-N,3-dimethyl-2-((R)-1-methylpiperidine-2-carboxamido)pentanamido)-4-methylpentyl)thiazole-4-carboxamido)-5-(3-(5-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)pentanamido)-4-(phosphonooxy)phenyl)-2-methylpentanoic acid (239).**



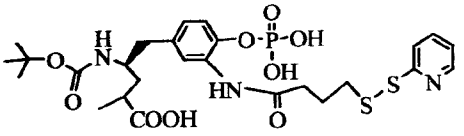
Compound **235** (102 mg, 0.171 mmol) in 1,4-dioxane (4 ml) was added conc. HCl (1 ml, 37%) and the mixture was stirred for 30 min, evaporated to dryness to afford the crude compound **237**. To the crude compound in DMA (5 ml) were added compound **234** (110 mg, 0.173 mmol) and DIPEA (30 ul, 0.172 mmol), and the mixture was stirred for overnight, evaporated and purified by SiO_2 chromatography (1% HOAc in water/acetone, 1:9 ~ 1:4) to afford the title compound **239** (123.2 mg, 71%). MS ESI: m/z-: $[M-H]^-$, calcd for $C_{47}H_{67}N_7O_{14}PS$, 1016.42, Found, 1016.42.

Example 53. **(4R)-4-(2-((1R,3R)-1-acetoxy-3-((2S,3S)-N,3-dimethyl-2-((R)-1-methylpiperidine-2-carboxamido)pentanamido)-4-methylpentyl)thiazole-4-carboxamido)-5-(3-(3-(2-(2-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)ethoxy)ethoxy)-propanamido)-4-(phosphonooxy)phenyl)-2-methylpentanoic acid (240)**



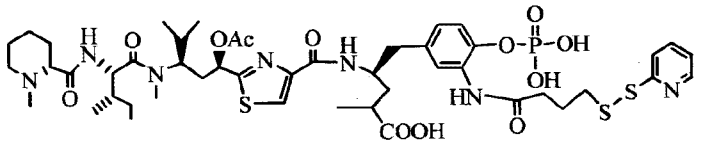
Compound **236** (108 mg, 0.154 mmol) in 1,4-dioxane (4 ml) was added conc. HCl (1 ml, 37%) and the mixture was stirred for 30 min, evaporated to dryness to afford the crude compound **238**. To the crude compound in DMA (5 ml) were added compound **234** (110 mg, 0.173 mmol) and DIPEA (30 ul, 0.172 mmol), and the mixture was stirred for overnight, evaporated and purified by SiO_2 chromatography (1% HOAc in water/acetone, 1:9 ~ 1:4) to afford the title compound **240** (131.2 mg, 76%). MS ESI: m/z-: $[M-H]^-$, calcd for $C_{51}H_{75}N_7O_{17}PS$, 1120.47, Found, 1120.48.

Example 54. **(4R)-4-(tert-butoxycarbonylamino)-2-methyl-5-(4-(phosphonooxy)-3-(4-(pyridin-2-yldisulfanyl)butanamido)phenyl)pentanoic acid (244).**



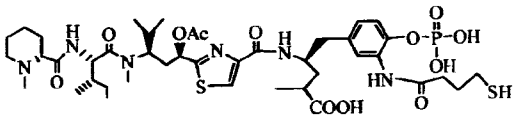
To a solution of compound **190** (548.3 mg, 1.311 mmol) in DMF (10 ml) were added succinimidyl 4-(pyridin-2-yl)disulfanyl)-butyrate (550.2mg, 1.687 mmol) and DIPEA (0.18 ml, 1.03 mmol). The mixture was stirred overnight, evaporated and purified by SiO_2 chromatography (1% HOAc in water/acetone, 1:9 ~ 1:4) to afford the title compound **244** (660.2 mg, 80%). MS ESI: m/z-: $[M-H]^-$, calcd for $C_{26}H_{36}N_3O_9PS_2$, 628.16, Found, 628.16.

Example 55. **(4R)-4-(2-((1R,3R)-1-acetoxy-3-((2S,3S)-N,3-dimethyl-2-((R)-1-methylpiperidine-2-carboxamido)pentanamido)-4-methylpentyl)thiazole-4-carboxamido)-2-methyl-5-(4-(phosphonooxy)-3-(4-(pyridin-2-yldisulfanyl)butanamido)phenyl)pentanoic acid(248)**



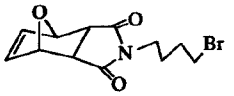
Compound **244** (110.5 mg, 0.175 mmol) in 1,4-dioxane (4 ml) was added conc. HCl (1 ml, 37%) and the mixture was stirred at 4°C for 30 min, evaporated to dryness to afford the crude compound **246**. To the crude compound in DMA (5 ml) were added compound **234** (110 mg, 0.173 mmol) and DIPEA (30 ul, 0.172 mmol). The mixture was stirred for overnight, evaporated and purified by SiO_2 chromatography (1% HOAc in water/acetone, 1:9 ~ 1:4) to afford the title compound **248** (129.1 mg, 71%). MS ESI: m/z-: $[M-H]^-$, calcd for $C_{47}H_{68}N_7O_{12}PS_3$, 1048.38, Found, 1048.38.

Example 56. **(4R)-4-(2-((1R,3R)-1-acetoxy-3-((2S,3S)-N,3-dimethyl-2-((R)-1-methylpiperidine-2-carboxamido)pentanamido)-4-methylpentyl)thiazole-4-carboxamido)-5-(3-(4-mercaptobutanamido)-4-(phosphonooxy)phenyl)-2-methylpentanoic acid (248b).**



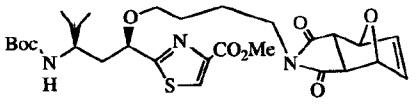
Compound **248** (30 mg, 0.0285 mmol) in a mixture of DMA (2 ml) and NaH_2PO_4 (0.1 M, pH 7) was added dithiothreitol (20 mg, 0.129 mmol). The mixture was stirred for 2h, evaporated and purified by SiO_2 chromatography (1% HOAc in water/CH_3CN, 1:9 ~ 1:4) to afford the title compound **248b** (22 mg, 85%). MS ESI: m/z-: $[M-H]^-$, calcd for $C_{42}H_{64}N_6O_{12}PS_2$, 939.38, Found, 939.38.

Example 57. **4-(4-bromobutyl)-10-oxa-4-azatricyclo[5.2.1.0^{2,6}]dec-8-ene-3,5-dione (271).**



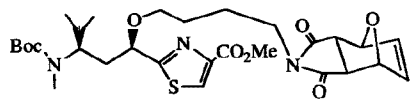
10-oxa-4-azatricyclo[5.2.1.0^{2,6}]dec-8-ene-3,5-dione (6.0 g, 36.35 mmol) and NaH (1.50 g, 37.50 mmol, 60% in oil) were stirred in DMA (60 ml) for 1 h, then 1,4-dibromobutane (35.0 g, 162.10 mmol) and NaI (0.50 g, 3.33 mmol) were added. The mixture was stirred overnight, quenched with CH_3OH (0.5 ml), evaporated, purified on SiO_2 column (EtOAc/Hexane, 1:8) to afford the title compound (9.34 g, 86%). MS ESI: m/z+: $[M+Na]^+$, calcd for $C_{12}H_{14}BrNaNO_3$, 322.02, Found, 322.02.

Example 58. **Methyl 2-((1R,3R)-3-(tert-butoxycarbonylamino)-1-[4'-(3'',6''-endoxo-Δ-tetrahydrophthalimido)butyloxy]-4-methylpentyl)thiazole-4-carboxylate (272).**



Methyl 2-((1R,3R)-3-(tert-butoxycarbonylamino)-1-hydroxy-4-methylpentyl)thiazole-4-carboxylate (**70**) (1.0 g, 2.79 mmol) and NaH (120 mg, 3.00 mmol, 60% in oil) were stirred in THF (30 ml) for 20 min and compound **271** (1.00 g, 3.34 mmol) and NaI (50 mg, 0.33 mmol) were added. The mixture was stirred overnight, quenched with MeOH (0.5 ml), evaporated and purified on SiO_2 column (EtOAc/CH_2Cl_2, 1:10) to afford the title compound (1.36 g, 84%). MS ESI: m/z+: $[M+Na]^+$, calcd for $C_{28}H_{39}NaN_3O_8S$, 600.25, Found, 600.25.

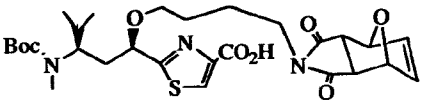
Example 59. **Methyl 2-((1R,3R)-3-(N,N-tert-butoxycarbonylmethylamino)-1-[4'-(3'',6''-endoxo-Δ-tetrahydrophthalimido)butyloxy]-4-methylpentyl)thiazole-4-carboxylate (273).**



Compound **272** (1.30 g, 2.25 mmol) and NaH (108 mg, 2.70 mmol, 60% in oil) stirred in DMF (80 ml) for 1 h was added CH_3I (460 mg, 3.24 mmol). The mixture was stirred overnight, evaporated and purified on SiO_2 column (EtOAc/CH_2Cl_2, 1:12 ~ 1:8) to afford the title

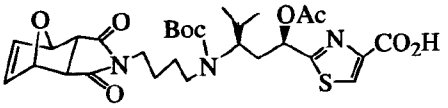
compound (1.01 g, 76%). MS ESI: m/z+: $[M+Na]^+$, calcd for $C_{29}H_{41}NaN_3O_8S$, 614.26, Found, 614.26.

Example 60. **2-((1R,3R)-3-(N,N-tert-butoxycarbonylmethyl-amino)-1-(4'-maleimido-butyloxy)-4-methylpentyl)thiazole-4-carboxylic acid (274).**



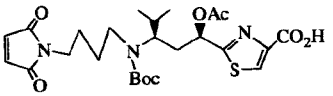
To the dryness compound (**273**) (900 mg, 1.52 mmol) in a mixture of 1,2-dichloroethane (30 ml) and toluene was added trimethyltin hydroxide (400 mg, 2.21 mmol). The mixture was stirred at 100 °C overnight, concentrated, purified on SiO_2 column eluted with $MeOH/CH_2Cl_2/HOAc$ (1:10:0.01) to afford the title compound (730 mg, 94%). ESI: m/z: [M+Na]+, calcd for $C_{24}H_{35}N_3NaO_7S$, 532.22, Found, 532.22.

Example 61. **Methyl 2-((1R,3R)-1-acetoxy-3-(N,N-(tert-butoxycarbonyl)(4'-(3'',6''-endoxo-Δ-tetrahydrophthalimido)butyl)amino)-4-methylpentyl)-thiazole-4-carboxylate (275)**



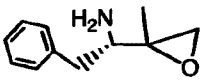
Compound **71** (1.50 g, 3.74 mmol) and NaH (180 mg, 4.50 mmol, 60% in oil) stirred in DMF (80 ml) for 1 h was added compound **271** (1.48 g, 4.94 mmol) and NaI (70 mg, 0.467 mmol). The mixture was stirred overnight, evaporated and purified on SiO_2 column ($EtOAc/CH_2Cl_2$, 1:10 ~ 1:6) to afford the title compound (1.60 g, 69%). MS ESI: m/z+: $[M+Na]^+$, calcd for $C_{30}H_{41}NaN_3O_9S$, 642.26, Found, 642.26.

Example 62. **2-((1R,3R)-1-acetoxy-3-(N,N-(tert-butoxycarbonyl)(4'-maleimidobutyl)amino)-4-methylpentyl)-thiazole-4-carboxylic acid (276)**



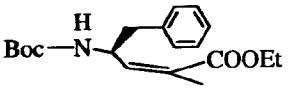
To the dryness compound (**275**) (800 mg, 1.29 mmol) in a mixture of 1,2-dichloroethane (40 ml) and toluene was added trimethyltin hydroxide (400 mg, 2.21 mmol). The mixture was stirred at 100 °C overnight, filtered through a short SiO_2 column, washed the column with $MeOH/CH_2Cl_2/HOAc$ (1:5:0.01) and evaporated to dryness. To the crude dryness mixture in pyridine (15 ml) was added Ac_2O (0.3 ml, 3.17 mmol) at 0 °C. The mixture was stirred at RT overnight, evaporated and purified on SiO2 column eluted with $MeOH/CH_2Cl_2/HOAc$ (1:10:0.01) to afford the title compound (578.4 mg, 74%). ESI: m/z: [M+Na]+, calcd for $C_{29}H_{39}N_3NaO_9S$, 628.24, Found, 628.24.

Example 63. **Phenylalanine-ketoepoxide**



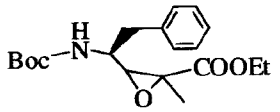
N-Boc phenylalanine-ketoepoxide (Sun, L. et al, J. Mol. Catalysis A: Chem., 2005, 234 (1-2), 29-34) (300 mg, 1.08 mmol) in 1,4-dioxane (8 mL) at 0 ^{0}C was added hydrochloric acid (37%, 2 mL). The mixture was stirred for 1 hour at which time TLC showed complete consumption of starting material. The resulting solution was diluted with toluene (10 ml), evaporated and crystallized with EtOH/Hexane to yield HCl salt of the title compound (201 mg, 87%). ESI: m/z: [M+H]+, calcd for $C_{11}H_{16}NO$, 178.12, Found, 178.12.

Example 64. **(S, E)-Ethyl 5-phenyl-4-(tert-butoxy-carbonylamino)-2-methylpent-2-enoate (327)**



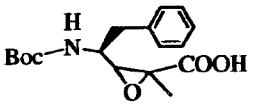
L-t-Boc-phenylalanine methyl ester **326** (5.60 g, 20.05 mmol) in CH_2Cl_2 (80 ml) at -78 °C was added dropwise DIBAL (40 ml, 40 mmol, 1.0 M) in CH_2Cl_2. After stirred at -78 °C for 45 min, the ylide solution prepared from 1-(1-ethoxycarbonyl ethyl)-triphenylphosphonium bromide (18.0 g, 40.64 mmol) and KOtBu (5.00g, 44.64 mmol) in CH_2Cl_2 (80 ml) at RT was added at -78 °C. After stirred at -78 °C for 1 h and RT over night, the mixture was poured into 1 L of NaH_2PO_4 (sat.) solution with vigorously stirring. Separated and the aqueous phase was extracted with CH_2Cl_2. The organic layers were dried over Na_2SO_4, concentrated and purified by SiO_2 chromatography (EtOAc/Hexane, 1:7 ~ 1:5) to afford 5.50 g (83% yields) of the title compound. ESI: m/z: $[M+Na]^+$, calcd for $C_{19}H_{27}NNaO_4$, 356.19, Found, 356.20.

Example 65. **Ethyl 3-((S)-1-(tert-butoxycarbonylamino)-2-phenylethyl)-2-methyloxirane-2-carboxylate (328)**



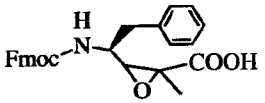
The compound **327** (5.0 g, 15.0 mmol) in CH_2Cl_2 (80 ml) was added 3-chloroperoxy-benzoic acid (5.5 g, 22.3 mmol) and the mixture was stirred overnight, diluted with NaHCO3 (25 ml, sat.), separated and extracted the aqueous solution with CH_2Cl_2. The organic layers were combined, dried over Na_2SO_4, filtered, evaporated and purified on SiO2 column (1:4 EtOAc/Hexane) to afford 4.71 g (90% yield) of the title compound. ESI: m/z: [M+Na]+, calcd for $C_{19}H_{27}NNaO_5$, 372.19, Found, 372.20.

Example 66. **3-((S)-1-(tert-butoxycarbonylamino)-2-phenylethyl)-2-methyloxirane-2-carboxylic acid (329)**



To a solution of LiOH (5.0 g, 208.7 mmol) in H_2O (60 mL) was added to a solution of compound (**328**) (4.70 g, 13.45 mmol) in THF (100 mL). After stirred for 1 h, the mixture was concentrated, poured into H_2O (150 mL) and the mixture was adjusted to pH ~ 4 with 4M HCl aq. The mixture was extracted with EtOAc, dried over Na_2SO_4, concentrated and purified by SiO_2 chromatography (MeOH/DCM/HOAc 1:10:0.01) to afford (3.97 g, 92%) of the title compound. ESI: m/z: $[M+Na]^+$, calcd for $C_{17}H_{23}NNaO_5$, 344.16, Found 344.16.

Example 67. **3-((S)-1-(((9H-fluoren-9-yl)methoxy)carbonylamino)-2-phenylethyl)-2-methyloxirane-2-carboxylic acid (331)**



To a solution of compound (**329**) (3.90g, 12.14 mmol) in CH_2Cl_2 (40 ml) at 0°C, was added TFA (10 ml) and the mixture was stirred at 0°C for 30 min, diluted with toluene, evaporated to dryness to form the crude TFA salt of compound **330**. In a solution of Na_2CO_3 (5.0 g, 47.16 mmol) in mixture of H_2O (60 mL) and ethanol (30 ml) were added the crude compound **330** and Fmoc-Cl (3.70 g, 14.30 mmol). After stirred for 6 h, the mixture was concentrated, poured into H_2O (150 mL) and the mixture was adjusted to pH ~ 4 with 4M HCl aq. The mixture was extracted with EtOAc, dried over Na_2SO_4, concentrated and purified by SiO_2 chromatography (MeOH/DCM/HOAc 1:10:0.01) to afford 3.87 g (72% yields in 2 steps) of the title compound. ESI: m/z: $[M+Na]^+$, calcd for $C_{27}H_{25}NNaO_5$, 466.17, Found 466.17.

Example 68. **General Solution Peptide Coupling Procedure**:

The HCl salt of an amine (1 eq) was dissolved in CH_2Cl_2 or DMF (0.2 M) and cooled to 4 °C in an ice bath, followed by the addition of the appropriate Boc-protected amino acid (1.3 eq), EDC (2 eq), or TBTU (2 eq), or PyBrOP (2 eq), HOBt (1.5 eq) and DIPEA (3.5 eq). The reaction was allowed to slowly warm to room temperature and stirred for 15 h, after which it was diluted with EtOAc and washed successively with aqueous solutions of 1M HCl, saturated sodium bicarbonate, water and saturated sodium chloride. The organic layer was dried with Na2SO4, filtered and concentrated under reduced pressure. Purification by column chromatography (0% to 20% MeOH:CH_2Cl_2) yielded Boc protected peptide.

Example 69. **General Boc Deprotection Procedure**:

The Boc protected amino acid was dissolved in 20% TFA in CH_2Cl_2 or 4 M HCl in dioxane and stirred 30 min, or until the reaction was deemed complete by TLC. The solution was

then concentrated under reduced pressure to give the TFA or HCl salt of the peptide. The TFA salt of the peptide was coevaporated with 2% HCl in CH_2Cl_2/Toluene for 3~ 4 times to generate the HCl salt.

Example 70. **General Solid Phase Peptide Synthesis (SPPS) procedure**:

Boc SPPS was used Merrifield resin or modified PAM resin or MBHA resin. Fmoc SPPS was used Wang resin, or 2-chlorotrityl resin, or HMPB, MBHA resin. The pre-treatment of the resin (pre-swell) and the first loading of an amino compound were followed the manufacture's labels or notes. Resin bound Boc protected amino acids were deprotected with 20% TFA in CH_2Cl_2 or 3M HCl in dioxane for 30 minutes and washed with DMF, MeOH, 50% DIPEA (iPr_2Net) in CH_2Cl_2 and CH_2Cl_2. For steps involving the deprotection of multiple free amines, this step was repeated once before acylation to ensure completeness of reaction. Resin bound Fmoc protected amino acids were deprotected with 20% piperidine in DMF for 30 minutes and washed with DMF, MeOH and CH_2Cl_2. For steps involving the deprotection of multiple free amines, this step was repeated once before acylation to ensure completeness of reaction. The free amine beads were then suspended in a solution of the protected amino acid (3 eq per eq of free amine), TBTU or PyBrOP (3 eq per eq of free amine) and DIPEA (5 eq per eq of free amine) and mixed for 4 h, and then washed with DMF, MeOH and CH_2Cl_2. For steps involving the acylation of multiple free amines, the coupling procedure was repeated once before deprotection to ensure completeness of reaction. These steps were repeated until the desired peptide was synthesized.

Example 71. **General Cleavage of Peptides from Wang Resin or 2-Chlorotrityl resin**:

The peptide bound with Wang resin was mixed with 50% TFA in CH_2Cl_2 and tri-isopropylsilane (1~5%) or the peptide bound with 2-Chlorotrityl resin was mixed with 1% TFA in CH_2Cl_2. Mixed for 2 h and then filtered. The resin was washed with CH_2Cl_2 (3x30 ml), Methanol (3x30 ml), which were combined with the filtrate and evaporated to almost dryness. Cold Et_2O was then added to precipitate the desired deprotected peptide.

Example 72. **General Cleavage of Peptides from Merrifield, MBHA or PAM resin:**

The peptide bound with the resins was mixed with HF/Me_2S/anisole (10:1:1) or CH_3SO_3H/Me_2S/ anisole (20:1:1), or HF/anisole/Me2S/p-thiocresol (10:1:1:0.2) for peptide containing Cys. Mixed for 2 h, concentrated under a stream of N2, diluted with TFA and filtered. The resin was washed with CH_2Cl_2 (3x30 ml), Methanol (3x30 ml), which were combined with the filtrate and condensed under reduced pressure to almost dryness. Cold Et_2O was then added to precipitate the desired deprotected peptide.

Example 73. **Chromatographic purification**:

The crude peptide mixture was then purified through SiO_2 column chromatography (10% to 25% MeOH in CH_2Cl_2) or by a reverse phase HPLC eluted with gradient from 100% of water (optionally containing 1% HOAc) to 30% of water (containing 1% HOAc)/70% methanol in 1h, pooled the fraction, evaporated to give the desired protected.

Example 74. **Conjugate Preparation.**

A binding molecule, preferably an antibody can be conjugated to an antimitotic agents of this prevention through amide, or thiol ether or disulfide bond linkage. In a experiment of generating free thiols on an antibody, the antibodies (mAbs) (>5 mg/mL) in PBS containing 50 mM sodium borate, pH 8.0, were treated with dithiothreitol (10 mM final) at 35 °C for 30 min. After gel filtration (G-25, PBS containing 1mM EDTA), thiol determination using Ellman's reagent [5,5'-dithiobis(2-nitrobenzoic acid)] indicated that there were approximately eight thiols per mAb. The free thiols were also introduced through conjugation of Traut's Reagent (2-Iminothiolane) (Jue, R., et al. Biochem. 1978, 17 (25): 5399-5405), or through conjugation of SATP (*N*-succinimidyl-S-acetylthiopropionate) or SAT(PEG)4 linkers at pH 7 ~8, followed by releasing SH group with hydroxylamine treatment (Duncan, R, et al, *Anal. Biochem.* 1983, **132**, 68-73, Fuji, N. et al, *Chem. Pharm. Bull.* 1985, **33**, 362-367). On average, 5 ~ 8 of free thiols were introduced on mAbs.

To the mAbs containing free thiols at 4 °C were added the drug bearing maleimide or bromoacetamide (0.5 M sodium borate buffer pH 9 was required to promote mAb alkylation with bromoacetamide) (1.2~ 1.5 equiv of drug derivatives /SH group ratio) in cold DMA (2 ~20% v/v). After 1~2 h, the reactions were quenched with excess cysteine; the conjugates were concentrated by centrifugal ultrafiltration, gel filtered (G-25, PBS), and sterile filtered. Protein concentration and drug loading were determined by spectral analysis at 280 and 252 nm, respectively. Size-exclusion HPLC was used to determine percent monomer of each conjugate prepared, and RP-HPLC established that there was less than 0.5% unconjugated cysteine-quenched drug. The resulting conjugate was monomeric and contained, on the average, 3.2–4.2 antimitotic agents linked per antibody molecule for these thiol ether linked conjugation.

For the conjugation through DMPS, SMDP, SMPT, SPP, SPDP, SPDB, SMCC, or SM(PEG)n linkers. A solution of mAb (>5 mg/mL) in aqueous buffer (50 mM PBS, 50 mM NaCl, 1 mM EDTA) at pH 6.5~7.5 was incubated for 2 h with a 6- to 10-fold molar excess of a linker. The reaction mixture was purified via a Sephadex™ G25 gel filtration column to remove low molecular weight material. (The concentration of the antibody was determined spectrophotometrically if the linkers contained a pyridylthio. The coefficients for the antibody ε_{280nm} =2067550 M^{-1} cm^{-1}. An aliquot of the modified antibody was treated with an excess (>20 equiv) of dithiothreitol and the release of pyridine-2-thione determined using the known extinction

coefficients of $\varepsilon_{343\,nm} = 8080\ M^{-1}\ cm^{-1}$ and $\varepsilon_{280\,nm} = 5100\ M^{-1}\ cm^{-1}$ for pyridine-2-thione). The modified antibody was treated with 1.2 ~ 1.5 equiv of an antimitotic agent bearing a thiol group. The reaction mixture was incubated for 5~18 h at RT. The reaction mixture was purified via a Sephadex G25 gel filtration column to remove the unconjugated drug and other low molecular weight species. The concentration of the conjugate was determined spectrophotometrically at 280 and 252 nm. The resulting conjugate was monomeric and contained, on the average, 3.2–4.5 antimitotic agent molecules linked per antibody molecule.

Example 75. **In Vitro Cytotoxicity Assays.**

BJAB (Burkitt's lymphoma), BT-474 (breast carcinoma) cells, Namalwa (human Burkitt's lymphoma), Ramos (human Burkitt's lymphoma), COLO 205 (human colon adenocarcinoma), and A375 (human malignant melanoma,) were from ATCC. The breast tumor line KPL-4 was from Dr. J. Kurebayashi (*Kurebayashi, J. et al. Br J Cancer 1999; 79: 707–17).*The cultures were maintained in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum (FBS). All cell lines were cultured in a humidified incubator at 37°C, 6% CO_2. The cytotoxicity study was performed using a clonogenic assay similar to a reference described (Franken, et al, Nature Protocols 1, 2315 - 2319 (2006)). The test cell lines were plated into 6-well culture dishes at a constant number of 5000 cells per well. The cells were incubated with varying concentrations (1 pM to 50 nM) of the test-agent (the antimitotic agents or their conjugates) for 72 h. The medium was then aspirated from the plates and replaced with fresh medium. The cultures were allowed to grow and form colonies for a total of 7 to 10 days after plating. The cultures were then fixed and stained with 0.2% crystal violet in 10% formalin /PBS, and the colonies were counted. Plating efficiency of non-treated cells (medium alone) was determined by dividing the number of colonies counted by the number of cells plated. The surviving fraction of cells exposed to a toxic agent was determined by dividing the number of colonies in wells that were exposed to the agent by the number of colonies in the control wells.

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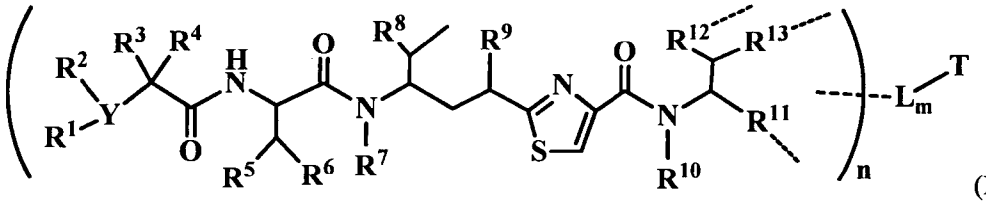
The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A conjugate which is:



(I')

or a pharmaceutically acceptable salt or solvate thereof,

wherein T is a cell-binding agent; L is a releasable linker; ----- is a linkage bond that L connects to an atom in a moiety inside the bracket at either R^{11}, R^{12} or R^{13}; n is 1 to 20 and m is 1 to 10;

wherein the cell-binding agent T is an antibody, a single chain antibody, an antibody fragment that binds to a target cell, a monoclonal antibody, a single chain monoclonal antibody, a monoclonal antibody fragment that binds to the target cell, a chimeric antibody, a chimeric antibody fragment that binds to the target cell, a domain antibody, a domain antibody fragment that binds to the target cell, an adnectin that mimics antibody, DARPins, a lymphokine, a hormone, a vitamin, a growth factor, a colony stimulating factor, a nutrient-transport molecule, a binding peptide, a protein, a small molecule attached to albumin, a polymer, a dendrimer, a liposome, a nanoparticle, a vesicle, or a viral capsid;

wherein the releasable linker L has a formula of: $-W_w-(Aa)_r-V_v-$, wherein: -W- is a stretcher unit, which links the moiety inside the bracket to an amino acid unit (Aa) when present, or V when Aa is not present, and W is independently a self-immolative spacer, a peptidyl unit, a hydrazone, a disulfide, a thioether, an ester, or an amide bond; w is 0 or 1, provided that w and r cannot both be 0 at the same time; wherein Aa is independently a natural or unnatural amino acid unit; r is independently an integer ranging from 0 to 12; $(Aa)_r$ represents a natural or unnatural amino acid, dipeptide, tripeptide, tetrapeptide, pentapeptide, hexapeptide, heptapeptide, octapeptide, nonapeptide, decapeptide, undecapeptide or dodecapeptide unit;

wherein V is a spacer unit and is independently O, NH, S, a C_1 to C_8 alkylene, a C_2 to C_8 heteroalkylene, alkenylene, or alkynylene; a C_3 to C_8 aryl, heterocyclic, carbocyclic, cycloalkyl, alkylcycloalkyl, heterocycloalkyl, heteroaralkyl, heteroalkylcycloalkyl, or alkylcarbonyl; or $(CH_2CH_2O)_r$, wherein r is an integer ranging from 0 to 12; and v is 0, 1 or 2;

wherein R^1, R^2, R^3, and R^4 are independently H, a C_1 to C_8 alkyl; a C_2 to C_8 heteroalkyl or heterocyclic; a C_3 to C_8 aryl, an Ar-alkyl, a cycloalkyl, an alkylcycloalkyl, a heterocycloalkyl, a

heteroalkylcycloalkyl, a carbocyclic, or an alkylcarbonyl; or R^1 and R^2, R^2 and R^3, or R^3 and R^4 form a 3 to 7 membered carbocyclic, cycloalkyl, heterocyclic, or heterocycloalkyl ring system;

wherein Y is N or CH;

wherein R^5, R^6, R^8 and R^{10} are independently H, or a C_1 to C_4 alkyl or heteroalkyl, or R^5 and R^6 form a 3 to 7 membered carbocyclic, cycloalkyl, heterocyclic, or heterocycloalkyl ring system;

wherein R^7 is independently H, R^{14}, $-R^{14}C(=O)X^1R^{15}$, or $-R^{14}X^1R^{15}$; wherein X^1 is O, S, S-S, NH, or NR^{14};

wherein R^9 is independently H, -OH, $-OR^{14}$, $-OC(=O)R^{14}$-, $-OC(=O)NHR^{14}$-, or $-OC(=O)\ R^{14}SSR^{15}$-;

wherein R^{11} is independently H, R^{14}, $-R^{14}C(=O)R^{16}$, $-R^{14}X^2R^{16}$, or $-R^{14}C(=O)NHR^{14}$, wherein X^2 is -O-, -S-, -NH-, $-N(R^{14})$-, $-O-R^{14}$-, $-S-R^{14}$-, or $-S(=O)-R^{14}$-;

wherein R^{12} is independently R^{14}, -OH, -SH, $-NH_2$, =NH, $=NNH_2$, $-NH(R^{14})$, $-OR^{14}$, $-COR^{16}$, $C(O)NH_2$, $C(O)NHR^{14}$, $-SR^{14}$, $-S(=O)R^{14}$, $-P(=O)(OR^{16})_2$, $-OP(=O)(OR^{16})_2$, $-CH_2OP(=O)(OR^{16})_2$, or $-SO_2R^{16}$;

wherein R^{13} is a C_1 to C_{10} alkyl, heteroalkyl, alkyl acid, alkyl amide, alkyl amine, or Ar; wherein Ar is an aromatic or hetero aromatic group, composed of one or several rings, and comprising four to ten carbon atoms; the hetero aromatic group is an aromatic group that has one or several carbon atoms replaced by hetero atoms; Ar is an aromatic group, wherein one or several H atoms are optionally replaced independently by R^{17}, F, Cl, Br, I, OR^{16}, SR^{16}, $NR^{16}R^{17}$, $N=NR^{16}$, $N=R^{16}$, $NR^{16}R^{17}$, NO_2, $SOR^{16}R^{17}$, SO_2R^{16}, SO_3R^{16}, OSO_3R^{16}, $PR^{16}R^{17}$, $POR^{16}R^{17}$, $PO_2R^{16}R^{17}$, $OP(O)(OR^{17})_2$, $OCH_2OP(O)(OR^{17})_2$, $OC(O)OP(O)(OR^{17})_2$, $PO(OR^{16})(OR^{17})$, $OP(O)(OR^{17})OP(O)(OR^{17})_2$, $OC(O)R^{17}$ or $OC(O)NHR^{17}$;

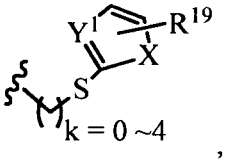
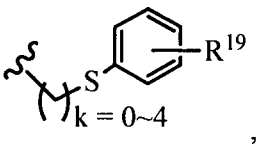
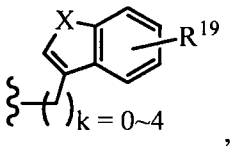
wherein R^{14} and R^{15} are independently a C_1 to C_8 alkyl; a C_2 to C_8 alkenyl, alkynyl or heterocyclic; a C_3 to C_8 aryl, heterocycloalkyl, heteroalkylcycloalkyl, heteroalkyl, carbocyclic, cycloalkyl, heteroaralkyl, or alkylcarbonyl, or one to four amino acid units;

wherein R^{16} and R^{17} are independently H, OH, a C_1 to C_8 alkyl; a C_2 to C_8 alkenyl, alkynyl, or heterocyclic; a C_3 to C_8 aryl, cycloalkyl, alkylcycloalkyl, carbocyclic, heteroalkyl, heterocycloalkyl, heteroalkylcycloalkyl, heteroaralkyl, or alkylcarbonyl; a C_4 to C_{12} glycoside, or a pharmaceutical salt thereof;

or R^{12} and R^{13} form a 3 to 7 membered carbocyclic, cycloalkyl, heterocyclic, or heterocycloalkyl ring system;

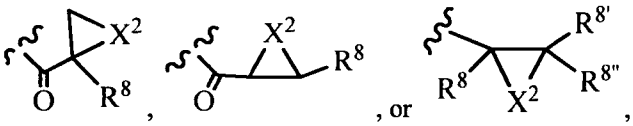
provided that: (i) R^{12} is H when R^{10} is not H,

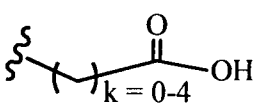
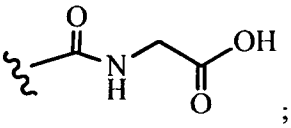
or (ii) either R^{10} or R^{12} is H, or both R^{10} and R^{12} are H, when R^{13} is:




, wherein R^{19} is H, OH, NH_2, $OSO_2(OR^{18})$, $XCH_2OP(O)(OR^{18})_2$, $XPO(OR^{18})_2$, $XC(O)OP(O)(OR^{18})_2$, $XC(O)R^{18}$, $XC(O)NHR^{18}$; a C_1 to C_8 alkyl or carboxylic acid derivative; a C_2 to C_8 alkenyl, alkynyl, heterocyclic, or carboxyalkyl; a C_3 to C_8 aryl, or alkylcarbonyl; or a pharmaceutical salt thereof;

wherein R^{18} is H, a C_1 to C_8 alkyl or heteroalkyl; a C_2 to C_8 carboxyalkyl, alkenyl, alkynyl, or heterocyclic; or a C_3 to C_8 aryl or alkylcarbonyl; X is O, S, or NH; and Y^1 is N or CH;

or (iii) either R^{10} or R^{12} is H, or both R^{10} and R^{12} are H, when R^{11} is:

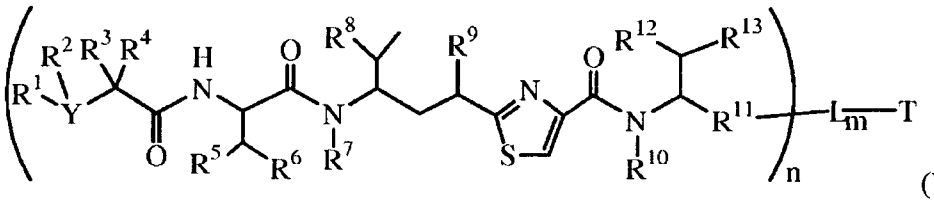


wherein X^2 is O, S, or N-R^8; R^8 is H, or a C_1 to C_6 alkyl or heteroalkyl; $R^{8'}$ and $R^{8''}$ are independently R^8,  or  ;

or (iv) either R^{10} or R^{12} is H, or both R^{10} and R^{12} are H, when R^2 and R^3 do not form a 3 to 7 membered carbocyclic, cycloalkyl, heterocyclic, or heterocycloalkyl ring system.

2. The conjugate according to claim 1 having the structure of:

A. Formula (V):


(V)

or a pharmaceutical acceptable salt or solvate thereof,

wherein T is the targeting or cell-binding agent; L is the releasable linker that connects to a molecule inside the bracket independently; n is 1 to 20 and m is 1 to 10;

the portion inside the round bracket is a potent antimitotic agent or drug wherein R^1, R^2, R^3, and R^4 are independently a C_1 to C_8 alkyl or heteroalkyl; a C_2 to C_8 heterocyclic, carbocyclic, alkylcycloalkyl, or heterocycloalkyl; a C_3 to C_8 aryl, Ar-alkyl, heteroalkylcycloalkyl, or alkylcarbonyl; or two R's can be 3 to 7 members of a carbocyclic, cycloalkyl, heterocyclic, or heterocycloalkyl ring system; and Y is N or CH; or R^1, R^3, and R^4

can be H; and R^2 can be absent; wherein Ar refers to an aromatic or hetero aromatic group, composed of one or several rings, comprising three to fourteen carbon atoms;

R^5, R^6, R^8 and R^{10} are independently H, or a C_1 to C_4 alkyl or heteroalkyl;

R^7 is independently H, R^{14}, $-R^{14}C(=O)X^1R^{15}$- or $-R^{14}X^1R^{15}$-, wherein R^{14} and R^{15} are independently a C_1 to C_8 alkyl or heteroalkyl; a C_2 to C_8 alkenyl, alkynyl; heterocyclic, carbocyclic, or cycloalkyl; or a C_3 to C_8 aryl, heterocycloalkyl, heteroaralkyl, heteroalkylcycloalkyl, or alkylcarbonyl; and X^1 is O, S, S-S, NH, or NR^{14};

R^9 is independently H, -OH, $-OR^{14}$, $-OC(=O)R^{14}$, or $-OC(=O)NHR^{14}$, $-OC(=O)R^{14}SSR^{15}$, $OP(=O)(OR^{14})$, or $OR^{14}OP(=O)(OR^{15})$, wherein R^{14} and R^{15} are independently a C_1 to C_8 alkyl or heteroalkyl; a C_2 to C_8 alkenyl, alkynyl, heterocyclic, or carbocyclic; or a C_3 to C_8 aryl, cycloalkyl, alkylcycloalkyl, heterocycloalkyl, heteroalkylcycloalkyl, heteroaralkyl, or alkylcarbonyl;

R^{11} is independently $-R^{14}$-, $-R^{14}C(=O)R^{17}$-, $-R^{14}X^2R^{17}$-, or $-R^{14}C(=O)X^2$-, wherein R^{17} is independently H, OH, a C_1 to C_8 alkyl; a C_2 to C_8 alkenyl, alkynyl, or heteroalkyl; a C_3 to C_8 aryl, arylene, heterocyclic, carbocyclic, or heterocycloalkyl; an amino acid, or two amino acid units; and X^2 is -O-, -S-, -NH-, $-N(R^{14})$-, $-O-R^{14}$-, $-S-R^{14}$-, $-S(=O)-R^{14}$-, or $-NHR^{14}$-; wherein R^{14} is a C_1 to C_8 alkyl or heteroalkyl; a C_2 to C_8 alkenyl or alkynyl; a C_3 to C_8 aryl, heterocyclic, carbocyclic, cycloalkyl, alkylcycloalkyl, heterocycloalkyl, heteroalkylcycloalkyl, heteroaralkyl, or alkylcarbonyl;

R^{12} is independently R^{14}, -OH, -SH, $-NH_2$, =NH, $=NNH_2$, $-NH(R^{14})$, $-OR^{14}$, COR^{16}, $COOR^{14}$, $C(O)NH_2$, $C(O)NHR^{14}$, $-SR^{14}$, $-S(=O)R^{14}$, $-P(=O)(OR^{16})_2$, $-OP(=O)(OR^{16})_2$, $-CH_2OP(=O)(OR^{16})_2$, or $-SO_2R^{16}$; wherein R^{14} is independently a C_1 to C_8 alkyl or heteroalkyl; a C_2 to C_8 alkenyl, alkynyl, hetero-cyclic, or carbocyclic; or a C_3 to C_8 aryl, cycloalkyl, alkylcycloalkyl, heterocycloalkyl, heteroaralkyl, heteroalkylcycloalkyl, or alkylcarbonyl; and R^{16} is H, OH, R^{14}, or 1 to 4 amino acid units;

R^{13} is a C_1 to C_{10} alkyl, heteroalkyl, alkyl acid, alkyl amide, or alkyl amine; or Ar; wherein Ar refers to an aromatic or hetero aromatic group, composed of one or several rings, comprising four to ten carbon atoms;

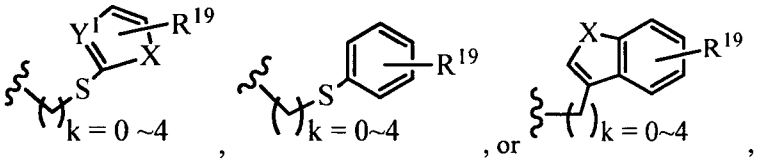
wherein R^{14} and R^{15} are independently a C_1 to C_8 alkyl; a C_2 to C_8 alkenyl, alkynyl, or heterocyclic; a C_3 to C_8 aryl, heterocycloalkyl, heteroalkylcycloalkyl, heteroalkyl, carbocyclic, cycloalkyl, heteroaralkyl, or alkylcarbonyl, or one to four amino acid units;

wherein R^{16} and R^{17} are independently H, OH, a C_1 to C_8 alkyl; a C_2 to C_8 alkenyl, alkynyl, or heterocyclic; a C_3 to C_8 aryl, cycloalkyl, alkylcycloalkyl, carbocyclic, heteroalkyl, heterocycloalkyl, heteroalkylcycloalkyl, heteroaralkyl, or alkylcarbonyl; a C_4 to C_{12} glycoside; or a pharmaceutical salt thereof;

or R^{12} and R^{13} form a 3 to 7 membered carbocyclic, cycloalkyl, heterocyclic, or heterocycloalkyl ring system;

provided that: (i) R^{12} is H when R^{10} is not H,

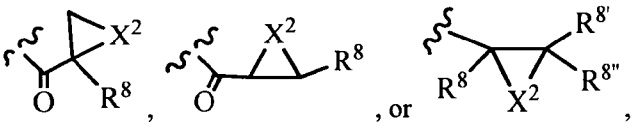
or (ii) either R^{10} or R^{12} is H, or both R^{10} and R^{12} are H, when R^{13} is:



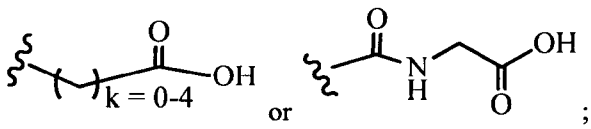
wherein R^{19} is H, OH, NH_2, $OSO_2(OR^{18})$, $XCH_2OP(O)(OR^{18})_2$, $XPO(OR^{18})_2$, $XC(O)OP(O)(OR^{18})_2$, $XC(O)R^{18}$, $XC(O)NHR^{18}$, a C_1 to C_8 alkyl or carboxylic acid derivative; a C_2 to C_8 alkenyl, alkynyl, heterocyclic, or carboxyalkyl; a C_3 to C_8 aryl or alkylcarbonyl; or a pharmaceutical salt thereof;

wherein R^{18} is H, a C_1 to C_8 alkyl or heteroalkyl; a C_2 to C_8 carboxyalkyl, alkenyl, alkynyl, or heterocyclic; or a C_3 to C_8 aryl or alkylcarbonyl; X is O, S, or NH; and Y^1 is N or CH;

or (iii) either R^{10} or R^{12} is H, or both R^{10} and R^{12} are H, when R^{11} is:

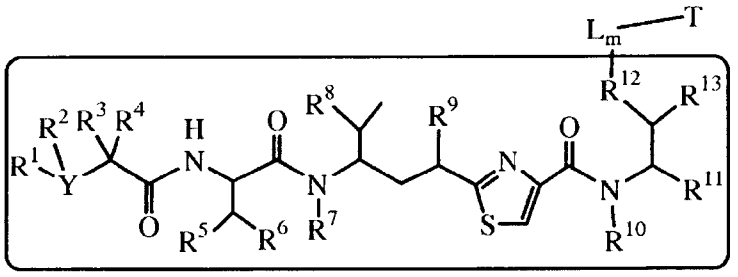


wherein X^2 is O, S, or N-R^8; R^8 is H, a C_1 to C_6 alkyl or heteroalkyl; $R^{8'}$ and $R^{8''}$ are independently R^8,



or (iv) either R^{10} or R^{12} is H, or both R^{10} and R^{12} are H, when R^2 and R^3 do not form a 3 to 7 membered carbocyclic, cycloalkyl, heterocyclic, or heterocycloalkyl ring system;

B. Formula (VI):



(VI)

or a pharmaceutical acceptable salt or solvate thereof,

wherein T is the targeting or cell-bindingagent; L is the releasable linker that connects to a molecule inside the rectangular box independently; n is 1 to 20 and m is 1 to 10;

the portion inside the round bracket is a potent antimitotic agent or drug wherein R^1, R^2, R^3, and R^4 are independently a C_1 to C_8 alkyl or heteroalkyl; a C_2 to C_8 heterocyclic, carbocyclic, alkylcycloalkyl, or heterocycloalkyl; a C_3 to C_8 aryl, Ar-alkyl, heteroalkylcycloalkyl, or alkylcarbonyl; two R's can be 3 to 7 members of a carbocyclic, cycloalkyl, or heterocyclic, heterocycloalkyl ring system; and Y is N or CH; or R^1, R^3, and R^4 can be H; and R^2 can be absent; wherein Ar refers to an aromatic or hetero aromatic group, composed of one or several rings, comprising three to fourteen carbon atoms;

R^5, R^6, R^8 and R^{10} are independently H, or a C_1 to C_4 alkyl or heteroalkyl;

R^7 is independently H, R^{14}, $-R^{14}C(=O)X^1R^{15}$ or $-R^{14}X^1R^{15}$, wherein R^{14} and R^{15} are independently a C_1 to C_8 alkyl or heteroalkyl; a C_2 to C_8 alkenyl, alkynyl; heterocyclic, carbocyclic, or cycloalkyl; or a C_3 to C_8 aryl, heterocycloalkyl, heteroaralkyl, heteroalkylcycloalkyl, or alkylcarbonyl; and X^1 is O, S, S-S, NH, or NR^{14};

R^9 is independently H, -OH, $-OR^{14}$, $-OC(=O)R^{14}$, $-OC(=O)NHR^{14}$, or $-OC(=O)R^{14}SSR^{15}$, wherein R^{14} and R^{15} are independently a C_1 to C_8 alkyl or heteroalkyl; a C_2 to C_8 alkenyl, alkynyl, heterocyclic, or carbocyclic; or a C_3 to C_8 aryl, cycloalkyl, alkylcycloalkyl, heterocycloalkyl, heteroalkylcycloalkyl, heteroaralkyl, or alkylcarbonyl;

R^{11} is independently H, R^{14}, $-R^{14}C(=O)R^{16}$, $-R^{14}X^2R^{16}$, or $-R^{14}C(=O)X^2-$, wherein X^2 is -O-,
-S-, -NH-, $-N(R^{14})-$, $-O-R^{14}-$, $-S-R^{14}-$, $-S(=O)-R^{14}-$, or $-NHR^{14}-$; R^{14} is a C_1 to C_8 alkyl or heteroalkyl; a C_2 to C_8 alkenyl, alkynyl, heterocyclic, or carbocyclic; or a C_3 to C_8 aryl, cycloalkyl, alkylcycloalkyl, heterocycloalkyl, heteroalkylcycloalkyl, heteroaralkyl, or alkylcarbonyl; and R^{16} is H, OH, R^{14} or one to four amino acid units;

R^{12} is independently $-R^{14}-$, -O-, -S-, -NH-, =N-, =NNH-, $-N(R^{14})-$, $-OR^{14}-$, -C(O)O-, -C(O)NH-, $-C(O)NR^{14}-$, $-SR^{14}-$, $-S(=O)R^{14}-$, $-NHR^{14}-$, $-CH_2OP(=O)(OR^{15})-$, $-P(=O)(OR^{15})-$, $-C(O)OP(=O)(OR^{15})-$, $-OP(=O)(OR^{15})O-$, or $-SO_2R^{14}-$; wherein R^{14} and R^{15} are independently a C_1 to C_8 alkyl or heteroalkyl; a C_2 to C_8 alkenyl or alkynyl; or a C_3 to C_8 aryl, heterocyclic, carbocyclic, cycloalkyl, alkylcycloalkyl, heterocycloalkyl, heteroaralkyl, heteroalkylcycloalkyl, or alkylcarbonyl;

or R^{12} is independently $-R^{14}-$, $-NH(R^{14})-$, $-OR^{14}-$, $-COR^{16}-$, $-COOR^{14}-$, $-C(O)NR^{14}-$, $-SR^{14}-$, $-S(=O)R^{14}-$, $-P(=O)(OR^{16})-$, $-OP(=O)(OR^{16})-$, $-OP(=O)(OR^{16})O-$, $-CH_2OP(=O)(OR^{16})-$, $-CH_2OP(=O)(OR^{16})O-$, or $-SO_2R^{16}-$; wherein R^{14} is independently a C_1 to C_8 alkyl or heteroalkyl; a C_2 to C_8 alkenyl, alkynyl, or hetero-cyclic, or carbocyclic; or a C_3 to C_8 aryl, cycloalkyl,

alkylcycloalkyl, heterocycloalkyl, heteroaralkyl, heteroalkylcycloalkyl, or alkylcarbonyl; and R^{16} is H, OH, R^{14} or one to four amino acid units;

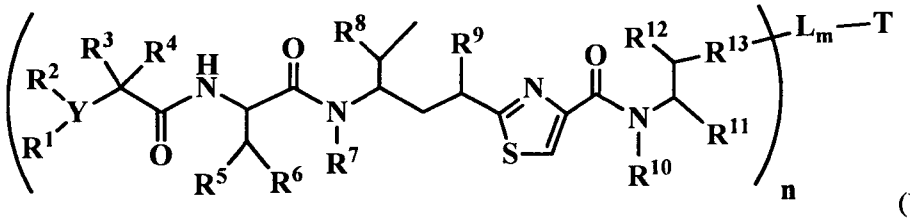
R^{13} is a C_1 to C_{10} alkyl, heteroalkyl, alkyl acid, alkyl amide, or alkyl amine; or Ar; wherein Ar refers to an aromatic or hetero aromatic group, composed of one or several rings, comprising four to ten carbon atoms;

wherein R^{14} and R^{15} are independently a C_1 to C_8 alkyl; a C_2 to C_8 alkenyl, alkynyl, or heterocyclic; a C_3 to C_8 aryl, heterocycloalkyl, heteroalkylcycloalkyl, heteroalkyl, carbocyclic, cycloalkyl, heteroaralkyl, or alkylcarbonyl, or one to four amino acid units;

wherein R^{16} and R^{17} are independently H, OH, a C_1 to C_8 alkyl; a C_2 to C_8 alkenyl, alkynyl, or heterocyclic; a C_3 to C_8 aryl, cycloalkyl, alkylcycloalkyl, carbocyclic, heteroalkyl, heterocycloalkyl, heteroalkylcycloalkyl, heteroaralkyl, or alkylcarbonyl; a C_4 to C_{12} glycoside; or a pharmaceutical salt thereof;

or R^{12} and R^{13} form a 3 to 7 membered carbocyclic, cycloalkyl, heterocyclic, or heterocycloalkyl ring system; or

C. Formula (VII)



(VII)

or a pharmaceutical acceptable salt or solvate thereof,

wherein T is the targeting or cell-bindingagent; L is the releasable linker that connects to a molecule inside the bracket independently; n is 1 to 20 and m is 1 to 10;

the portion inside the round bracket is a potent antimitotic agent or drug wherein R^1, R^2, R^3, and R^4 are independently a C_1 to C_8 alkyl or heteroalkyl; a C_2 to C_8 heterocyclic, carbocyclic, alkylcycloalkyl, or heterocycloalkyl; or a C_3 to C_8 aryl, Ar-alkyl, heteroalkylcycloalkyl, or alkylcarbonyl; or two R's can be 3 to 7 members of a carbocyclic, cycloalkyl, heterocyclic, or heterocycloalkyl ring system; and Y is N or CH; or R^1, R^3, and R^4 can be H; and R^2 can be absent; wherein Ar refers to an aromatic or hetero aromatic group, composed of one or several rings, comprising three to fourteen carbon atoms;

R^5, R^6, R^8 and R^{10} are independently H, a C_1 to C_4 alkyl or heteroalkyl;

R^7 is independently H, R^{14}, $-R^{14}C(=O)X^1R^{15}$- or $-R^{14}X^1R^{15}$, wherein R^{14} and R^{15} are independently a C_1 to C_8 alkyl or heteroalkyl; a C_2 to C_8 alkenyl, alkynyl; heterocyclic,

carbocyclic, or cycloalkyl; or a C_3 to C_8 aryl, heterocycloalkyl, heteroaralkyl, heteroalkylcycloalkyl, or alkylcarbonyl; and X^1 is O, S, S-S, NH, or NR^{14};

R^9 is independently H, -OH, $-OR^{14}$, $-OC(=O)R^{14}$, $-OC(=O)NHR^{14}$, $-OC(=O)R^{14}SSR^{15}$, $OP(=O)(OR^{14})$, or $OR^{14}OP(=O)(OR^{15})$, wherein R^{14} and R^{15} are independently a C_1 to C_8 alkyl or heteroalkyl; a C_2 to C_8 alkenyl, alkynyl, heterocyclic, or carbocyclic; or a C_3 to C_8 aryl, cycloalkyl, alkylcycloalkyl, heterocycloalkyl, heteroalkylcycloalkyl, heteroaralkyl, or alkylcarbonyl;

R^{11} is independently H, R^{14}, $-R^{14}C(=O)R^{16}$, $-R^{14}X^2R^{16}$, or $-R^{14}C(=O)X^2$, wherein X^2 is -O-, -S-, -NH-, $-N(R^{14})-$, $-O-R^{14}-$, $-S-R^{14}-$, $-S(=O)-R^{14}-$, or $-NHR^{14}-$; wherein R^{14} is a C_1 to C_8 alkyl or heteroalkyl; a C_2 to C_8 alkenyl, alkynyl, heterocyclic, or carbocyclic; or a C_3 to C_8 aryl, cycloalkyl, alkylcycloalkyl, heterocycloalkyl, heteroalkylcycloalkyl, heteroaralkyl, or alkylcarbonyl; and R^{16} is H, OH, R^{14} or one to four amino acid units;

R^{12} is independently H, R^{14}, -OH, -SH, $-NH_2$, =NH, $=NNH_2$, $-NH(R^{14})$, $-OR^{14}$, COR^{16}, $COOR^{14}$, $C(O)NH_2$, $C(O)NHR^{14}$, $-SR^{14}$, $-S(=O)R^{14}$, $-P(=O)(OR^{16})_2$, $-OP(=O)(OR^{16})_2$, $-CH_2OP(=O)(OR^{16})_2$, $-C(O)OP(=O)(OR^{16})$, or $-SO_2R^{16}$; wherein R^{14} is independently a C_1 to C_8 alkyl or heteroalkyl; a C_1 to C_8 alkenyl, alkynyl, heterocyclic, or carbocyclic; or a C_3 to C_8 aryl, cycloalkyl, alkylcycloalkyl, heterocycloalkyl, heteroaralkyl, heteroalkylcycloalkyl, or alkylcarbonyl; and R^{16} is H, OH, R^{14} or one to four amino acid units; and

R^{13} is a C_1 to C_{10} alkyl, heteroalkyl, alkyl acid, alkyl amide, or alkyl amine; or Ar; wherein Ar refers to an aromatic or hetero aromatic group, composed of one or several rings, comprising four to ten carbon atoms;

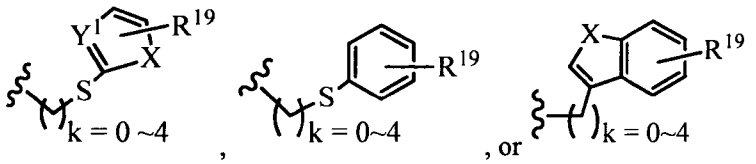
wherein R^{14} and R^{15} are independently a C_1 to C_8 alkyl; a C_2 to C_8 alkenyl, alkynyl, or heterocyclic; a C_3 to C_8 aryl, heterocycloalkyl, heteroalkylcycloalkyl, heteroalkyl, carbocyclic, cycloalkyl, heteroaralkyl, or alkylcarbonyl; or one to four amino acid units;

wherein R^{16} and R^{17} are independently H, OH, a C_1 to C_8 alkyl; a C_2 to C_8 alkenyl, alkynyl, or heterocyclic; a C_3 to C_8 aryl, cycloalkyl, alkylcycloalkyl, carbocyclic, heteroalkyl, heterocycloalkyl, heteroalkylcycloalkyl, heteroaralkyl, or alkylcarbonyl; a C_4 to C_{12} glycoside, or a pharmaceutical salt thereof;

or R^{12} and R^{13} form a 3 to 7 membered carbocyclic, cycloalkyl, heterocyclic, or heterocycloalkyl ring system;

provided that: (i) R^{12} is H when R^{10} is not H,

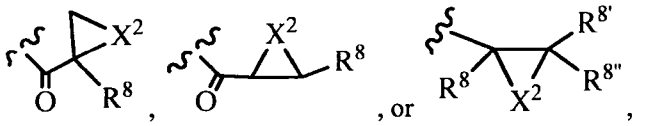
or (ii) either R^{10} or R^{12} is H, or both R^{10} and R^{12} are H, when R^{13} is:

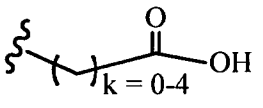
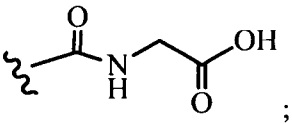


wherein R^{19} is H, OH, NH_2, $OSO_2(OR^{18})$, $XCH_2OP(O)(OR^{18})_2$, $XPO(OR^{18})_2$, $XC(O)OP(O)(OR^{18})_2$, $XC(O)R^{18}$, $XC(O)NHR^{18}$, a C_1 to C_8 alkyl, carboxylic acid derivative; a C_2 to C_8 alkenyl, alkynyl, heterocyclic, or carboxyalkyl; a C_3 to C_8 aryl or alkylcarbonyl; or a pharmaceutical salt thereof;

wherein R^{18} is H, a C_1 to C_8 alkyl or heteroalkyl; a C_2 to C_8 carboxyalkyl, alkenyl, alkynyl, or heterocyclic; or a C_3 to C_8 aryl or alkylcarbonyl; X is O, S, or NH; and Y^1 is N or CH;

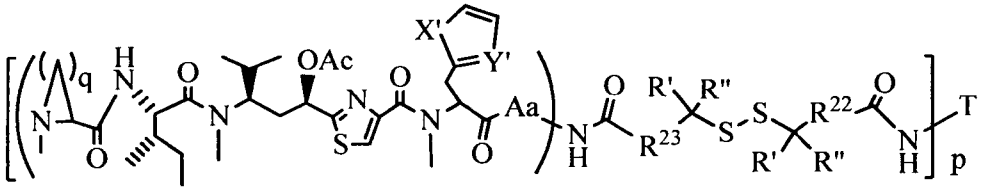
or (iii) either R^{10} or R^{12} is H, or both R^{10} and R^{12} are H, when R^{11} is:



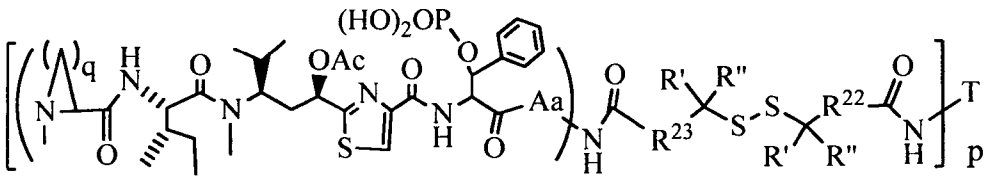
wherein X^2 is O, S, or N-R^8; R^8 is H, or a C_1 to C_5 alkyl or heteroalkyl; $R^{8'}$ and $R^{8''}$ are independently R^8,  or  ;

or (iv) either R^{10} or R^{12} is H, or both R^{10} and R^{12} are H, when R^2 and R^3 do not form a 3 to 7 membered carbocyclic, cycloalkyl, heterocyclic, or heterocycloalkyl ring system.

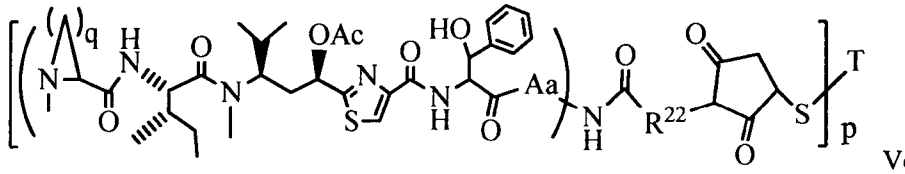
3. The conjugate according to claim 1, having a structure of Va, Vb, Vc, Vd, Ve, Vf, Vg, Vh,VIa, VIb, VIc, VIe, VIf, VIg, VIh, VIi, VIIa, VIIb, VIIc, VIId, VIIe, VIIf, VIIg, VIIh, VIIi, VIIj,VIIk, VIIl, VIIm, VIIn, VIIo, VIIp, VIIr, VIIs, or VIIt as shown below:



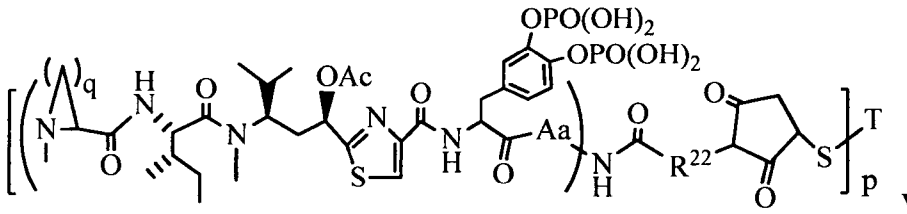
Va;



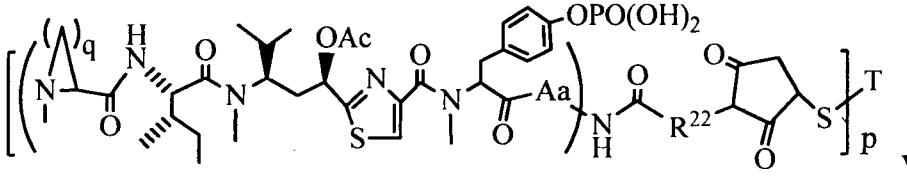
Vb;



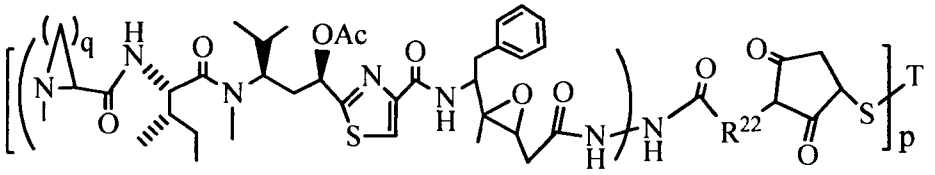
Vc;



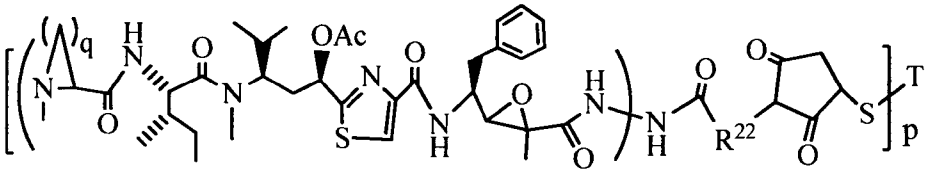
Vd;



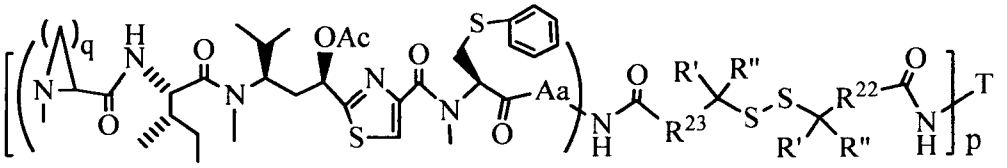
Ve;



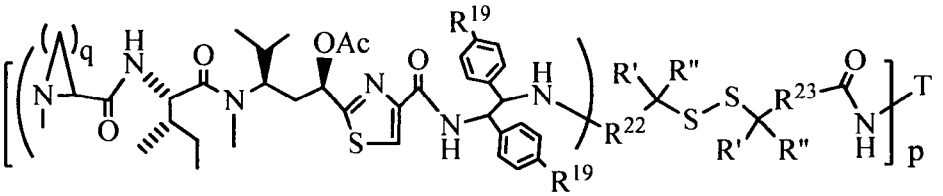
Vf;



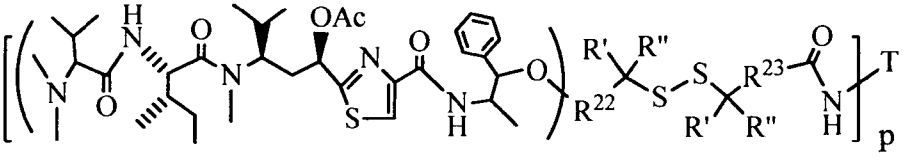
Vg;



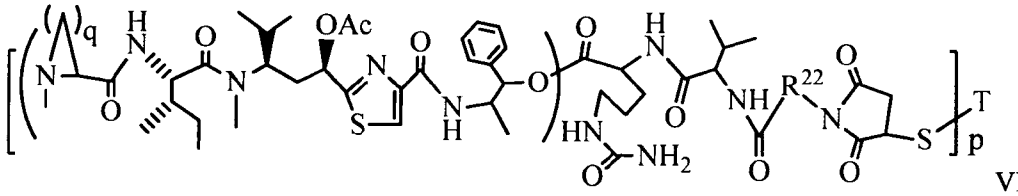
Vh;



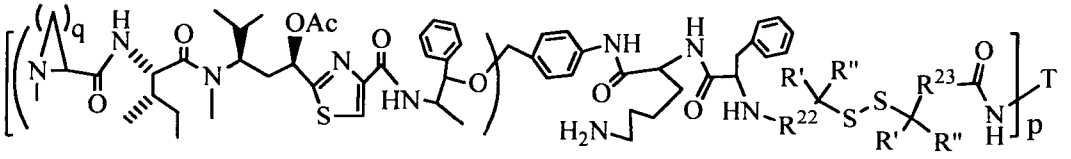
VIa;



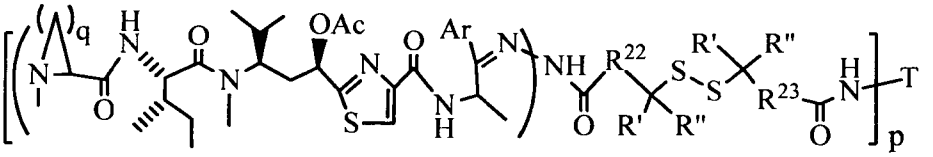
VIb;



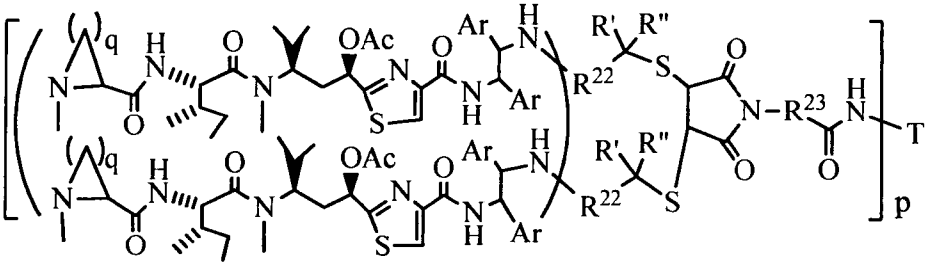
VIc;



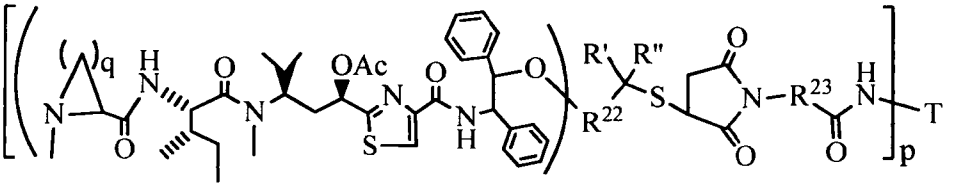
VId;



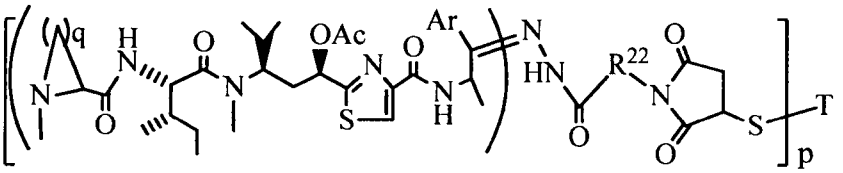
VIf;



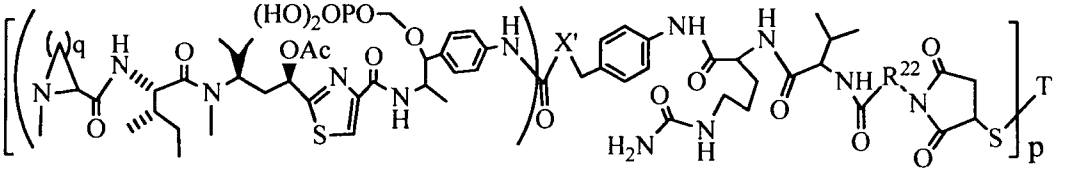
VIg;



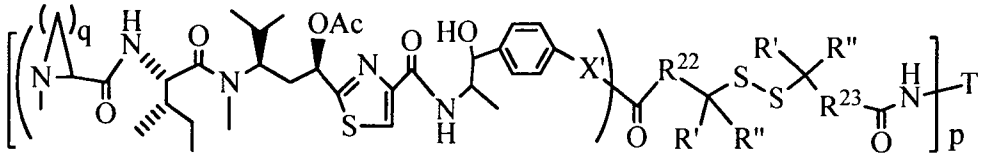
VIh;



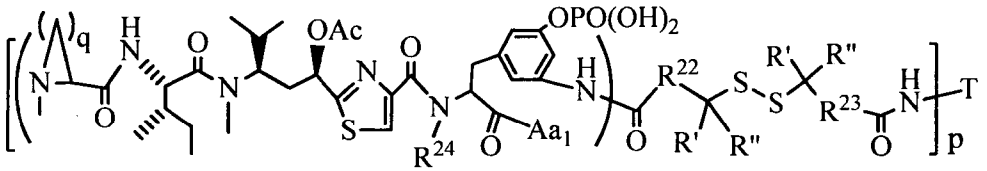
VIi;



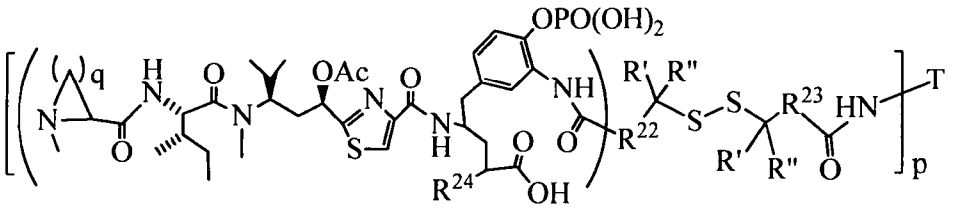
VIIa;



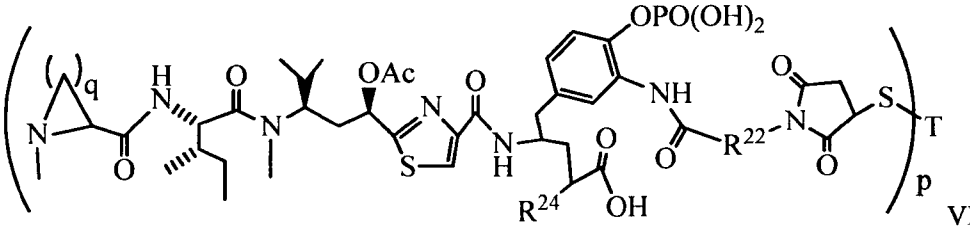
VIIb;



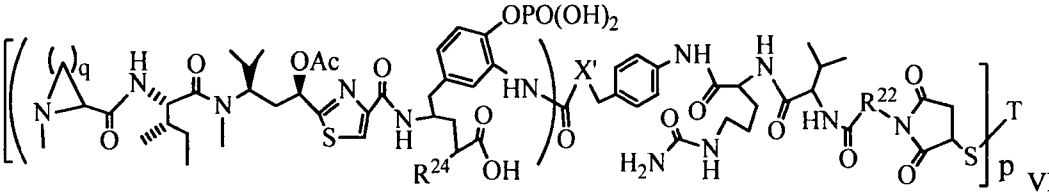
VIIc;



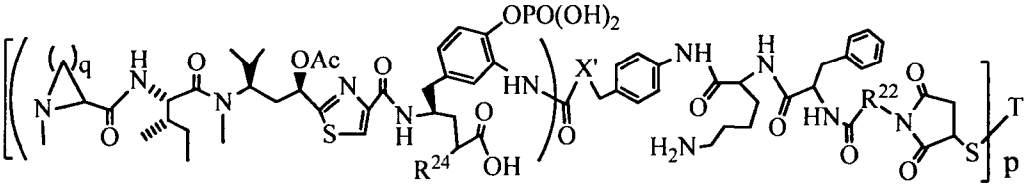
VIId;



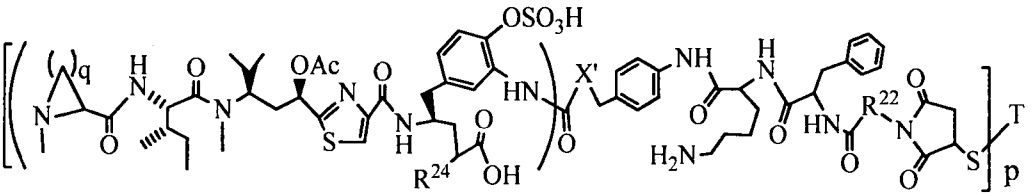
VIIe;



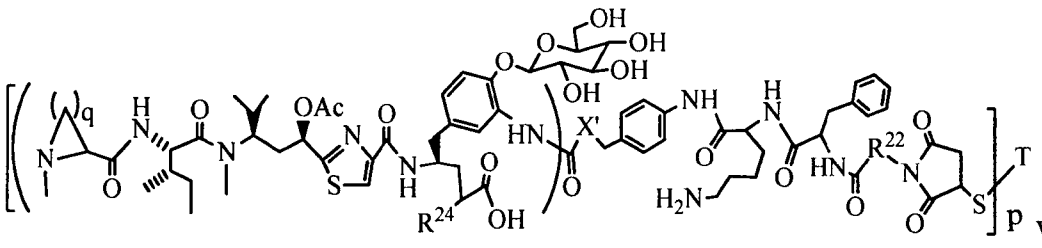
VIIf;



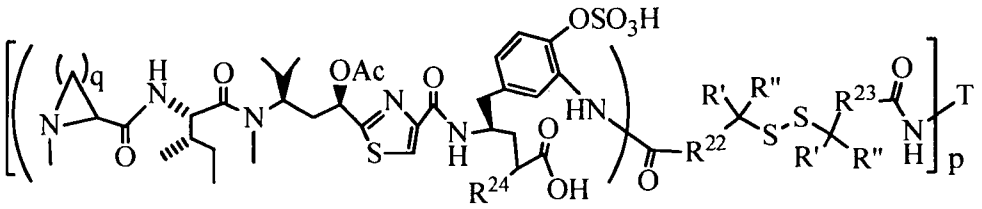
VIIg;



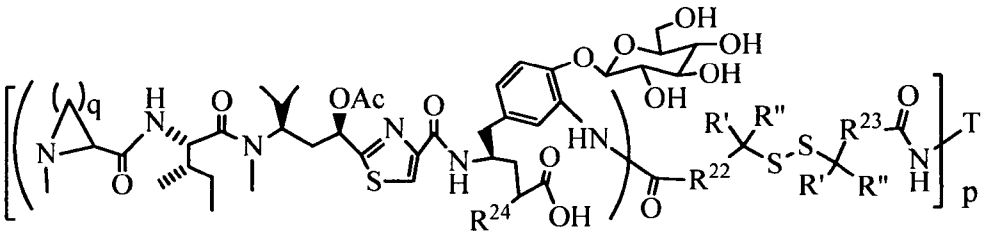
VIIh;



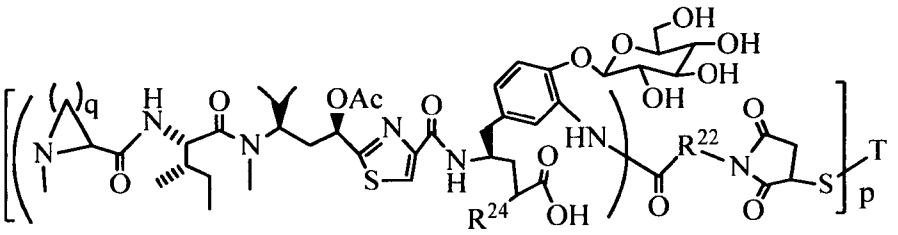
VIIi;



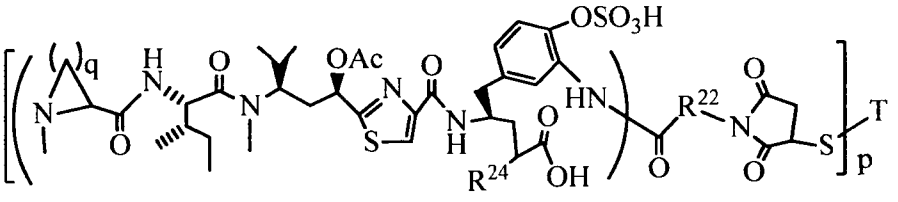
VIIj;



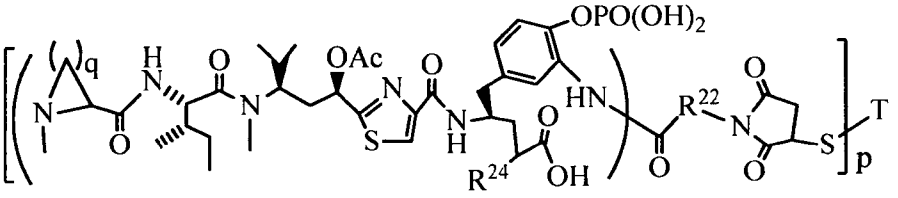
VIIk;



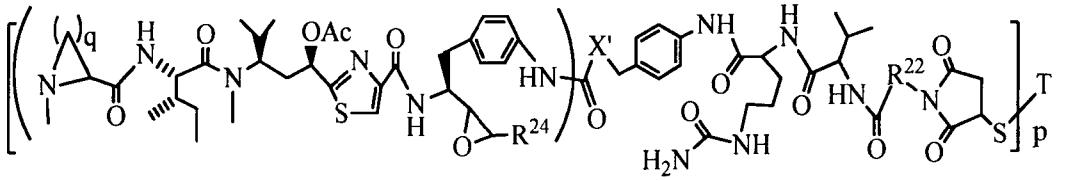
VIII;



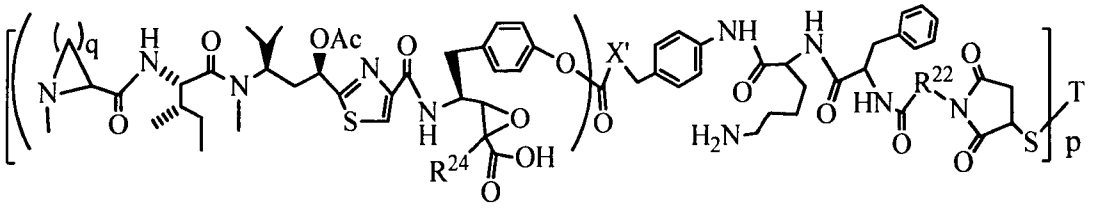
VIIm;



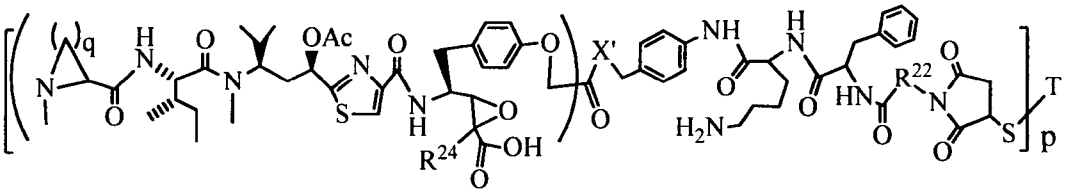
VIIn;



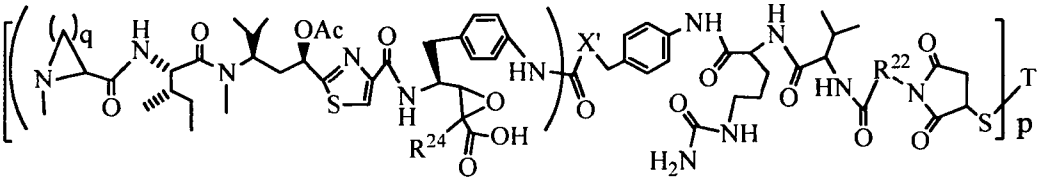
VIIo;



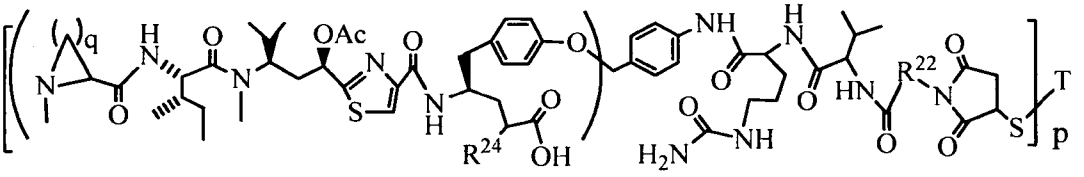
VIIp;



VIIr;



VIIs; or



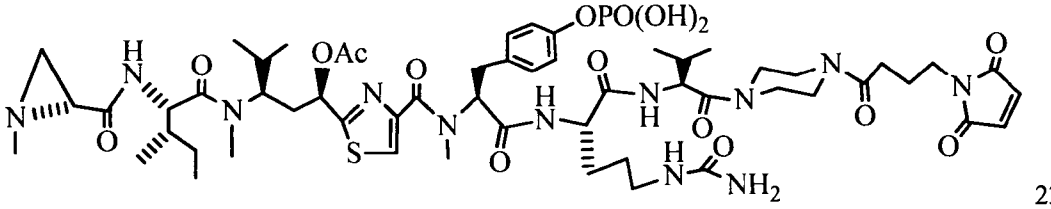
VIIt;

wherein Aa is a natural or an unnatural amino acid; p is 1 to 20; q is 1 to 5; X' and Y' are independently CH, O, S, NH, or NR^{24}; R^{22} and $R^{23}$are independently a C_1 to C_8 alkylene; a C_2 to C_8 alkenylene, alkynylene, or heteroalkylene; a C_3 to C_8 aryl, heterocyclic, carbocyclic, cycloalkyl, alkylcycloalkyl, heterocycloalkyl, Ar-alkyl, heteroalkylcycloalkyl, or heteroaralkyl; or -$(OCH_2CH_2)_s$-;

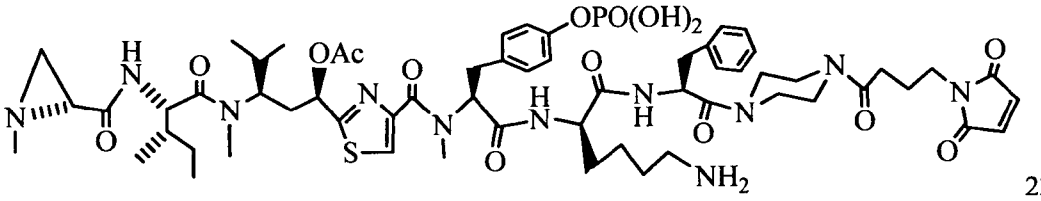
R^{24} is H or CH_3;

s is 1 to 20; R' and R'' are independently H or CH_3; and inside the round brackets is an antimitotic drug and inside the square brackets is an antimitotic drug conjugated to the cell binding agent T via the linker.

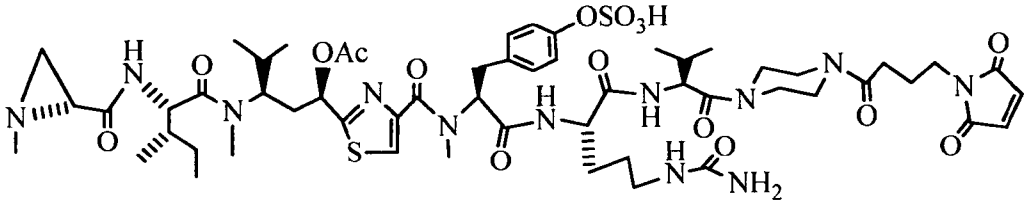
4. The conjugate according to claim 2, wherein the antimitotic agent has a structure of 232a, 232b, 232c, 232d, 232e, 232f, 232g, 232h, 232i, 232j, 232k, 232l, 232m, 232n, 232o, 232p, 232q, 232r, 232s, 232t, 221a, 221b, 233a, 233b, 233c, 233d, 222a, 241, 242, 247, 248, 206, 211, 346, 350, 354, 357, 361, 366, 371, or 376 as shown below:



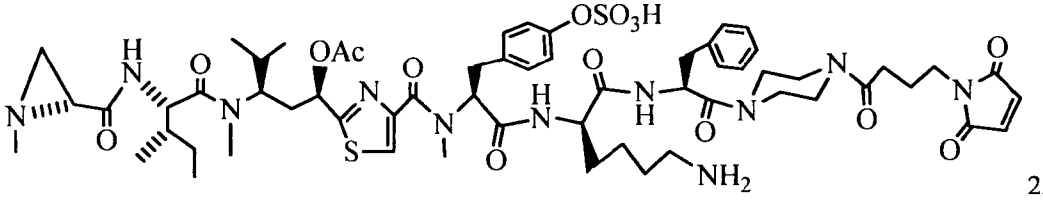
232a;



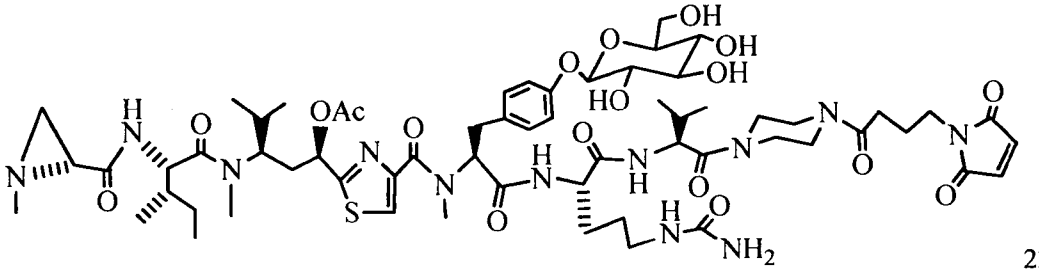
232b;



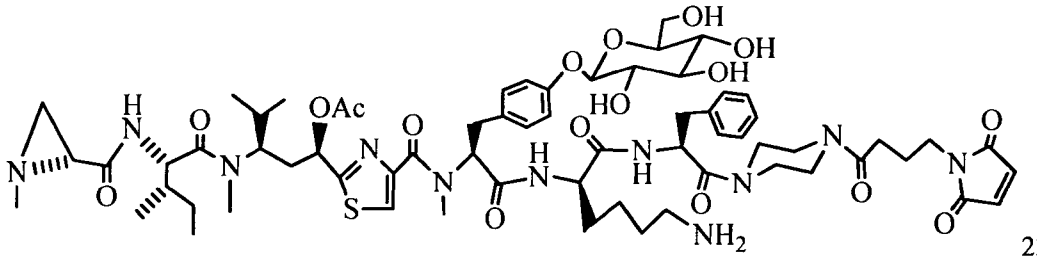
232c;



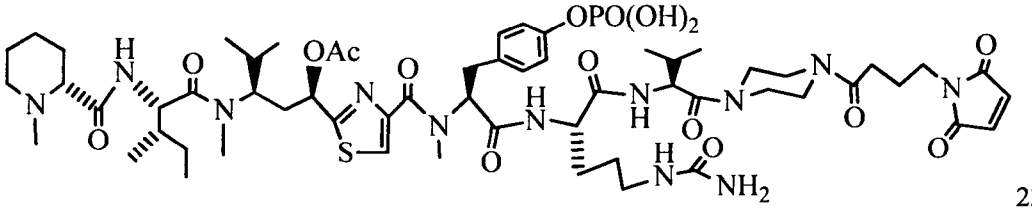
232d;



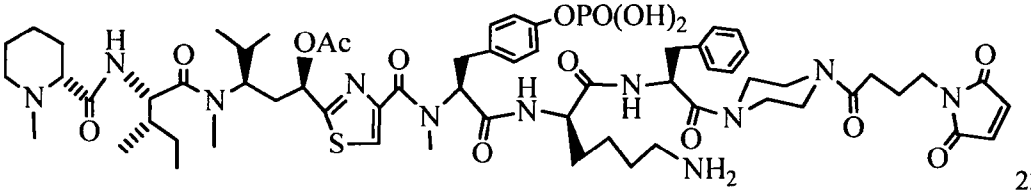
232e;



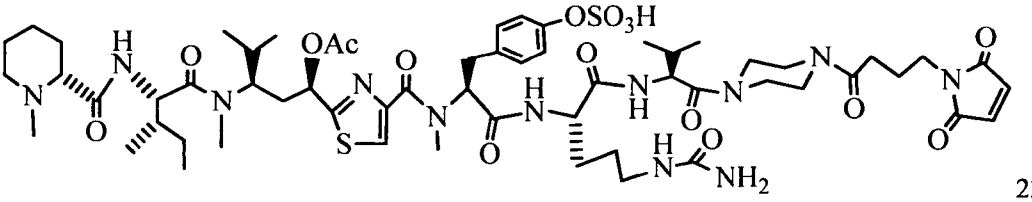
232f;



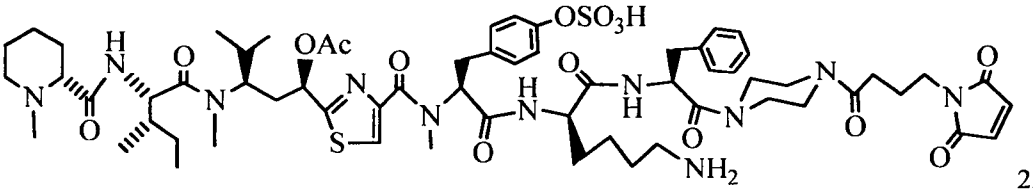
232g;



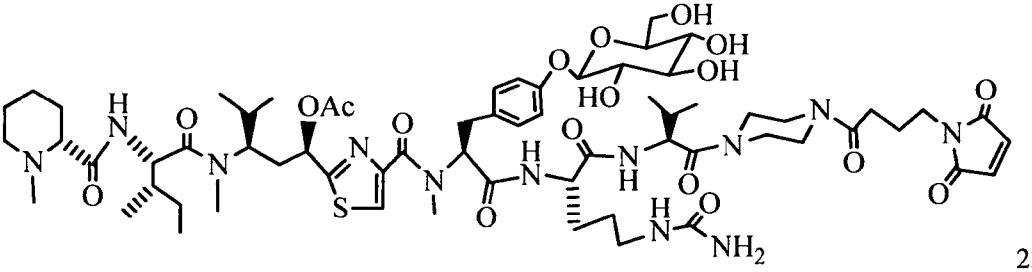
232h;



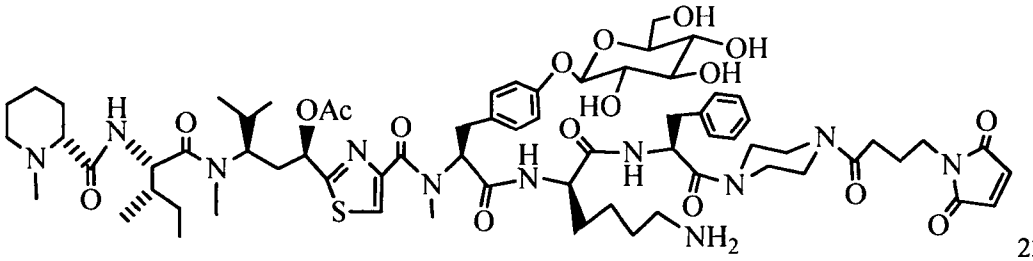
232i;



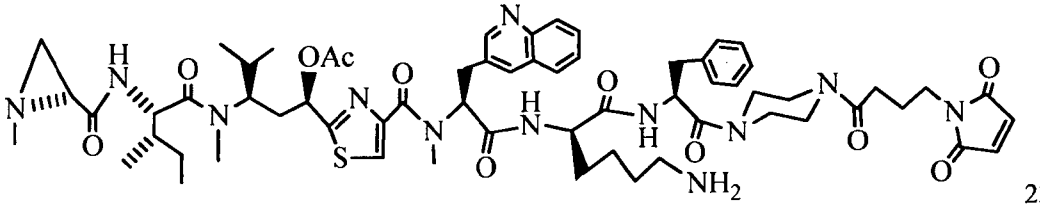
232j;



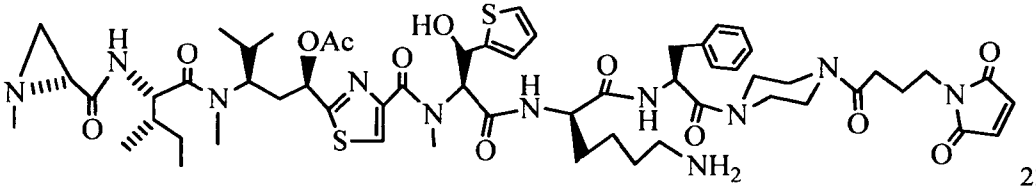
232k;



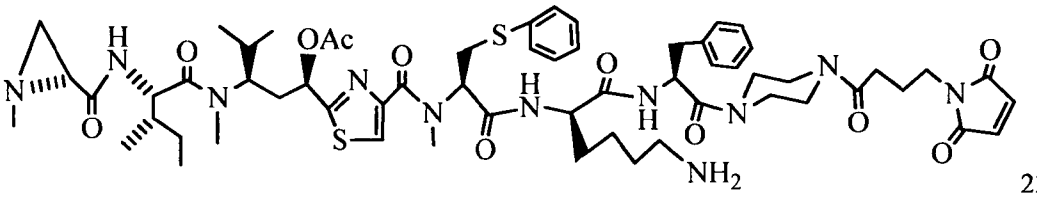
232l;



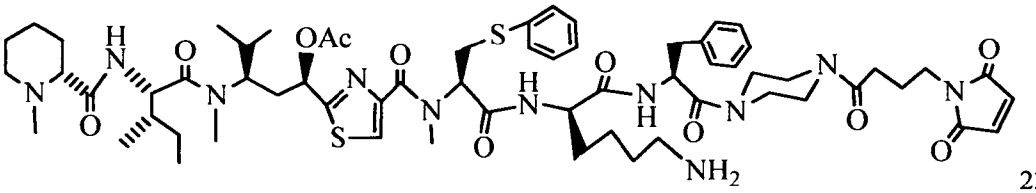
232m;



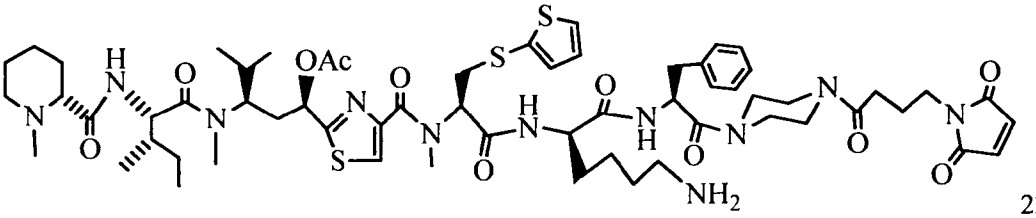
232n;



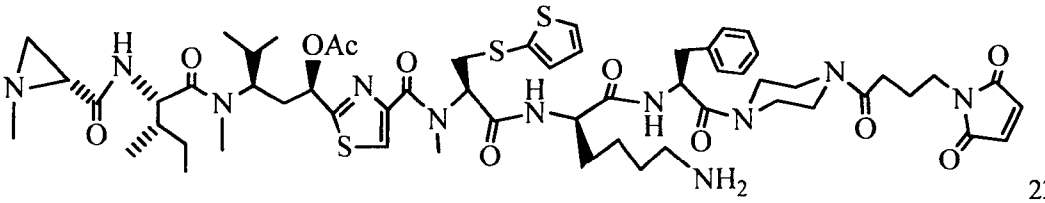
232o;



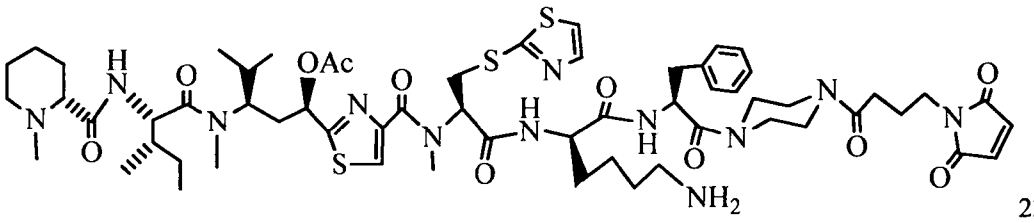
232p;



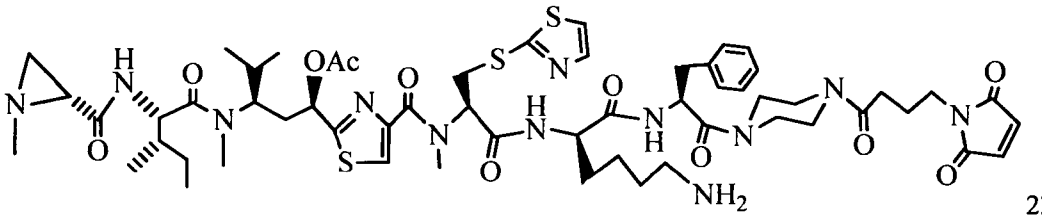
232q;



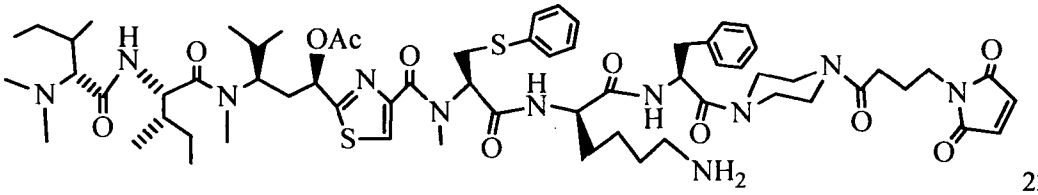
232r;



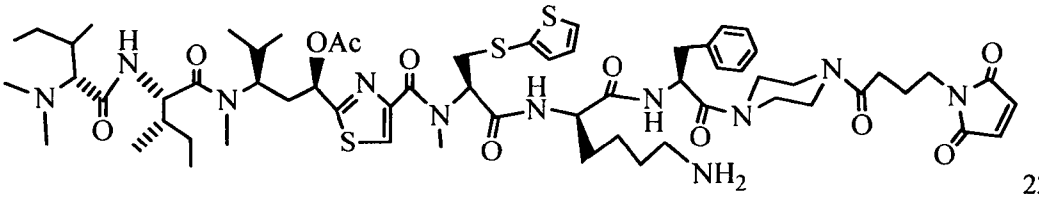
232s;



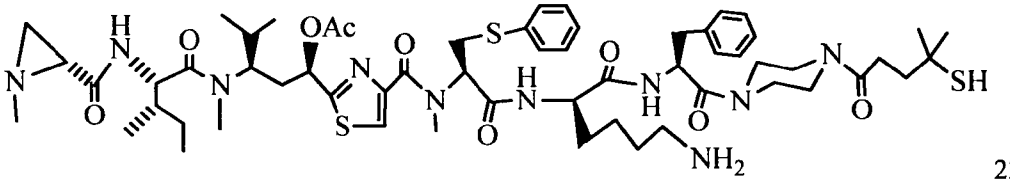
232t;



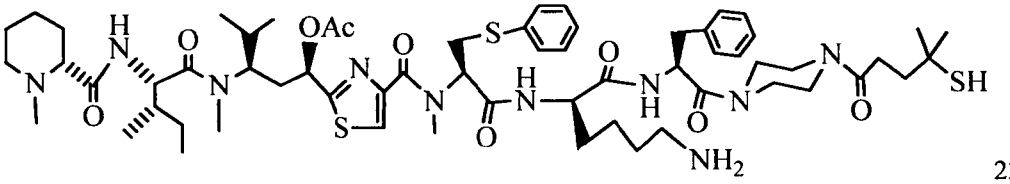
221a;



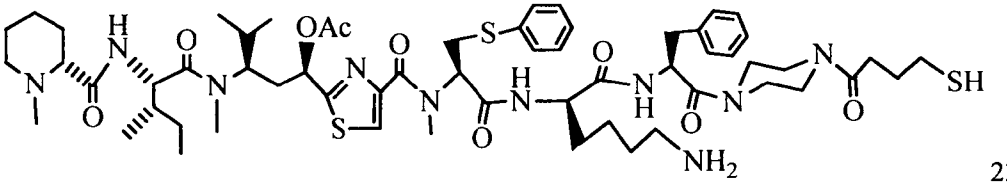
221b;



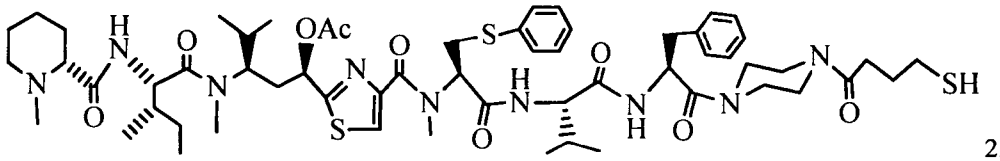
233a;



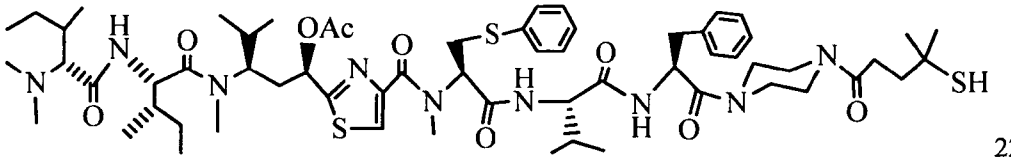
233b;



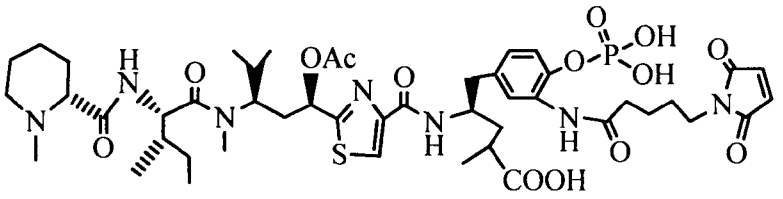
233c;



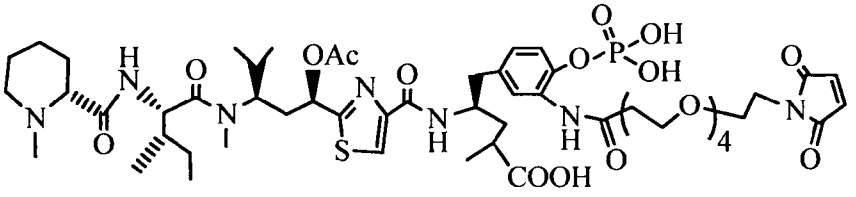
233d;



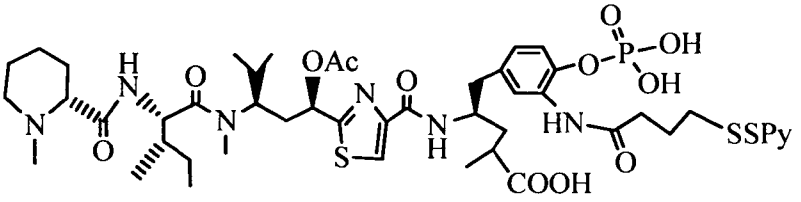
222a;



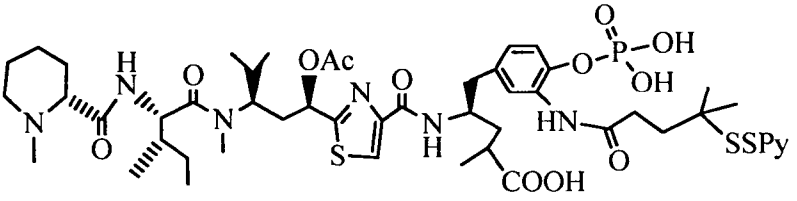
241;



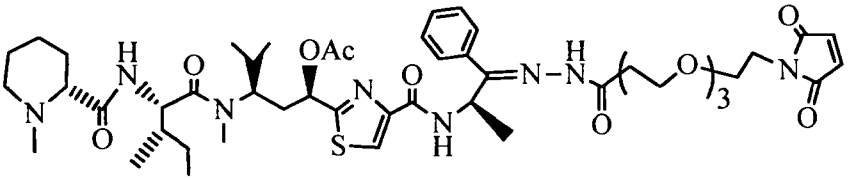
242;



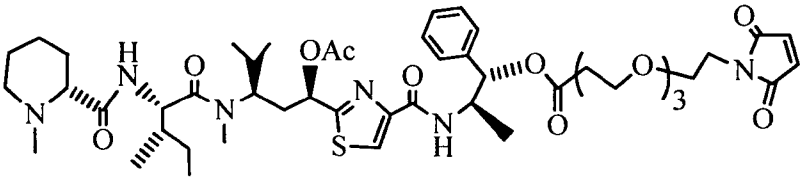
247;



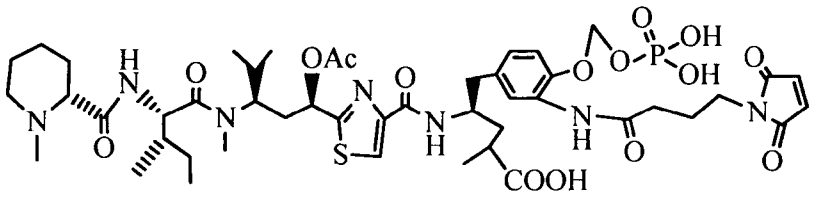
248;

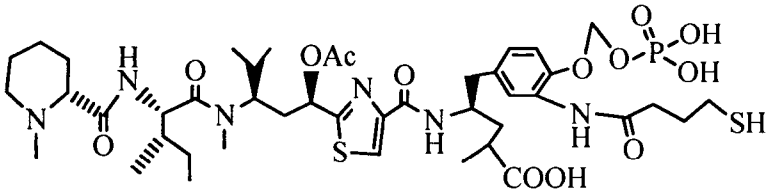


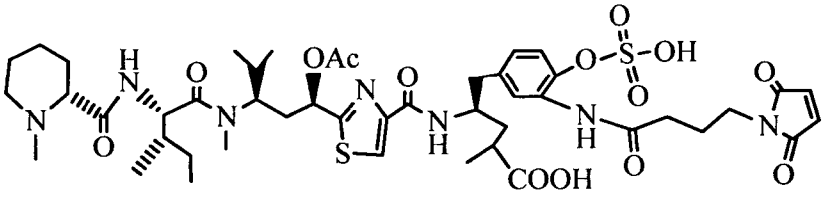
206;

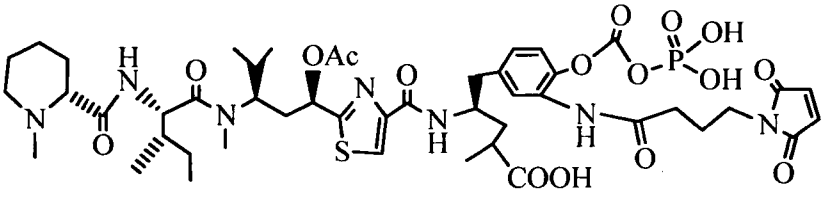


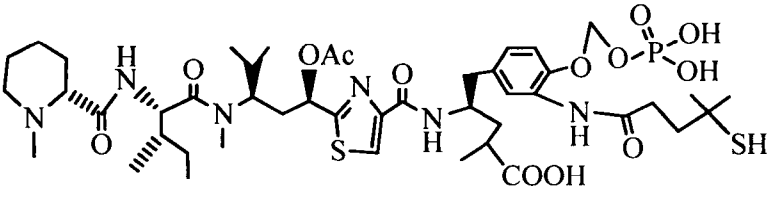
211;

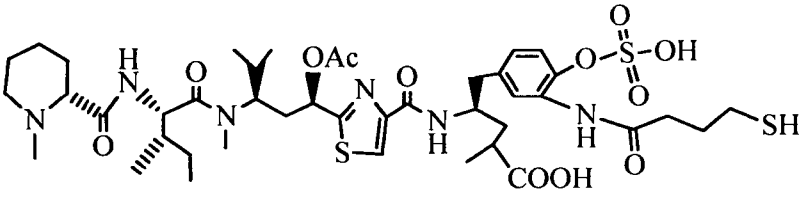

346;

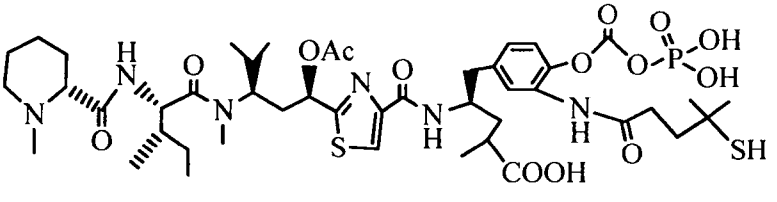

350;

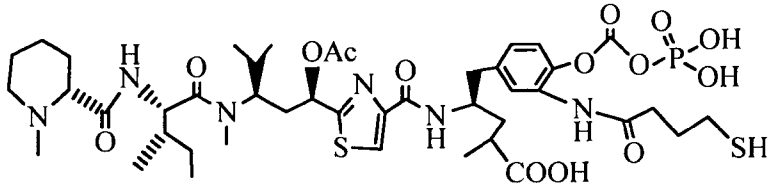

354;


357;


361;

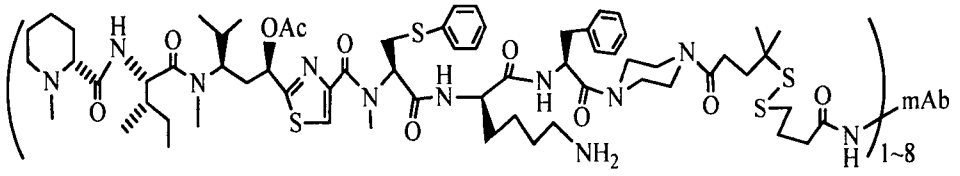

366;


371; or

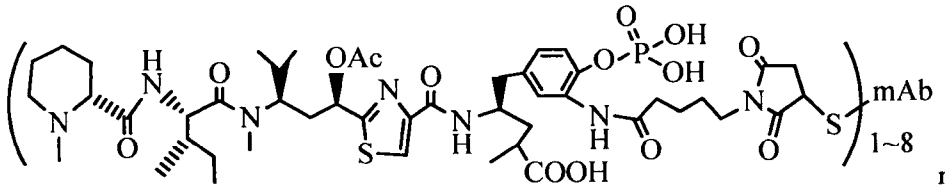


376.

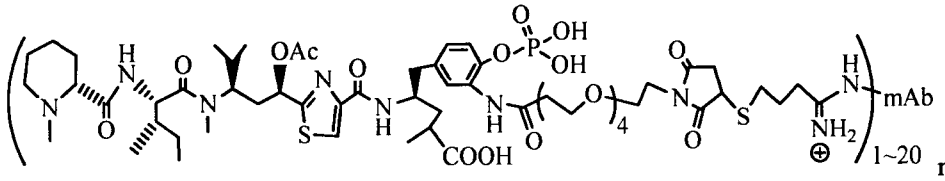
5. The conjugate according to claim 1 or 2, which is an antimitotic agent conjugated to an antibody and has a structure of mAb-TZ01, mAb-TZ04, mAb-TZ05, mAb-TZ07, mAb-TZ10a, mAb-TZ10b, mAb-TZ11, mAb-TZ12, mAb-TZ13, mAb-TZ14, mAb-TZ17, mAb-TZ19, mAb-TZ20, mAb-TZ21, mAb-TZ22, mAb-TZ23, mAb-TZ24, or mAb-TZ25, as shown below:



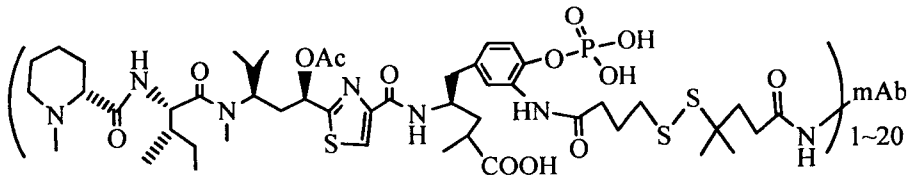
mAb-TZ01;



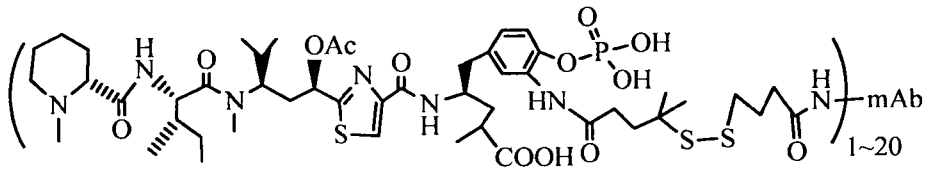
mAb-TZ04;



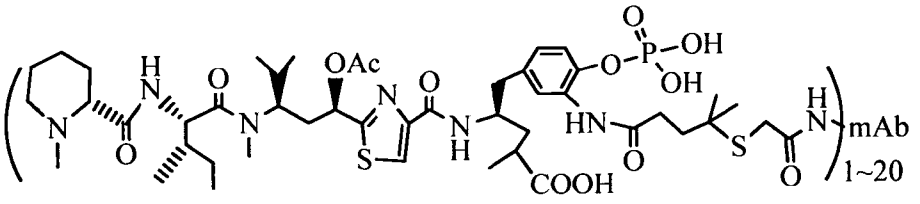
mAb-TZ05;



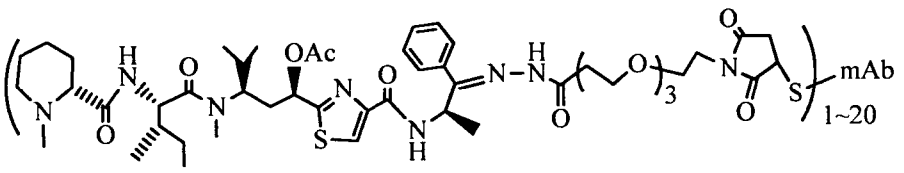
mAb-TZ07;



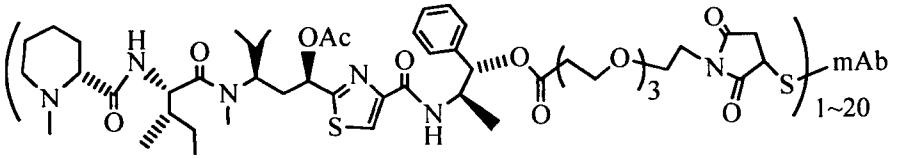
mAb-TZ10a;



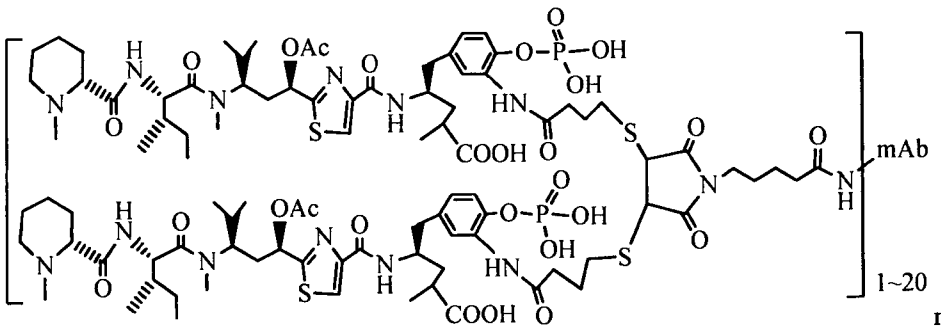
mAb-TZ10b;



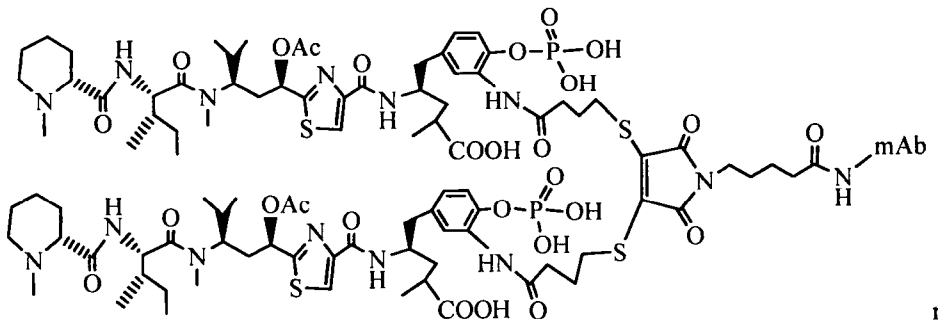
mAb-TZ11;



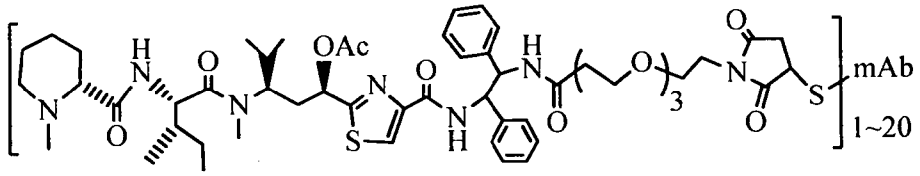
mAb-TZ12;



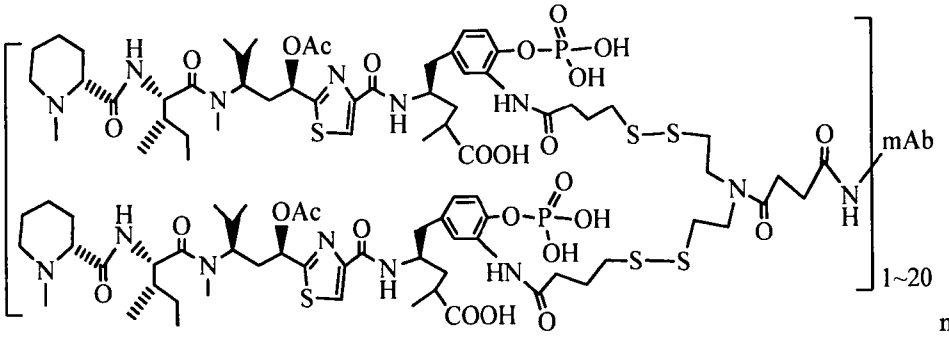
mAb-TZ13;



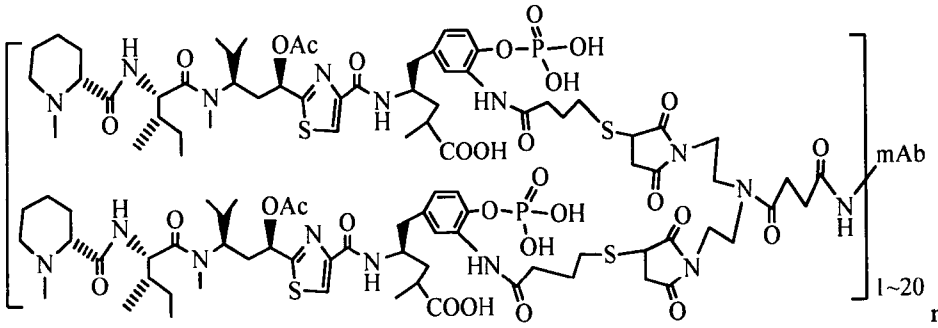
mAb-TZ14;



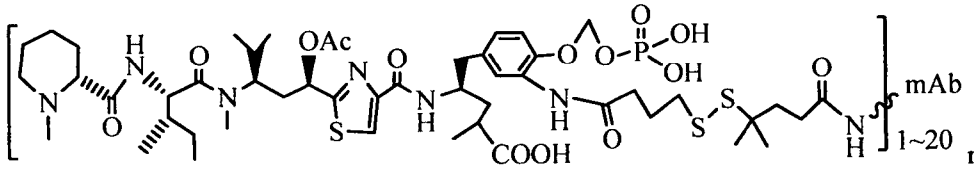
mAb-TZ17;



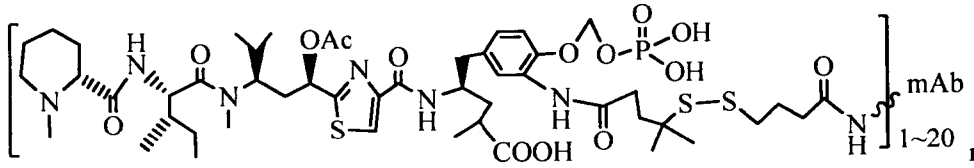
mAb-TZ19;



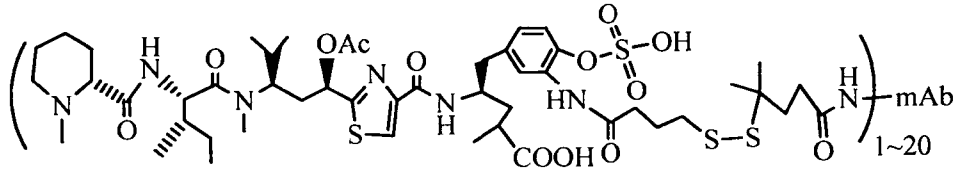
mAb-TZ20;



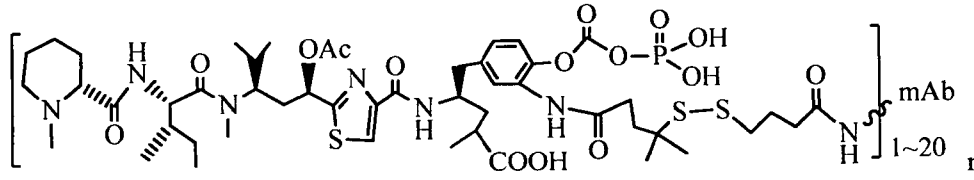
mAb-TZ21;



mAb-TZ22;

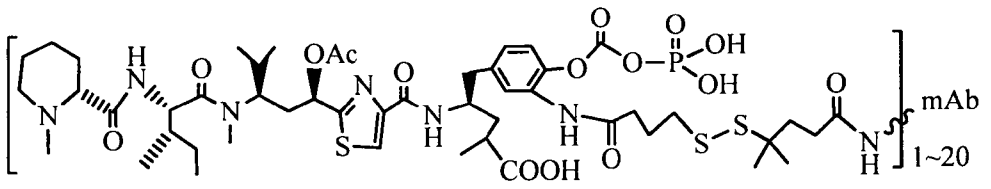


mAb-TZ23;



mAb-TZ24;

or



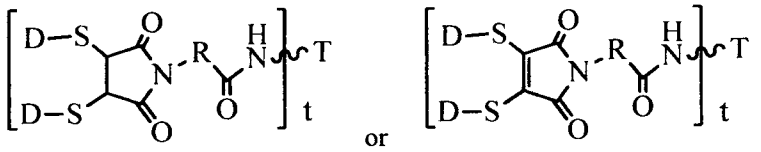
mAb-TZ25;

wherein mAb is an antibody.

6. The conjugate according to any one of claims 1 to5, wherein the targeting or cell-binding agent T is a cell-surface binding agent which binds to: (i) target cells from tumor cells, virus infected cells, microorganism infected cells, parasite infected cells, autoimmune cells, activated cells, myeloid cells, activated T-cells, B cells, or melanocytes, (ii) cells expressing one or more antigens of Apo2, ASLG659, BAFF-R, bone morphogenetic protein receptor (BMPR1B), IGF-IR, CA125, CanAg, E16, EGFR, EphA2 receptor, ErbB2, interleukin,MUC1, MUC16, Napi3b, VEGF, TF, MY9, anti-B4, EpCAM, FcRH2, C242, CD2, CD3, CD4, CD5, CD6, CD11, CD 11a, CD18, CD19, CD20, CD21, CD22, CD26, CD30, CD33, CD37, CD38, CD40, CD44, CD56, CD70, CD72, CD79, CD79a, CD79b, CD90, CD105, CD138, CRIPTO, endothelin B receptor (ETBR), EphA receptors, EphB receptors, EGFr, EGFRvIII, , FCRH1, HER2, HER2/neu, HER3, HLA-DOB, integrins, IRTA2, LIV-1, MPF, GEDA, Mesothelin, NaPi2b, PDL1, Sema 5b, PSCA, PSMA, six transmembrane epithelial antigen of prostate 1 (STEAP1), or STEAP2; or (iii) cells expressing insulin growth factor receptor, epidermal growth factor receptor, or folate receptor.

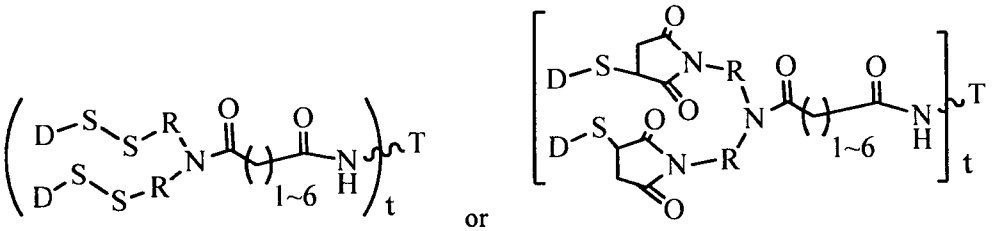
7. A conjugate having the following formula:

A.



or

B.



wherein the portion inside the square or round brackets comprises two cytotoxic agents and/or drugs ;

wherein R is a C_1 to C_8 alkylene or $C_2H_4(OC_2H_4)_s$, s is 1 to 20; t is 1 to 20;

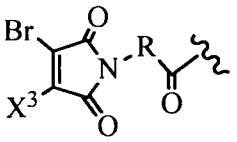
D is the cytotoxic agent or drug; the wavy line represents a linker and T is a targeting or cell-bindingagent; and

at least one D is the antimitotic agent as defined inclaim 2, 3, 4 or 5.

8. The conjugate according to claim7, comprising an additional cytotoxic agent or drug D which is a chemical compound useful in the treatment of cancer, or a pharmaceutically acceptable salt, acid or derivative thereof, wherein when only one of the antimitotic agents is present, the other D is the additional cytotoxic agent or drug that is the chemical compound useful in the treatment of cancer..

9. The conjugate according to claim8, wherein the chemical compound useful in the treatment of cancer is calicheamicin, auristatin, maytansinoid, dolastin, CC-1065 analog, doxorubicin, taxane, pyrrolobenzodiazepine dimer, or siRNA, or any combination thereof,

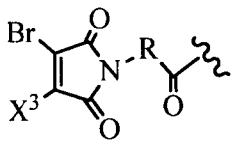
10. The conjugate according to claim 1, 2 or 3, which is prepared using a linker having a 3-bromo-maleimido, or 3,4-dibromo-maleimido terminus, of the following formula:



wherein R is a C_1 to C_8 alkylene or $C_2H_4(OC_2H_4)_s$, s is 1 to 20; and X^3 is H or Br; to link the antimitotic agent with the targeting or cell-binding agent T.

11. A conjugate precursor for use in preparing the linker as defined in claim10, which is a conjugate as defined in claim 1, 2 or 3, with one or both of the antimitotic agent and the targeting

or cell-binding agent T having a 3-bromo-maleimido, or 3,4-dibromo-maleimido terminus, of the following formula:



wherein R is a C_1 to C_8 alkylene or $C_2H_4(OC_2H_4)_s$, s is 1 to 20; X^3 is H or Br; and the wavy line represents the remainder of the conjugate precursor.

12. A pharmaceutical composition comprising a therapeutically effective amount of the conjugate as defined in any one of claims 1 to9, or a pharmaceutically acceptable salt thereof, and a carrier, diluent, or excipient therefor.

13. The pharmaceutical composition according to claim12, which is a pharmaceutical composition for the treatment or prevention of:

A.

a cancer, or an autoimmune disease, or an-infectious disease;

B.

a cancer, an autoimmune disease, or an infectious disease in which the pharmaceutical composition comprising a therapeutically effective amount of the conjugate as defined in any one of claims 1 to 9 and is to be used in combination with another therapeutic agent for treatment or prevention of a cancer, an autoimmune disease, or an infectious disease; or

C.

a cancer, an autoimmune disease, or an infectious disease in which the pharmaceutical composition comprising a therapeutically effective amount of a conjugate as defined in any one of claims 1 to 8 and is to be used concurrently with another therapeutic agent for treatment or prevention of a cancer, an autoimmune disease, or an infectious disease, wherein said therapeutic agent is a chemotherapeutic agent, an agent for radiation therapy, an immunotherapy agent, an anti-autoimmune disorder agents, an anti-infectious agents or an antibody-drug conjugate other than those defined in any one of claims 1 to9.

FIGURES:

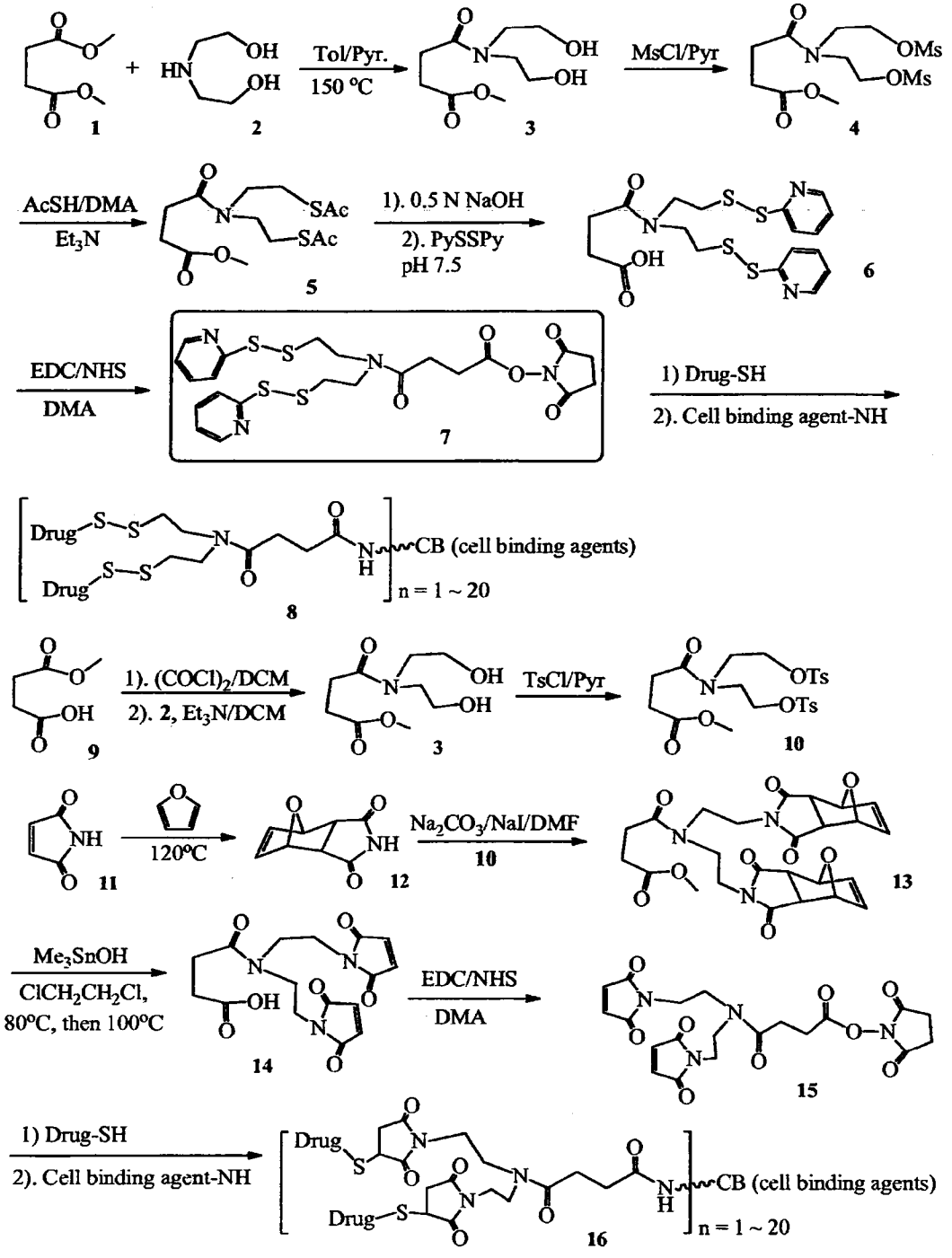


Figure 1.

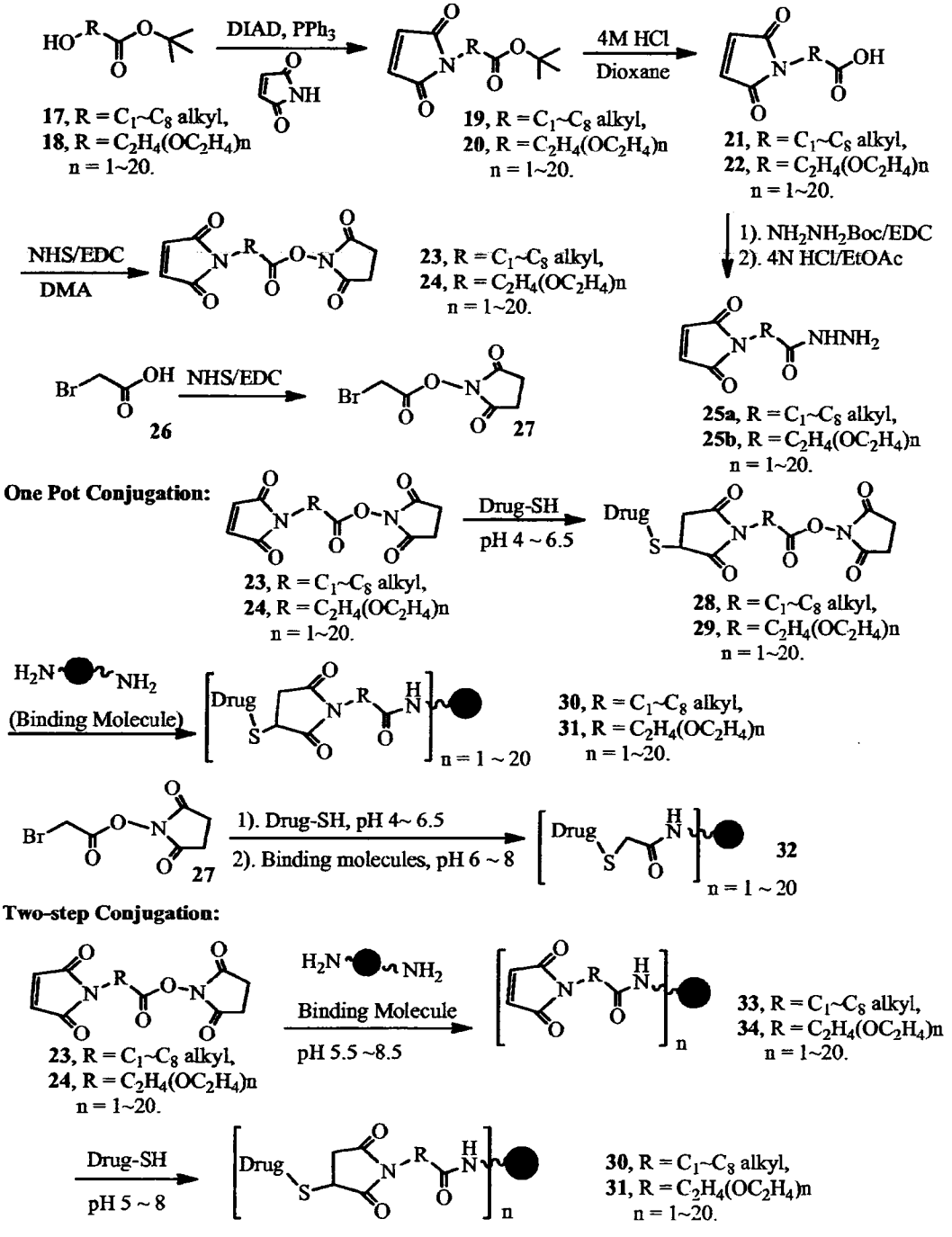

DIAD, PPh3
4M HCl
Dioxane
17, R = C1~C8 alkyl,
18, R = C2H4(OC2H4)n
n = 1~20.
19, R = C1~C8 alkyl,
20, R = C2H4(OC2H4)n
n = 1~20.
21, R = C1~C8 alkyl,
22, R = C2H4(OC2H4)n
n = 1~20.
NHS/EDC
DMA
23, R = C1~C8 alkyl,
24, R = C2H4(OC2H4)n
n = 1~20.
1). NH2NH2Boc/EDC
2). 4N HCl/EtOAc
NHS/EDC
26
27
25a, R = C1~C8 alkyl,
25b, R = C2H4(OC2H4)n
n = 1~20.
One Pot Conjugation:
Drug-SH
pH 4 ~ 6.5
23, R = C1~C8 alkyl,
24, R = C2H4(OC2H4)n
n = 1~20.
28, R = C1~C8 alkyl,
29, R = C2H4(OC2H4)n
n = 1~20.
(Binding Molecule)
n = 1 ~ 20
30, R = C1~C8 alkyl,
31, R = C2H4(OC2H4)n
n = 1~20.
1). Drug-SH, pH 4~ 6.5
2). Binding molecules, pH 6 ~ 8
27
32
n = 1 ~ 20
Two-step Conjugation:
23, R = C1~C8 alkyl,
24, R = C2H4(OC2H4)n
n = 1~20.
Binding Molecule
pH 5.5 ~8.5
33, R = C1~C8 alkyl,
34, R = C2H4(OC2H4)n
n = 1~20.
Drug-SH
pH 5 ~ 8
30, R = C1~C8 alkyl,
31, R = C2H4(OC2H4)n
n = 1~20.

Figure 2.

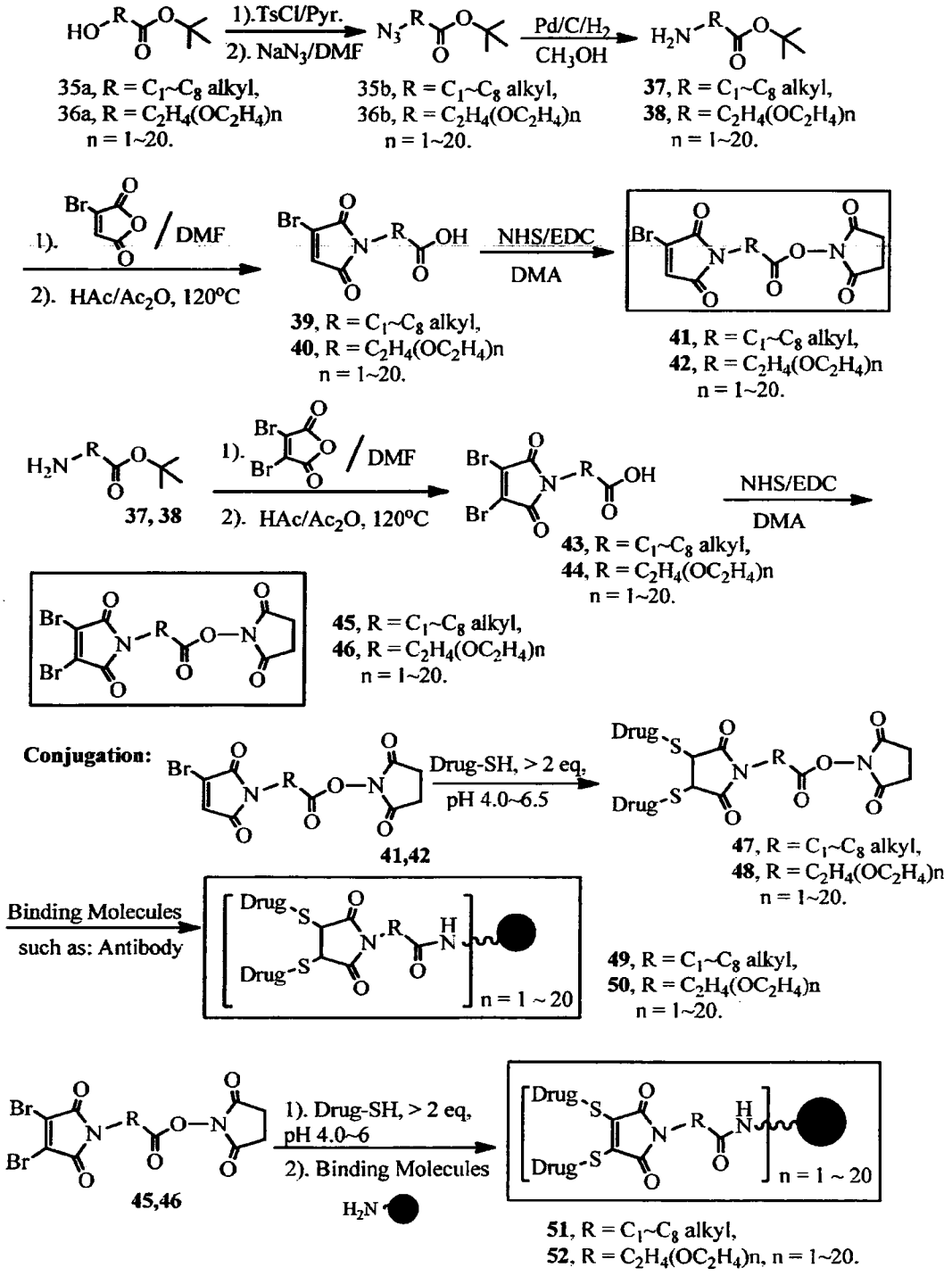

1).TsCl/Pyr.
2). NaN3/DMF
Pd/C/H2
CH3OH
35a, R = C1~C8 alkyl,
36a, R = C2H4(OC2H4)n
n = 1~20.
35b, R = C1~C8 alkyl,
36b, R = C2H4(OC2H4)n
n = 1~20.
37, R = C1~C8 alkyl,
38, R = C2H4(OC2H4)n
n = 1~20.
1).
DMF
2). HAc/Ac2O, 120°C
39, R = C1~C8 alkyl,
40, R = C2H4(OC2H4)n
n = 1~20.
NHS/EDC
DMA
41, R = C1~C8 alkyl,
42, R = C2H4(OC2H4)n
n = 1~20.
37, 38
43, R = C1~C8 alkyl,
44, R = C2H4(OC2H4)n
n = 1~20.
45, R = C1~C8 alkyl,
46, R = C2H4(OC2H4)n
n = 1~20.
Conjugation:
Drug-SH, > 2 eq,
pH 4.0~6.5
41,42
47, R = C1~C8 alkyl,
48, R = C2H4(OC2H4)n
n = 1~20.
Binding Molecules
such as: Antibody
n = 1 ~ 20
49, R = C1~C8 alkyl,
50, R = C2H4(OC2H4)n
n = 1~20.
1). Drug-SH, > 2 eq,
pH 4.0~6
2). Binding Molecules
45,46
51, R = C1~C8 alkyl,
52, R = C2H4(OC2H4)n, n = 1~20.

Figure 3.

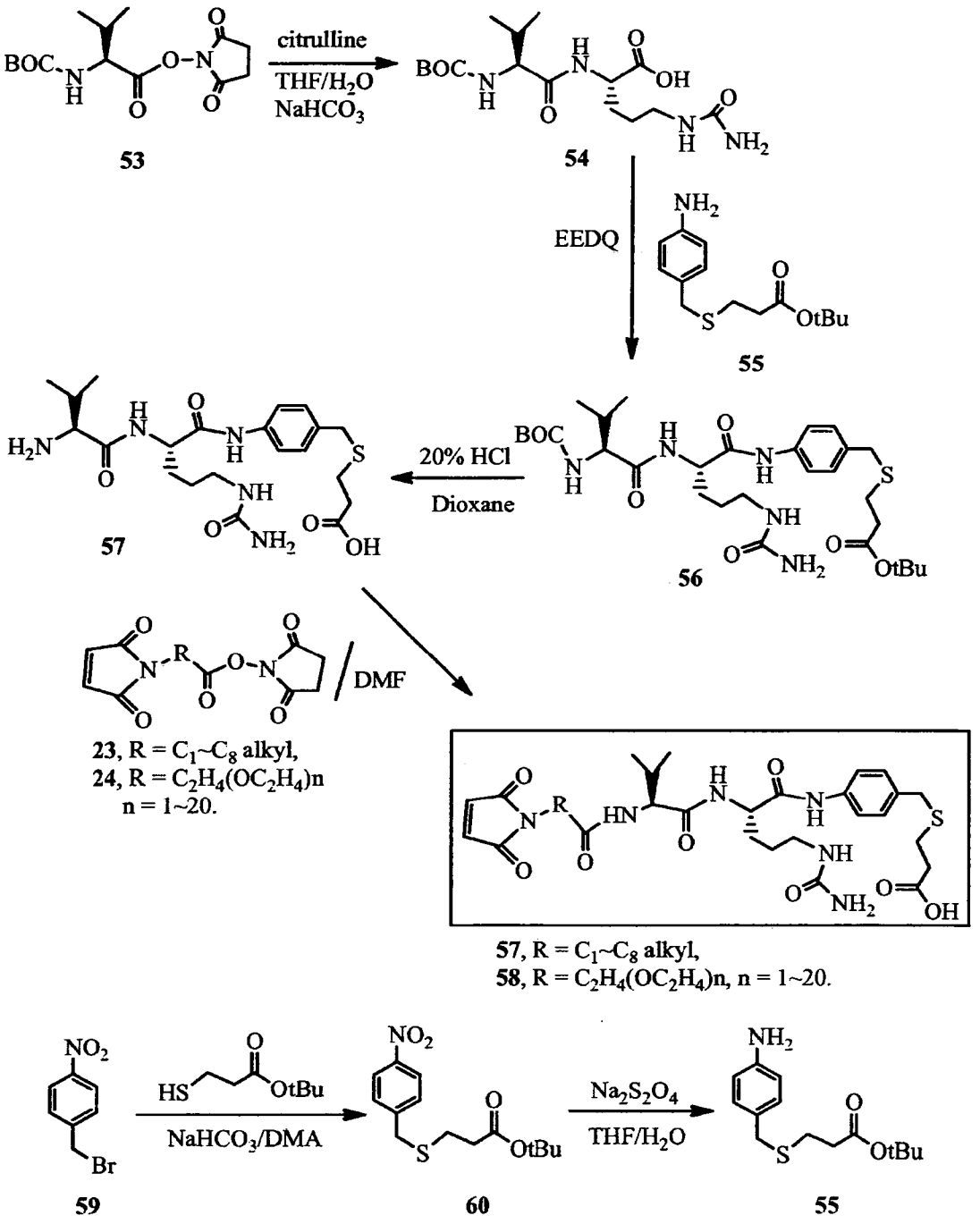

citrulline
THF/H2O
NaHCO3
53
54
EEDQ
55
20% HCl
Dioxane
56
57
DMF
23, R = C1~C8 alkyl,
24, R = C2H4(OC2H4)n
n = 1~20.
57, R = C1~C8 alkyl,
58, R = C2H4(OC2H4)n, n = 1~20.
NaHCO3/DMA
Na2S2O4
THF/H2O
59
60
55

Figure 4.

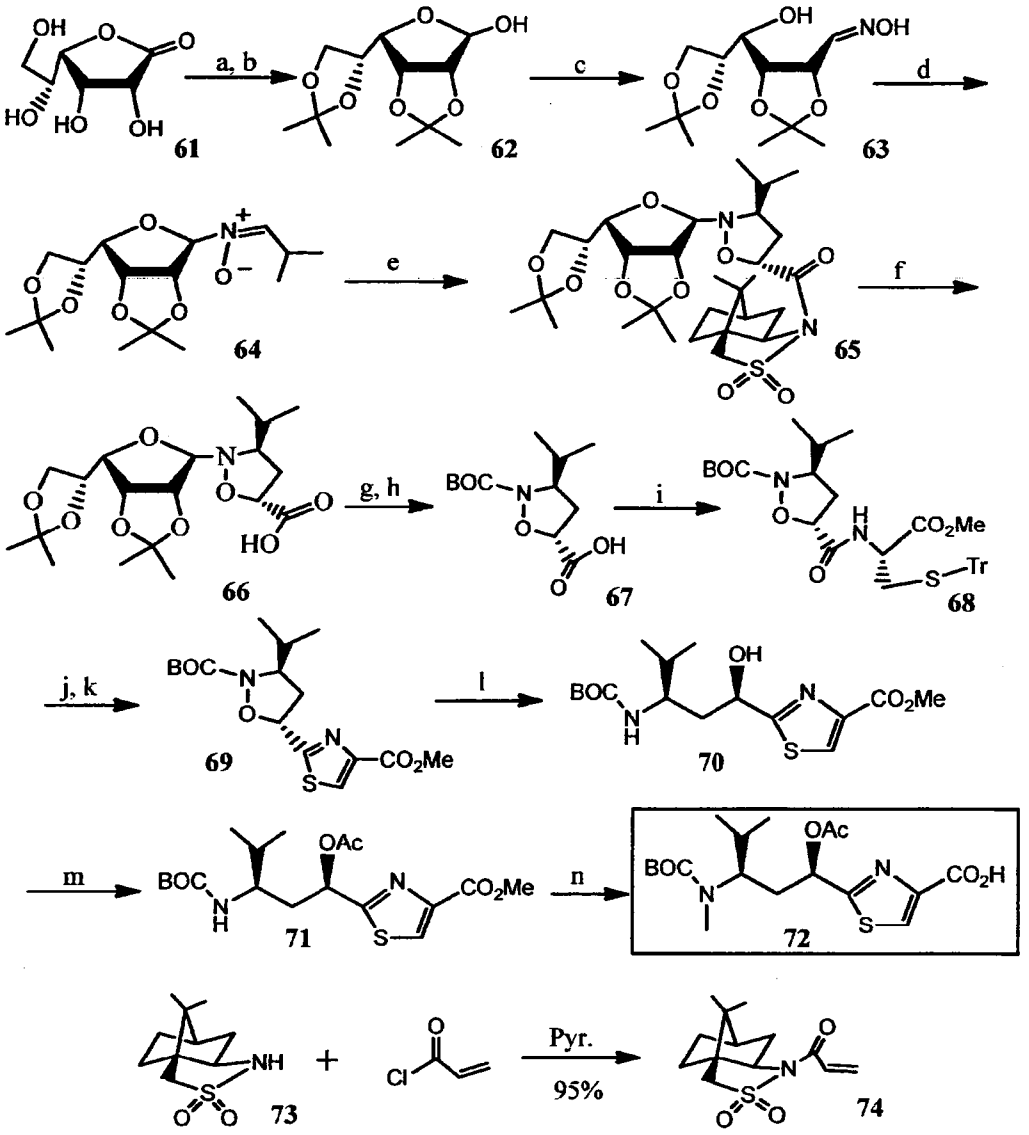


Figure 5.

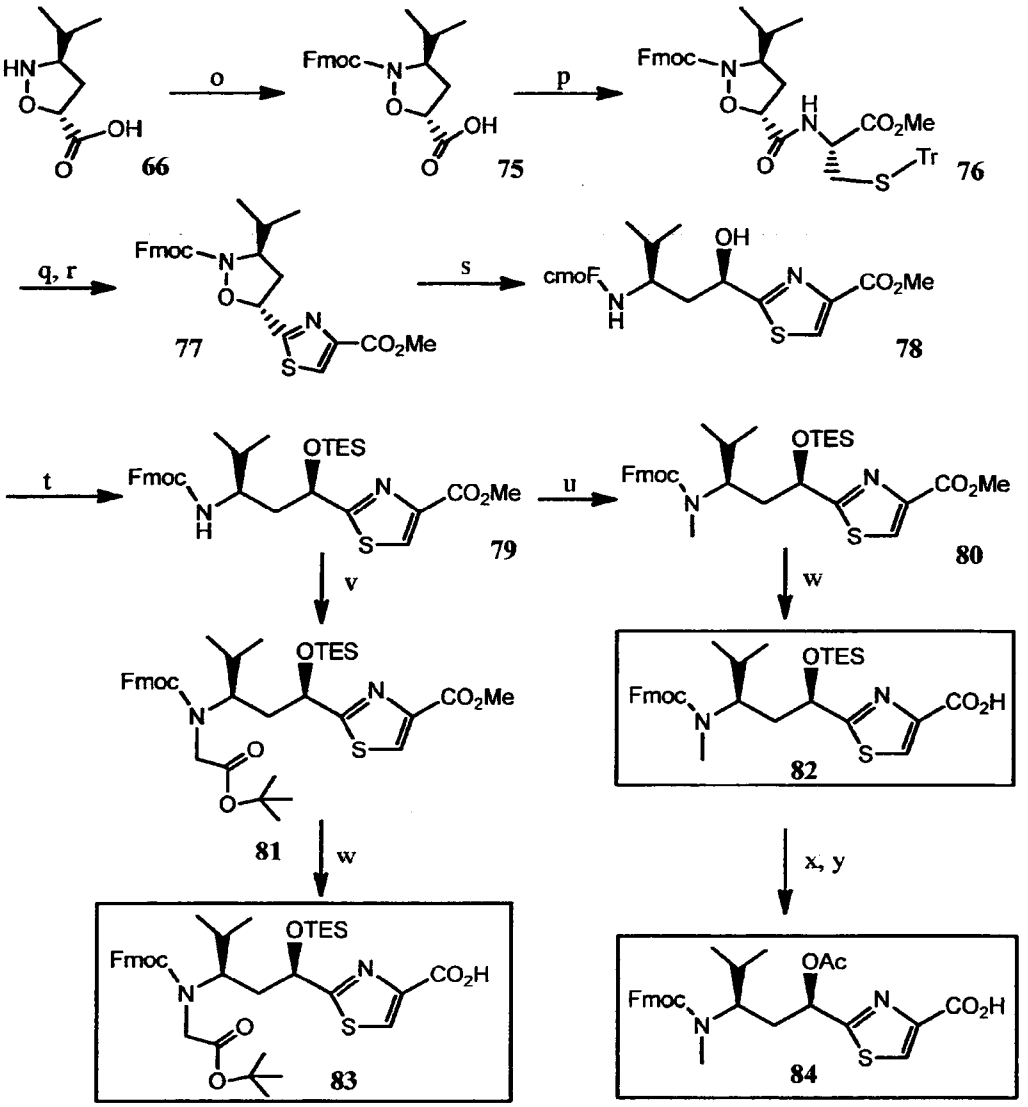

HN
O
OH
O
66
o
Fmoc
N
O
OH
O
75
p
Fmoc
N
O
H
N
CO2Me
O
Tr
S
76
q, r
Fmoc
N
O
N
S
CO2Me
77
s
cmoF
N
H
OH
N
S
CO2Me
78
t
Fmoc
N
H
OTES
N
S
CO2Me
79
u
Fmoc
N
OTES
N
S
CO2Me
80
v
Fmoc
N
OTES
N
S
CO2Me
O
O
81
w
Fmoc
N
OTES
N
S
CO2H
O
O
83
w
Fmoc
N
OTES
N
S
CO2H
82
x, y
Fmoc
N
OAc
N
S
CO2H
84

Figure 6.

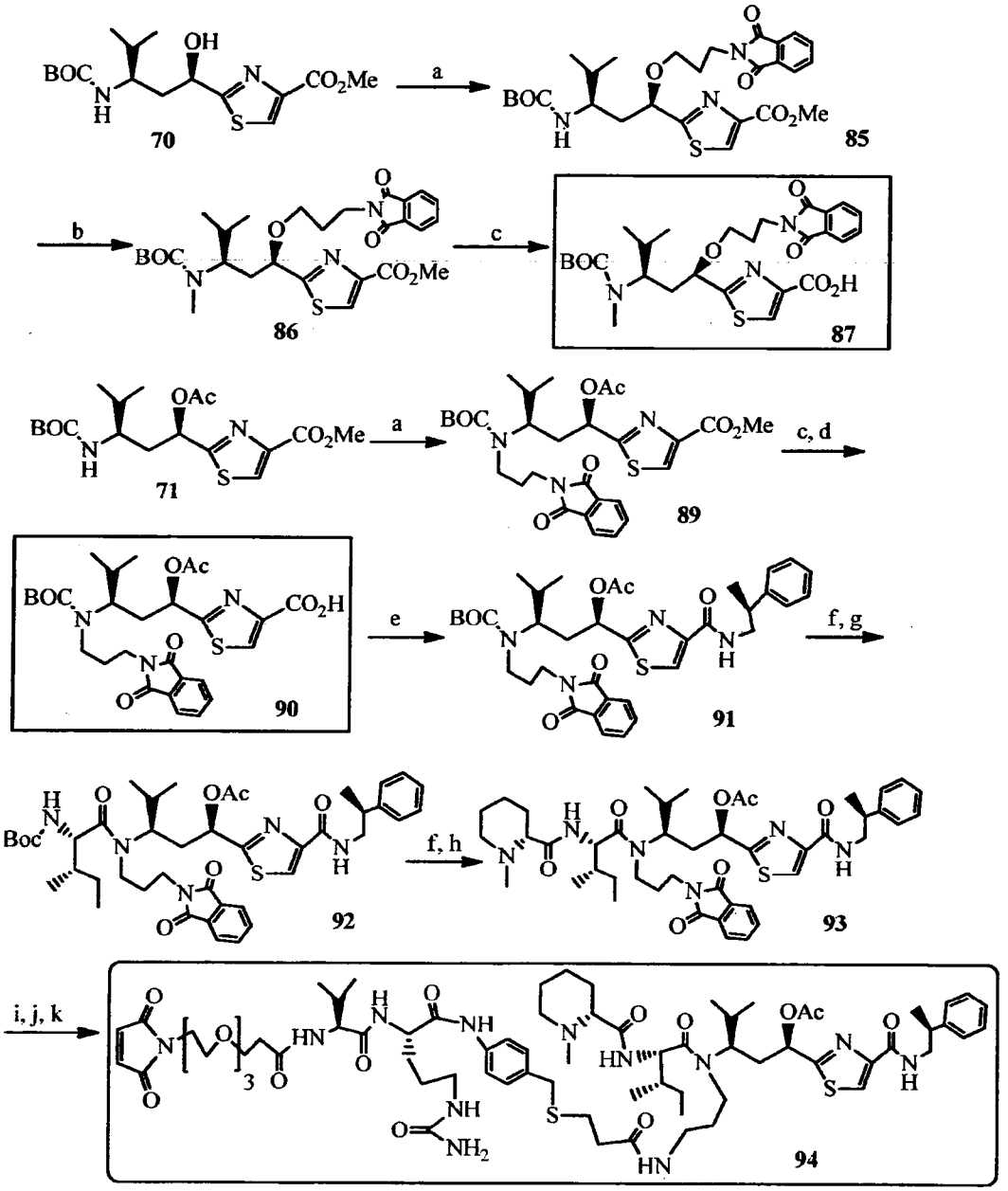


Figure 7.

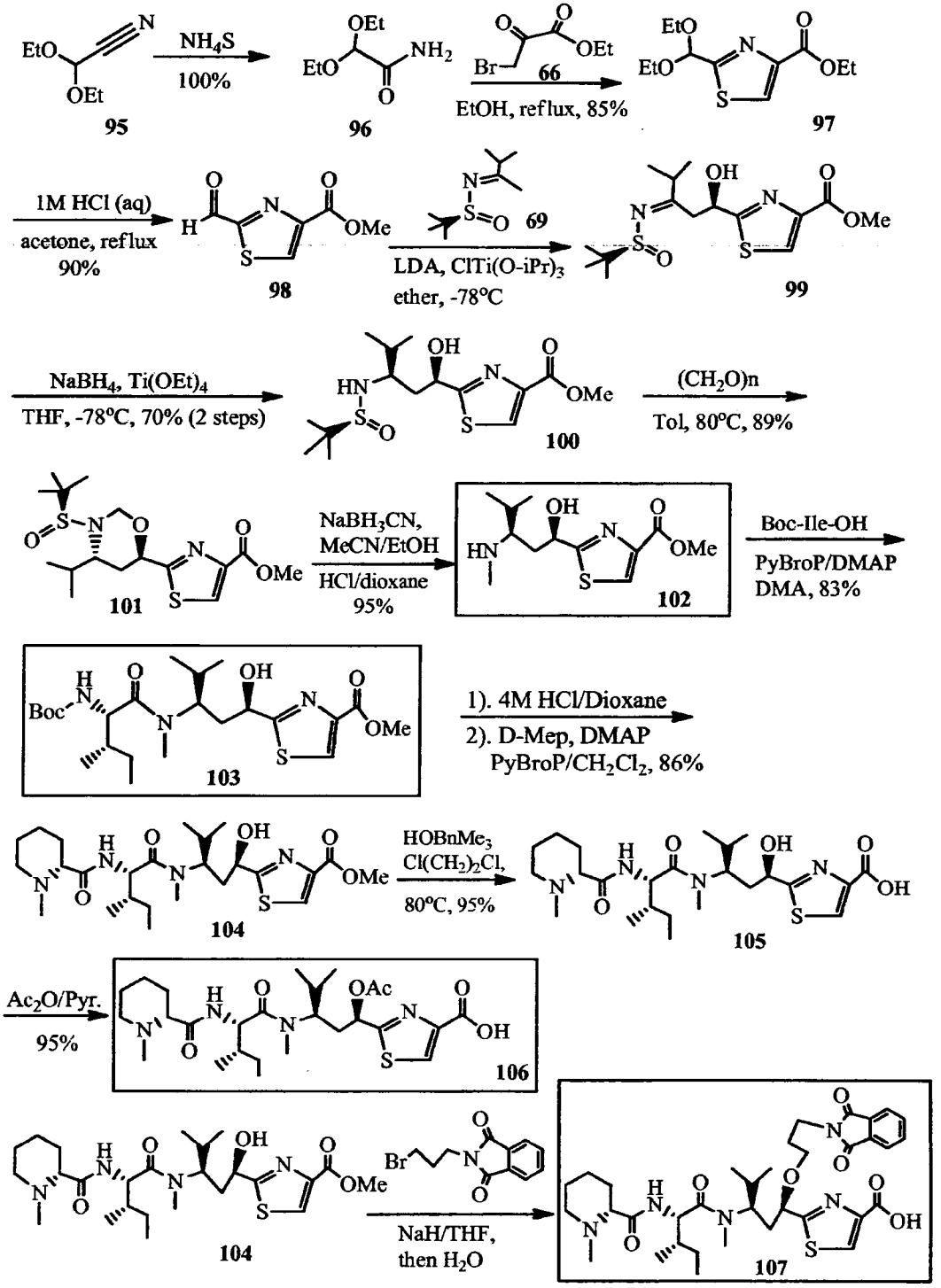

NH4S
100%
EtOH, reflux, 85%
66
95
96
97
1M HCl (aq)
acetone, reflux
90%
98
LDA, ClTi(O-iPr)3
ether, -78°C
69
99
NaBH4, Ti(OEt)4
THF, -78°C, 70% (2 steps)
100
(CH2O)n
Tol, 80°C, 89%
101
NaBH3CN,
MeCN/EtOH
HCl/dioxane
95%
102
Boc-Ile-OH
PyBroP/DMAP
DMA, 83%
103
1). 4M HCl/Dioxane
2). D-Mep, DMAP
PyBroP/CH2Cl2, 86%
104
HOBnMe3
Cl(CH2)2Cl,
80°C, 95%
105
Ac2O/Pyr.
95%
106
NaH/THF,
then H2O
107

Figure 8.

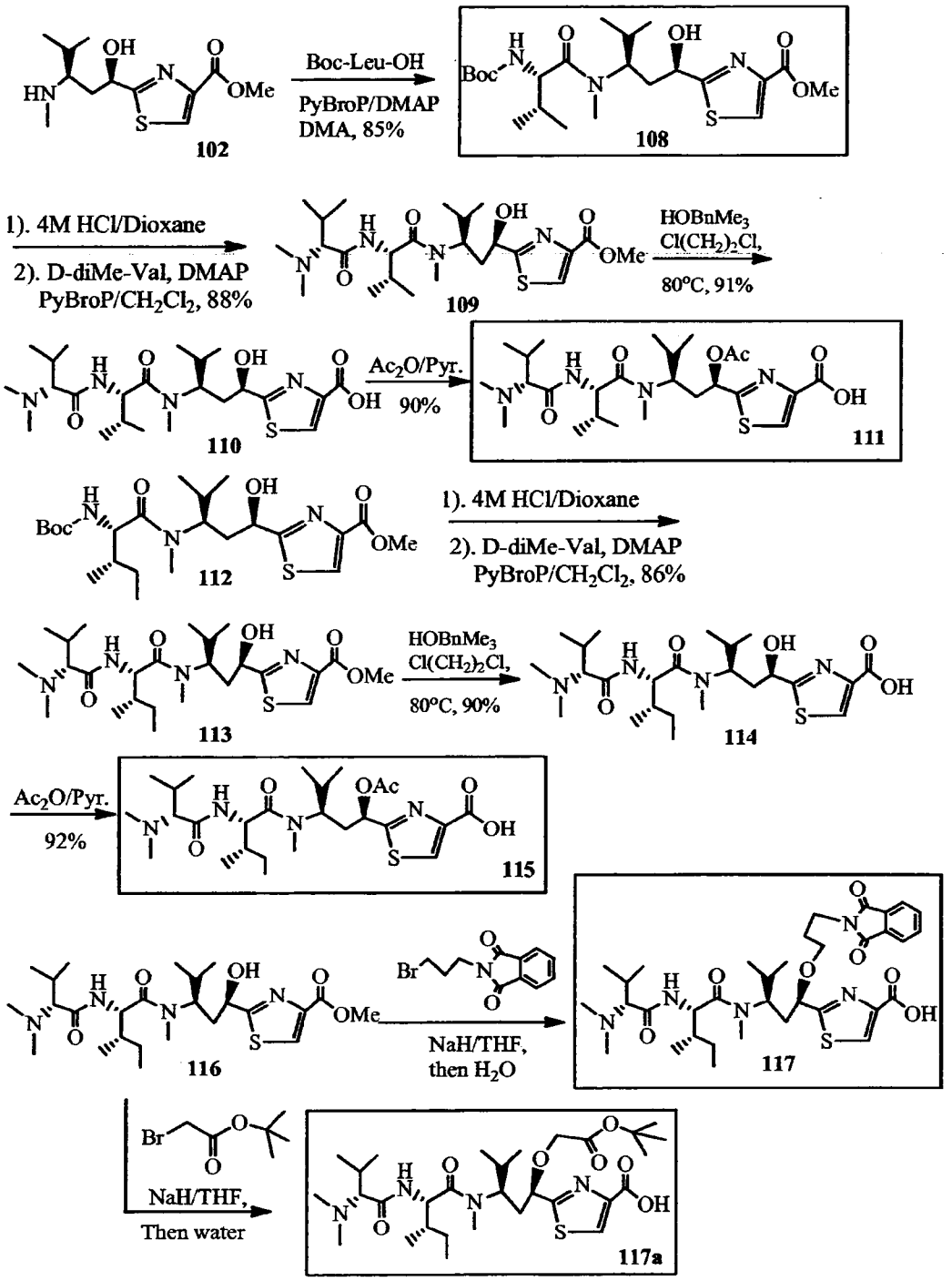

Boc-Leu-OH
PyBroP/DMAP
DMA, 85%
102
108
1). 4M HCl/Dioxane
2). D-diMe-Val, DMAP
PyBroP/CH2Cl2, 88%
109
HOBnMe3
Cl(CH2)2Cl,
80°C, 91%
110
Ac2O/Pyr.
90%
111
112
1). 4M HCl/Dioxane
2). D-diMe-Val, DMAP
PyBroP/CH2Cl2, 86%
113
HOBnMe3
Cl(CH2)2Cl,
80°C, 90%
114
Ac2O/Pyr.
92%
115
116
NaH/THF,
then H2O
117
NaH/THF,
Then water
117a

Figure 9.

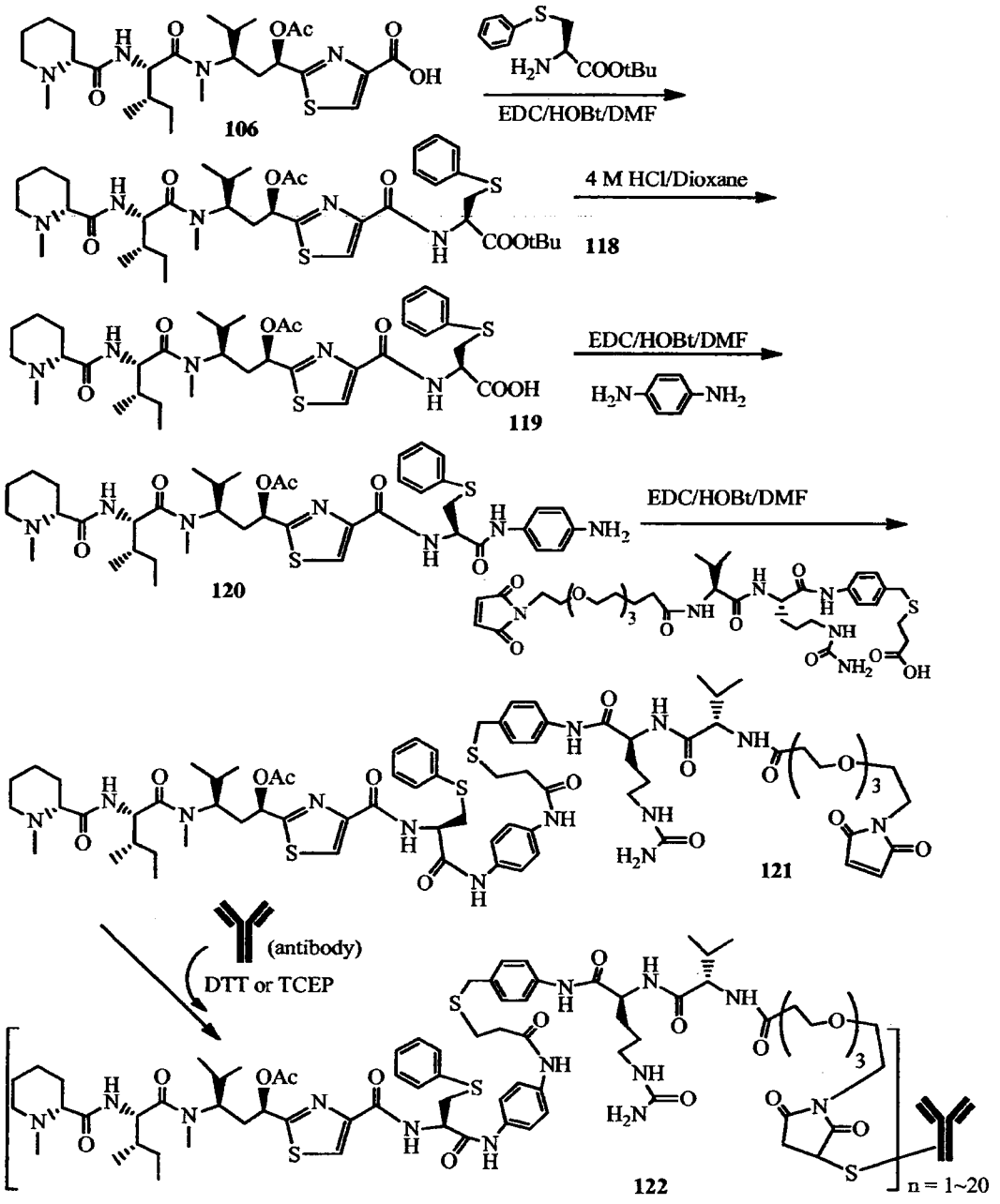

106
EDC/HOBt/DMF
4 M HCl/Dioxane
118
119
EDC/HOBt/DMF
120
EDC/HOBt/DMF
121
(antibody)
DTT or TCEP
122
n = 1~20

Figure 10.

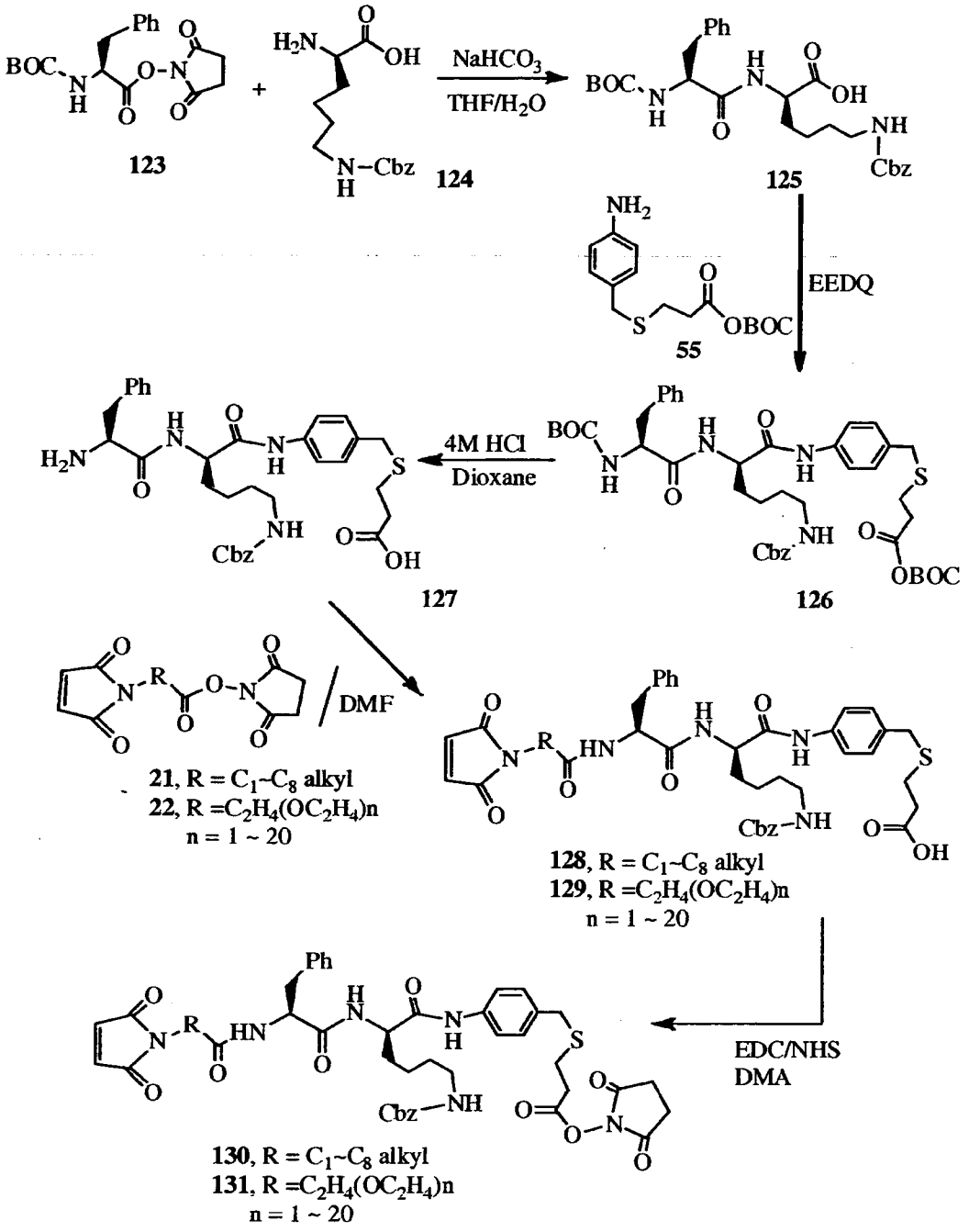

123
124
NaHCO3
THF/H2O
125
55
EEDQ
4M HCl
Dioxane
127
126
DMF
21, R = C1~C8 alkyl
22, R =C2H4(OC2H4)n
n = 1 ~ 20
128, R = C1~C8 alkyl
129, R =C2H4(OC2H4)n
n = 1 ~ 20
EDC/NHS
DMA
130, R = C1~C8 alkyl
131, R =C2H4(OC2H4)n
n = 1 ~ 20

Figure 11.

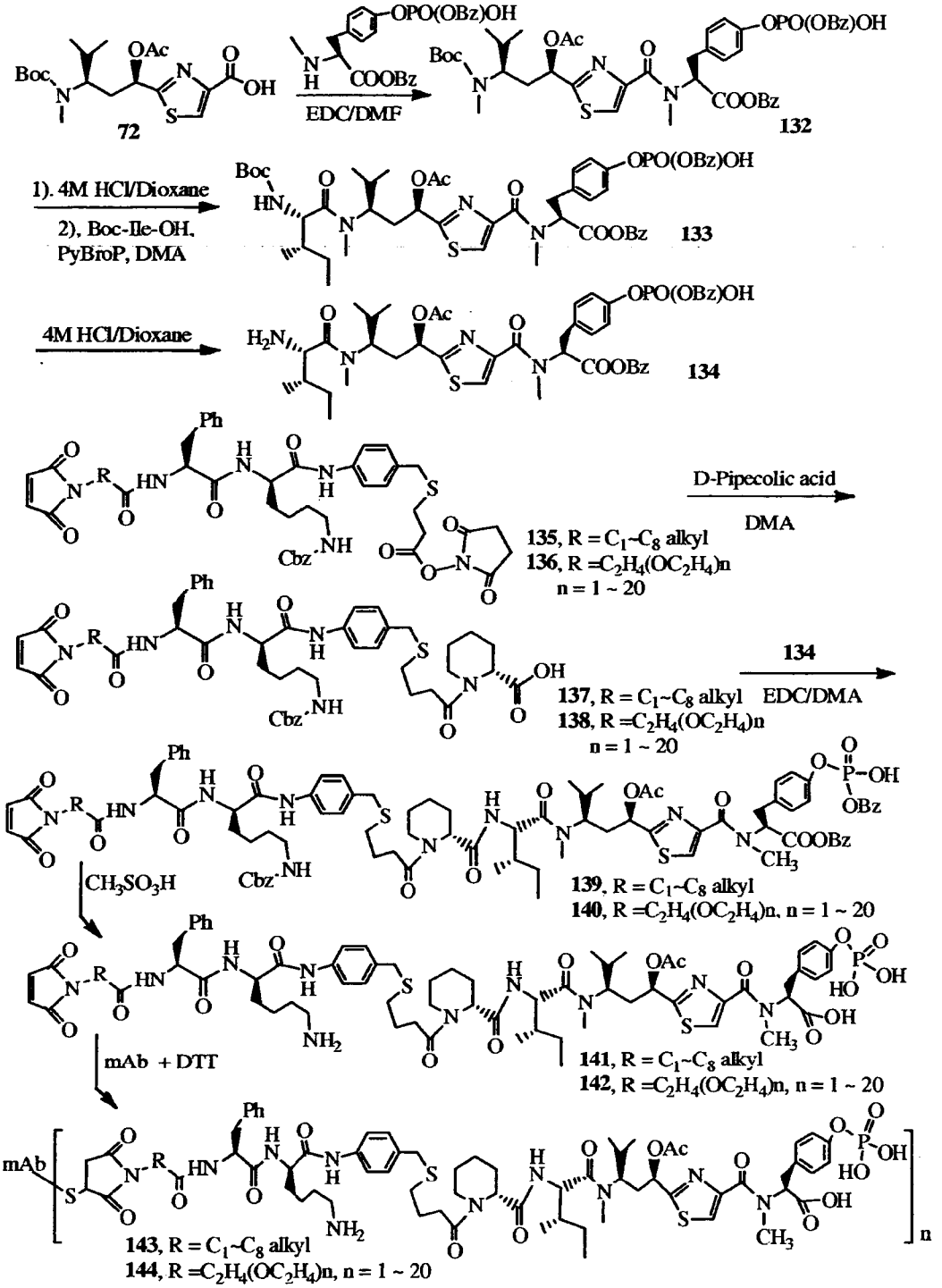

72
EDC/DMF
132
1). 4M HCl/Dioxane
2), Boc-Ile-OH,
PyBroP, DMA
133
4M HCl/Dioxane
134
D-Pipecolic acid
DMA
135, R = C1~C8 alkyl
136, R =C2H4(OC2H4)n
n = 1 ~ 20
134
EDC/DMA
137, R = C1~C8 alkyl
138, R =C2H4(OC2H4)n
n = 1 ~ 20
CH3SO3H
139, R = C1~C8 alkyl
140, R =C2H4(OC2H4)n, n = 1 ~ 20
mAb + DTT
141, R = C1~C8 alkyl
142, R =C2H4(OC2H4)n, n = 1 ~ 20
143, R = C1~C8 alkyl
144, R =C2H4(OC2H4)n, n = 1 ~ 20

Figure 12.

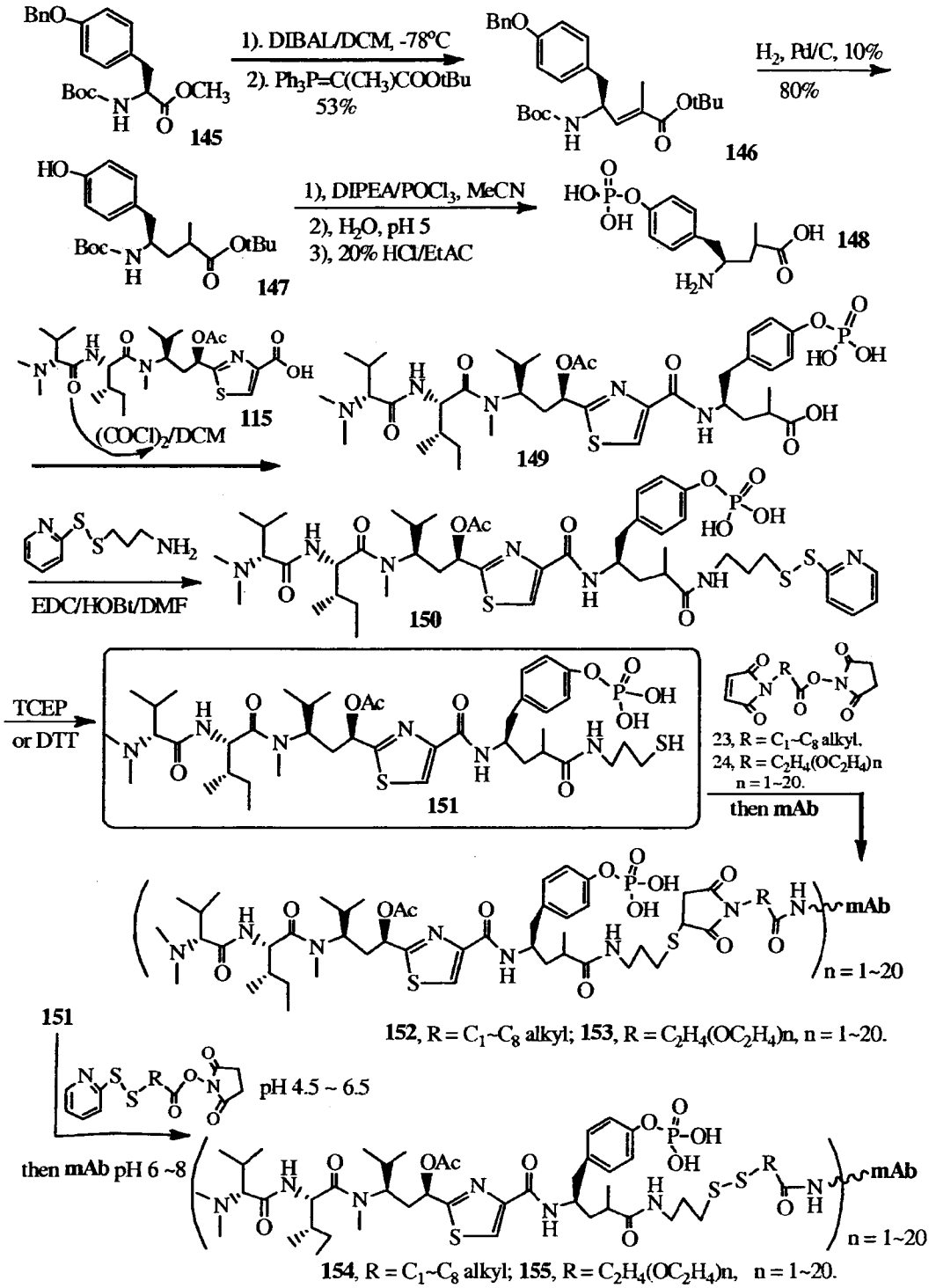

1). DIBAL/DCM, -78°C
2). Ph3P=C(CH3)COOtBu
53%
H2, Pd/C, 10%
80%
145
146
147
148
1), DIPEA/POCl3, MeCN
2), H2O, pH 5
3), 20% HCl/EtAC
115
149
(COCl)2/DCM
EDC/HOBt/DMF
150
TCEP
or DTT
151
23, R = C1~C8 alkyl.
24, R = C2H4(OC2H4)n
n = 1~20.
then mAb
mAb
n = 1~20
152, R = C1~C8 alkyl; 153, R = C2H4(OC2H4)n, n = 1~20.
pH 4.5 ~ 6.5
then mAb pH 6 ~8
154, R = C1~C8 alkyl; 155, R = C2H4(OC2H4)n, n = 1~20.

Figure 13.

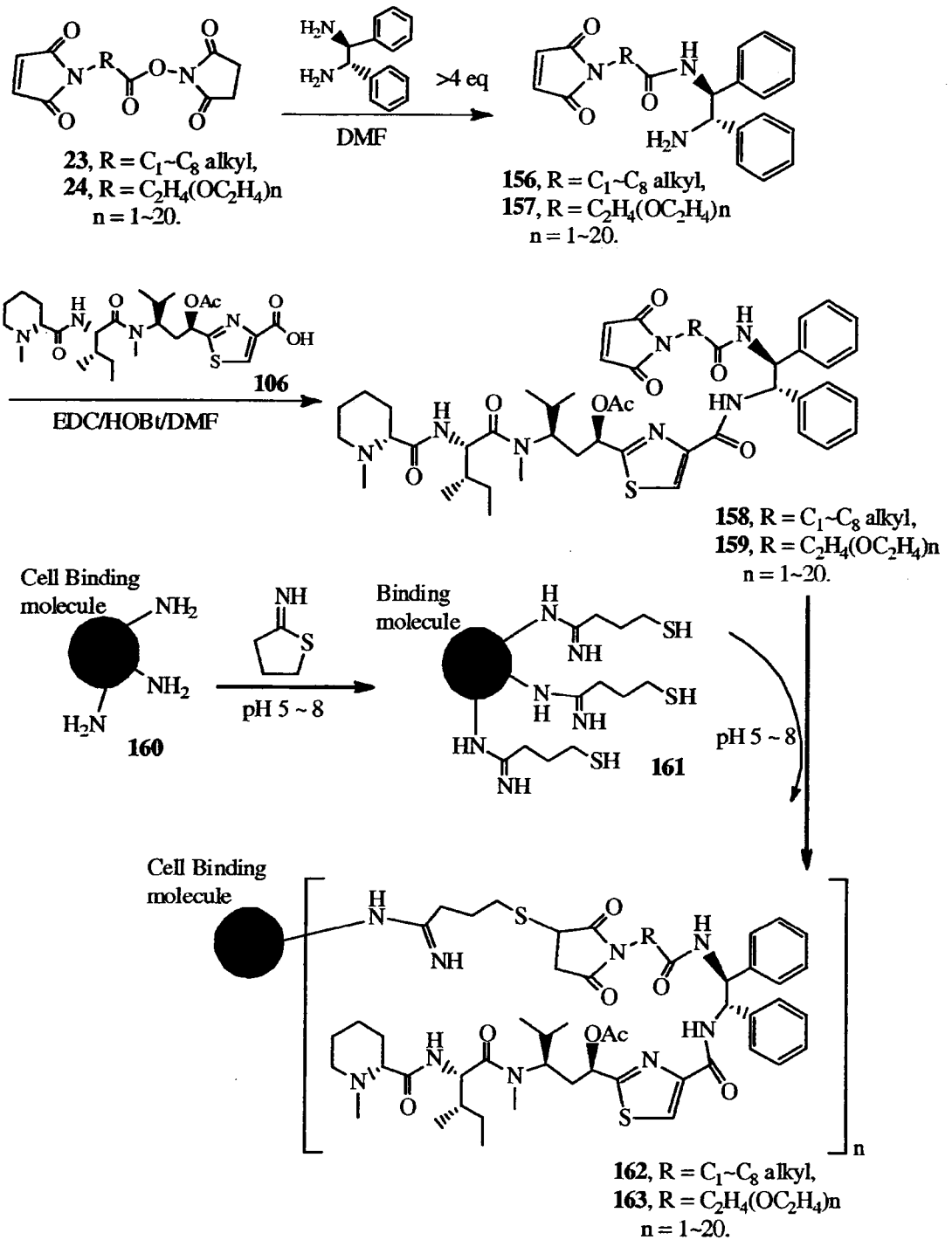

23, R = C1~C8 alkyl,
24, R = C2H4(OC2H4)n
n = 1~20.
>4 eq
DMF
156, R = C1~C8 alkyl,
157, R = C2H4(OC2H4)n
n = 1~20.
106
EDC/HOBt/DMF
158, R = C1~C8 alkyl,
159, R = C2H4(OC2H4)n
n = 1~20.
Cell Binding molecule
160
pH 5 ~ 8
Binding molecule
161
pH 5 ~ 8
Cell Binding molecule
162, R = C1~C8 alkyl,
163, R = C2H4(OC2H4)n
n = 1~20.

Figure 14.

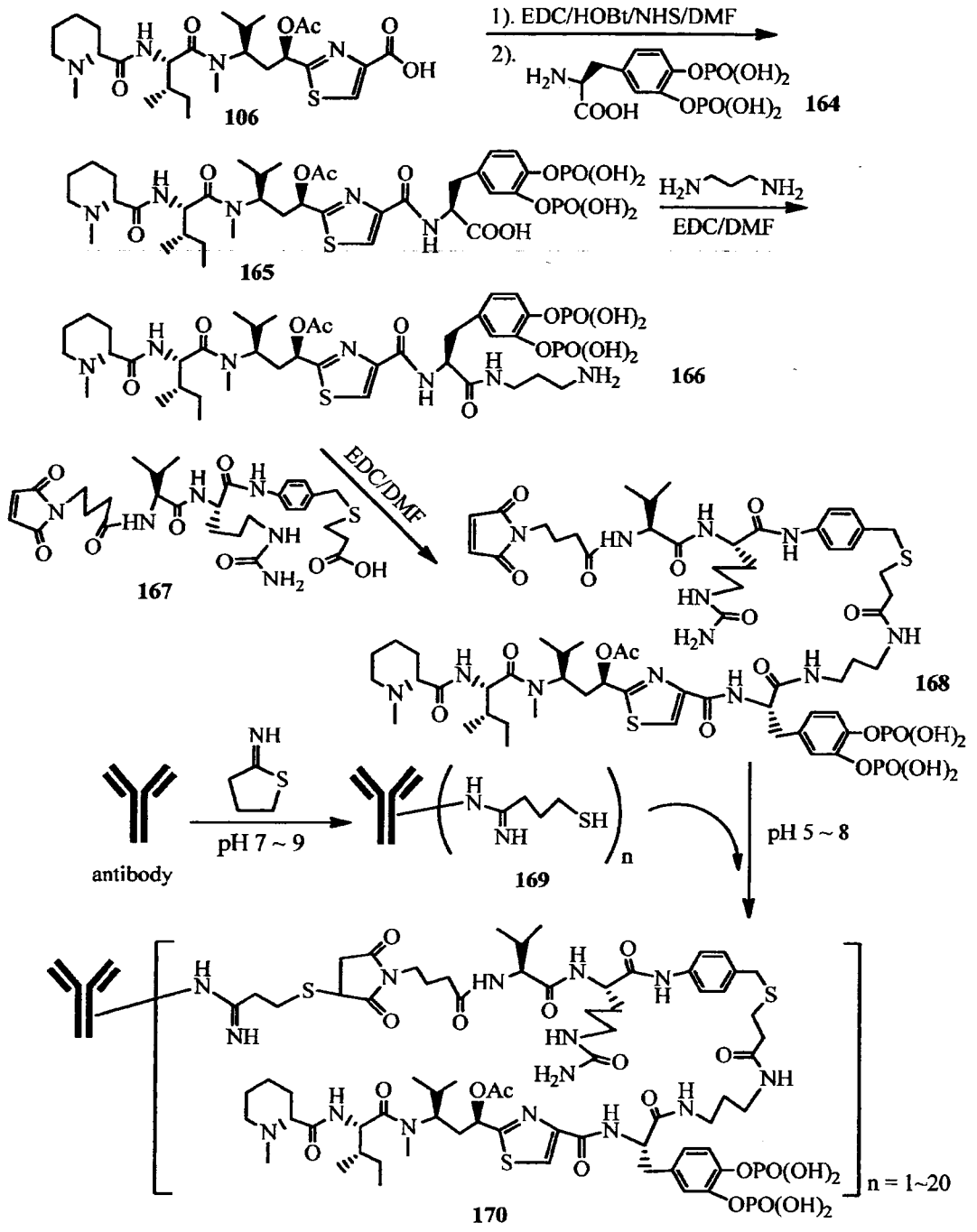

1). EDC/HOBt/NHS/DMF
2).
106
164
165
166
EDC/DMF
167
168
antibody
pH 7 ~ 9
169
pH 5 ~ 8
170
n = 1~20

Figure 15.

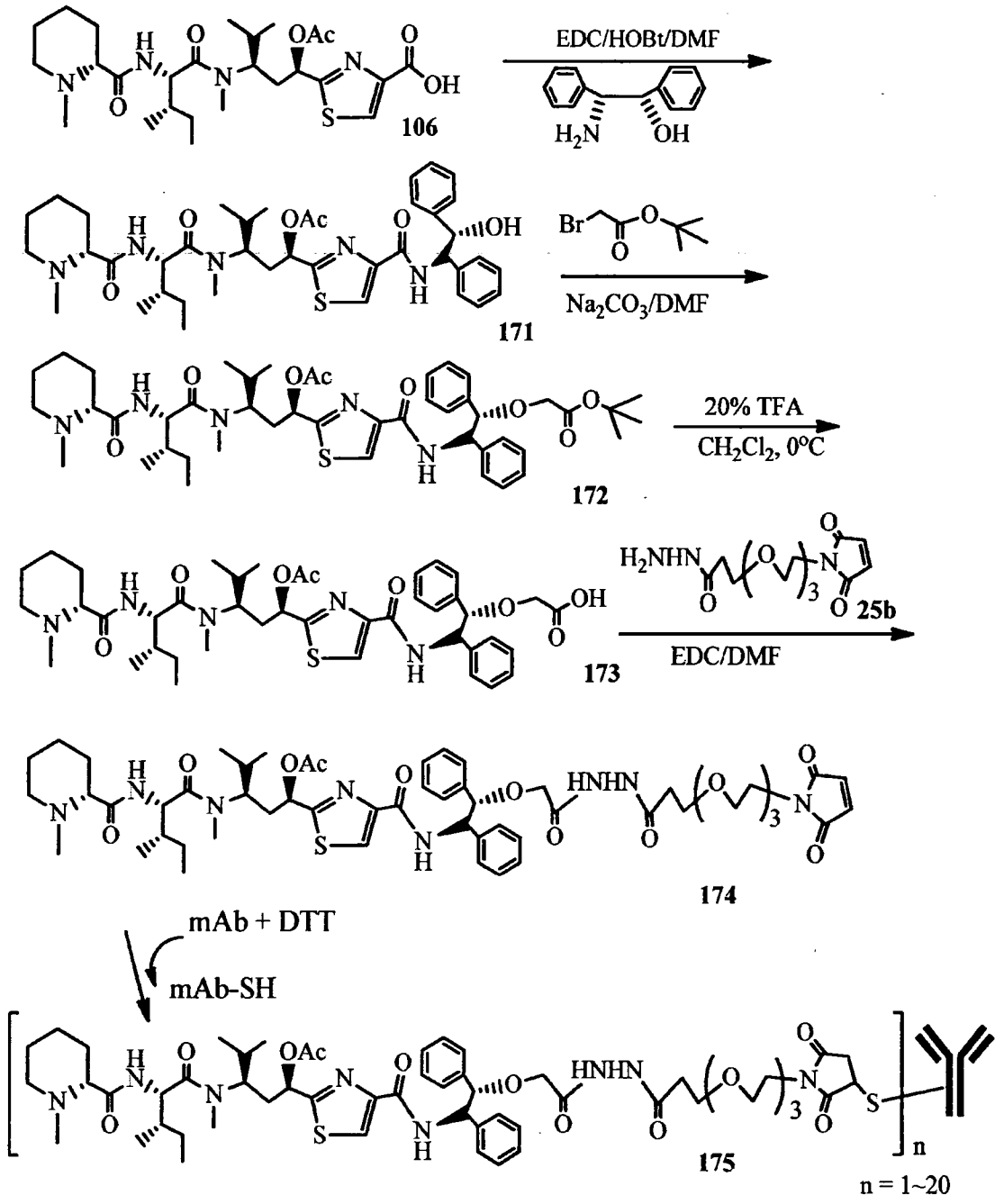

EDC/HOBt/DMF
106
171
Na2CO3/DMF
20% TFA
CH2Cl2, 0°C
172
25b
EDC/DMF
173
174
mAb + DTT
mAb-SH
175
n = 1~20

Figure 16.

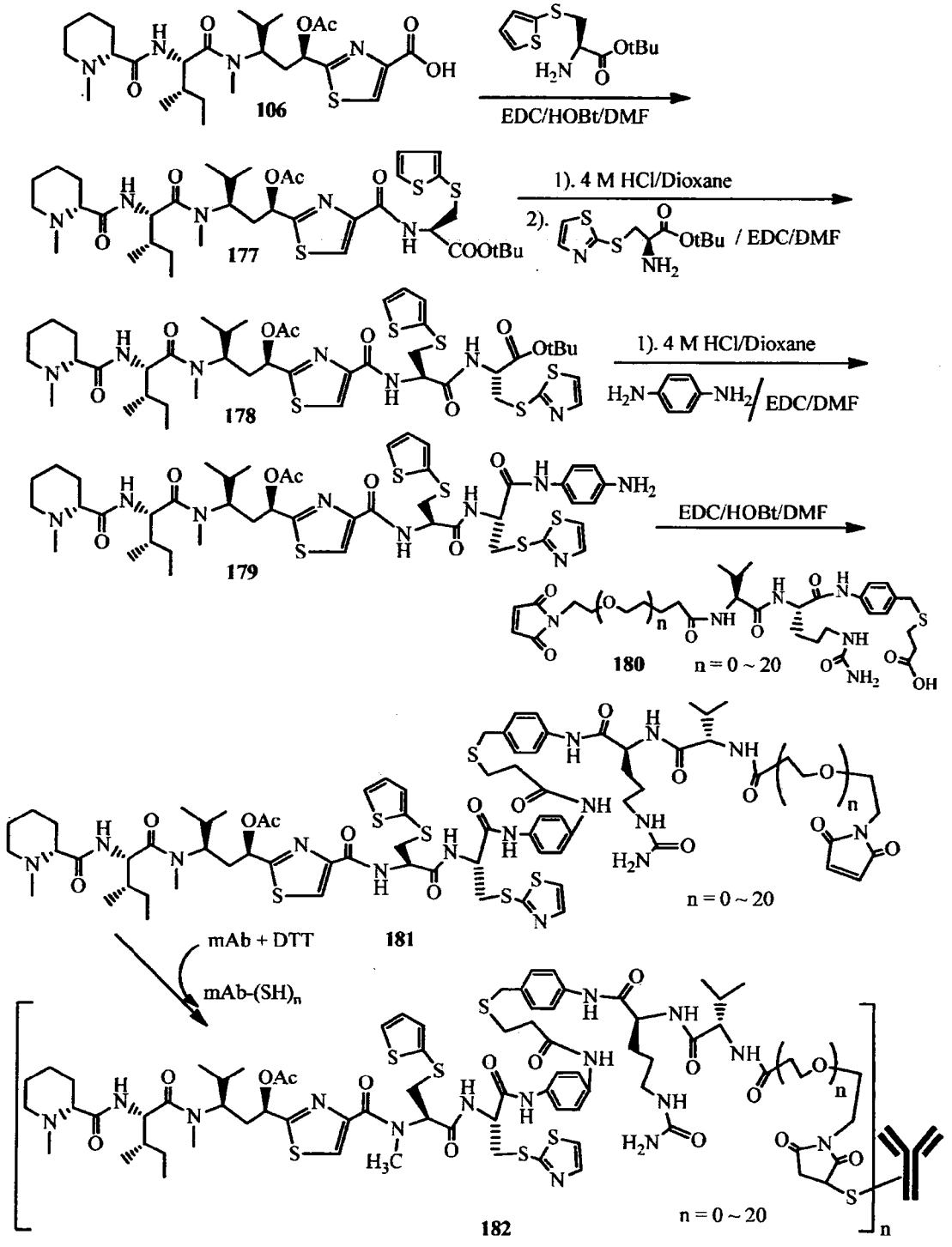

106
EDC/HOBt/DMF
177
1). 4 M HCl/Dioxane
2). / EDC/DMF
178
1). 4 M HCl/Dioxane
EDC/HOBt/DMF
179
180
n = 0 ~ 20
181
n = 0 ~ 20
mAb + DTT
mAb-(SH)n
182
n = 0 ~ 20

Figure 17.

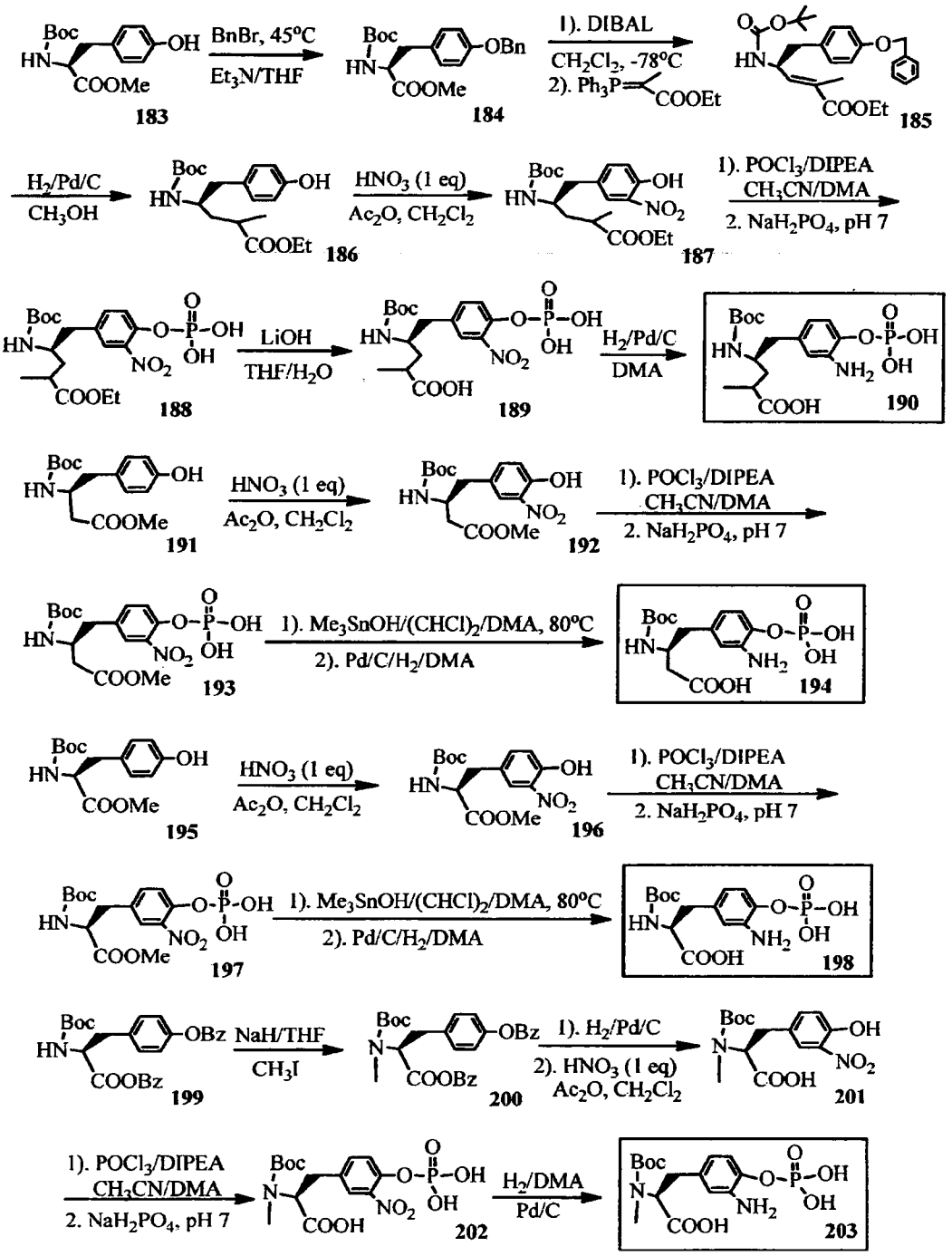

Boc
HN
OH
COOMe
183
BnBr, 45°C
Et3N/THF
OBn
184
1). DIBAL
CH2Cl2, -78°C
2). Ph3P=
COOEt
185
H2/Pd/C
CH3OH
HNO3 (1 eq)
Ac2O, CH2Cl2
NO2
186
187
1). POCl3/DIPEA
CH3CN/DMA
2. NaH2PO4, pH 7
LiOH
THF/H2O
188
189
H2/Pd/C
DMA
NH2
190
191
192
193
1). Me3SnOH/(CHCl)2/DMA, 80°C
2). Pd/C/H2/DMA
194
195
196
197
198
NaH/THF
CH3I
OBz
COOBz
199
200
201
202
203

Figure 18.

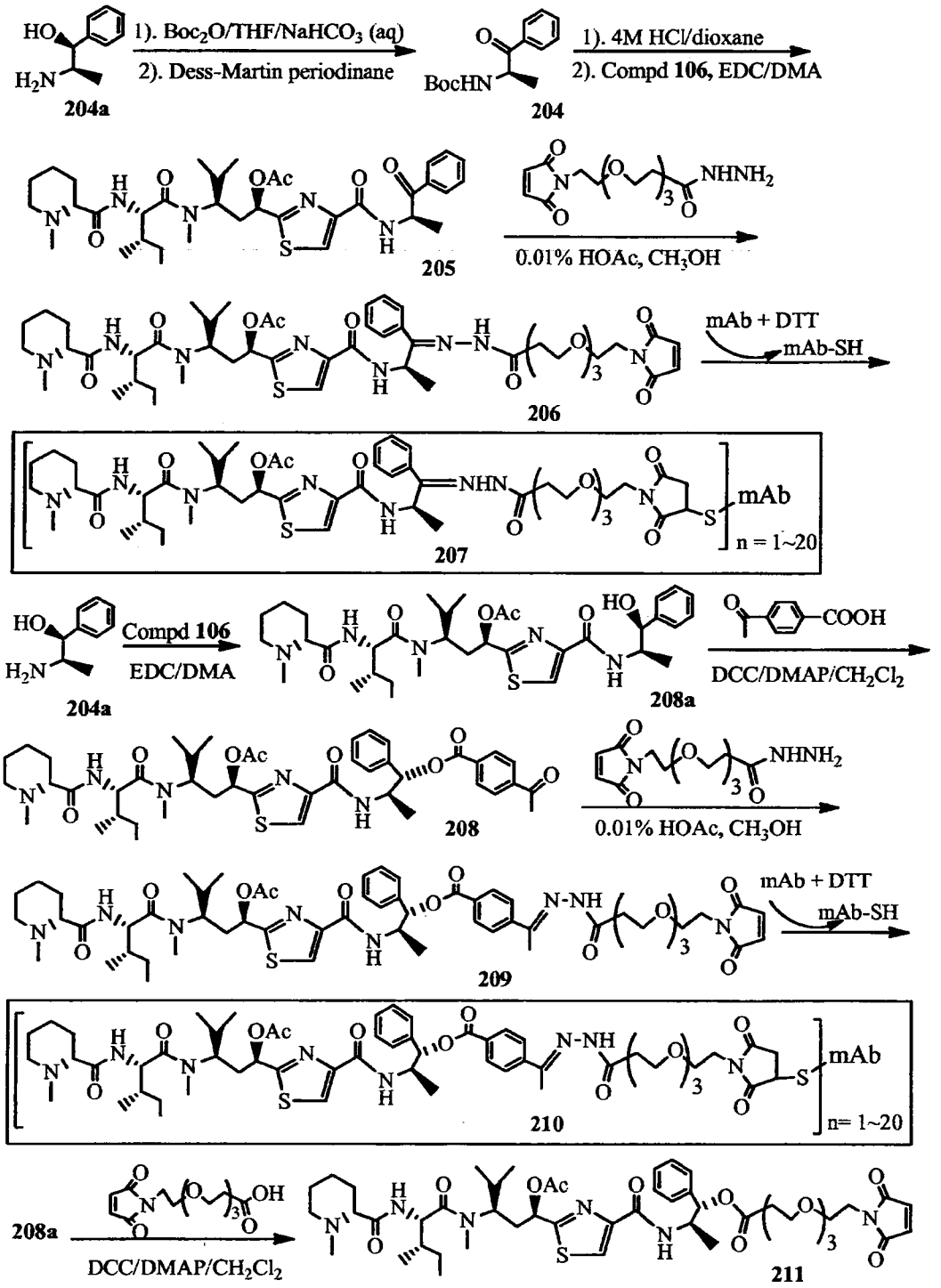

1). Boc2O/THF/NaHCO3 (aq)
2). Dess-Martin periodinane
1). 4M HCl/dioxane
2). Compd 106, EDC/DMA
204a
204
205
0.01% HOAc, CH3OH
206
mAb + DTT
mAb-SH
207
n = 1~20
Compd 106
EDC/DMA
208a
DCC/DMAP/CH2Cl2
208
209
210
n= 1~20
211

Figure 19.

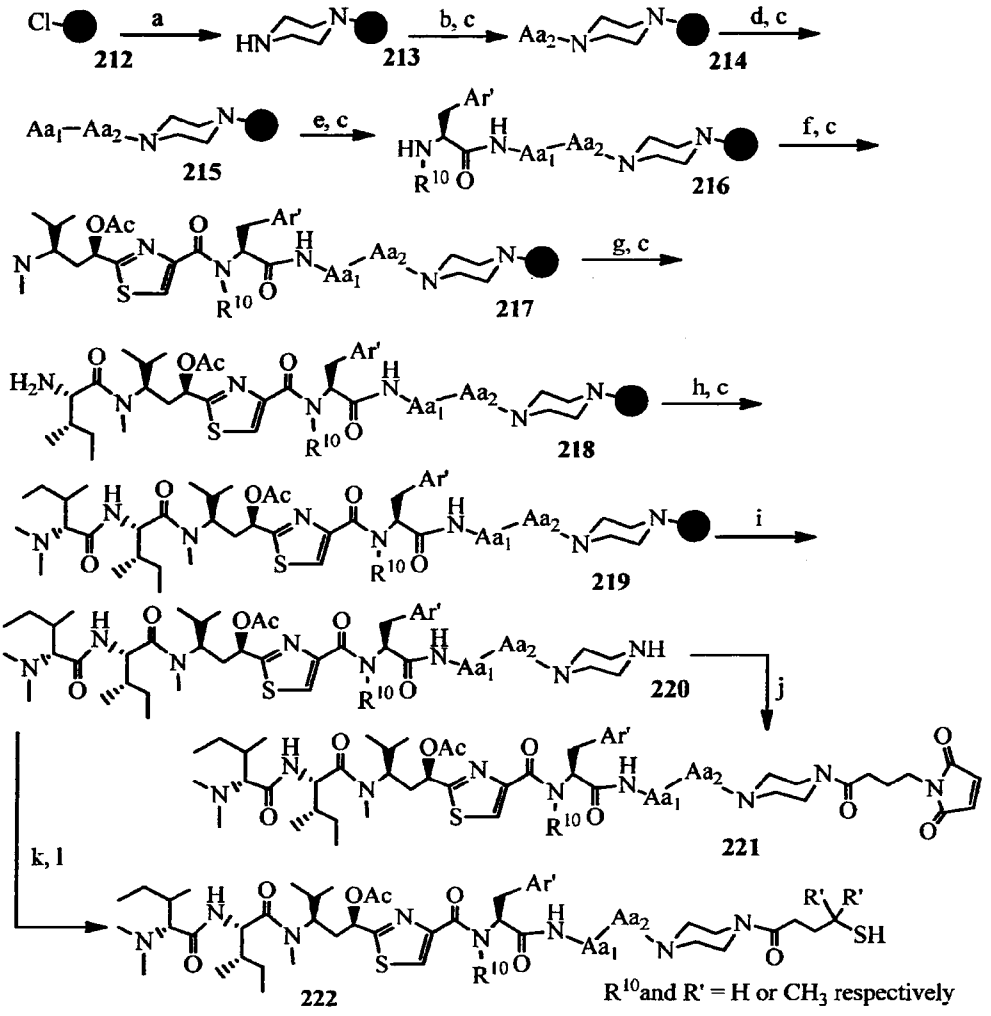

Cl
a
212
HN
213
b, c
Aa2
214
d, c
Aa1–Aa2
215
e, c
Ar'
HN
R10 O
216
f, c
OAc
S
217
g, c
H2N
218
h, c
219
i
NH
220
j
221
k, l
R'
SH
222
R10and R' = H or CH3 respectively

Figure 20.

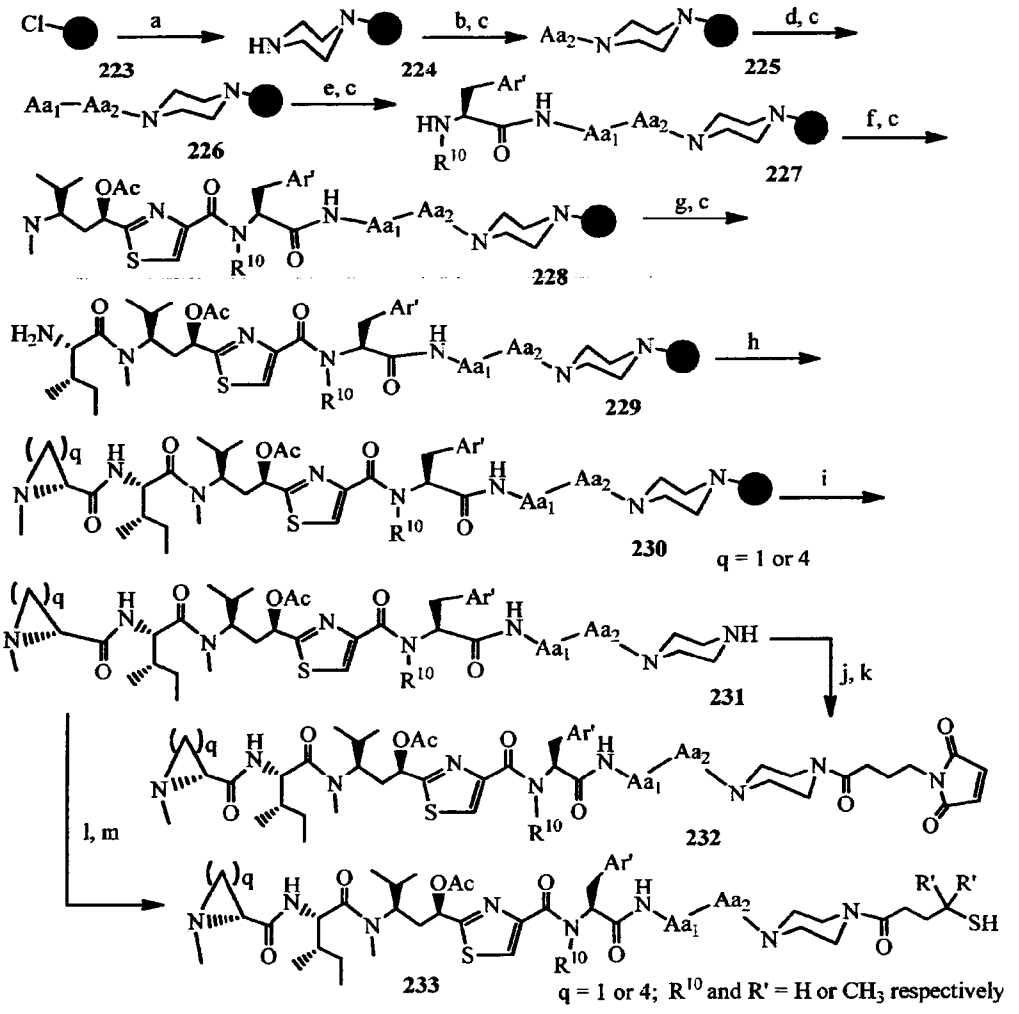

a
b, c
d, c
e, c
f, c
g, c
h
i
j, k
l, m
223
224
225
226
227
228
229
230
231
232
233
q = 1 or 4
q = 1 or 4; R10 and R' = H or CH3 respectively

Figure 21.

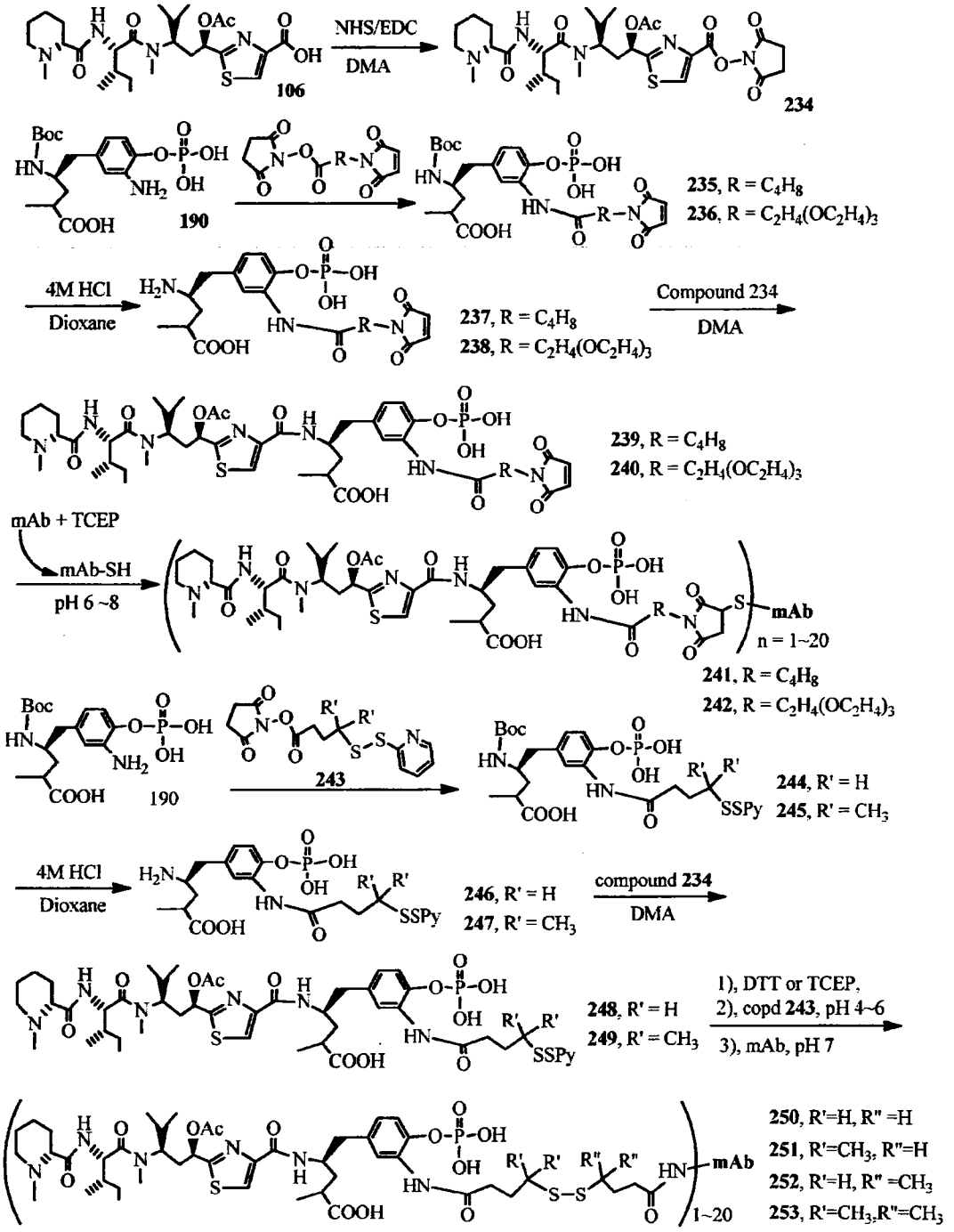

NHS/EDC
DMA
106
234
190
235, R = C4H8
236, R = C2H4(OC2H4)3
4M HCl
Dioxane
237, R = C4H8
238, R = C2H4(OC2H4)3
Compound 234
DMA
239, R = C4H8
240, R = C2H4(OC2H4)3
mAb + TCEP
mAb-SH
pH 6 ~8
mAb
n = 1~20
241, R = C4H8
242, R = C2H4(OC2H4)3
243
244, R' = H
245, R' = CH3
246, R' = H
247, R' = CH3
compound 234
DMA
248, R' = H
249, R' = CH3
1), DTT or TCEP,
2), copd 243, pH 4~6
3), mAb, pH 7
250, R'=H, R" =H
251, R'=CH3, R"=H
252, R'=H, R" =CH3
253, R'=CH3,R"=CH3
1~20

Figure 22.

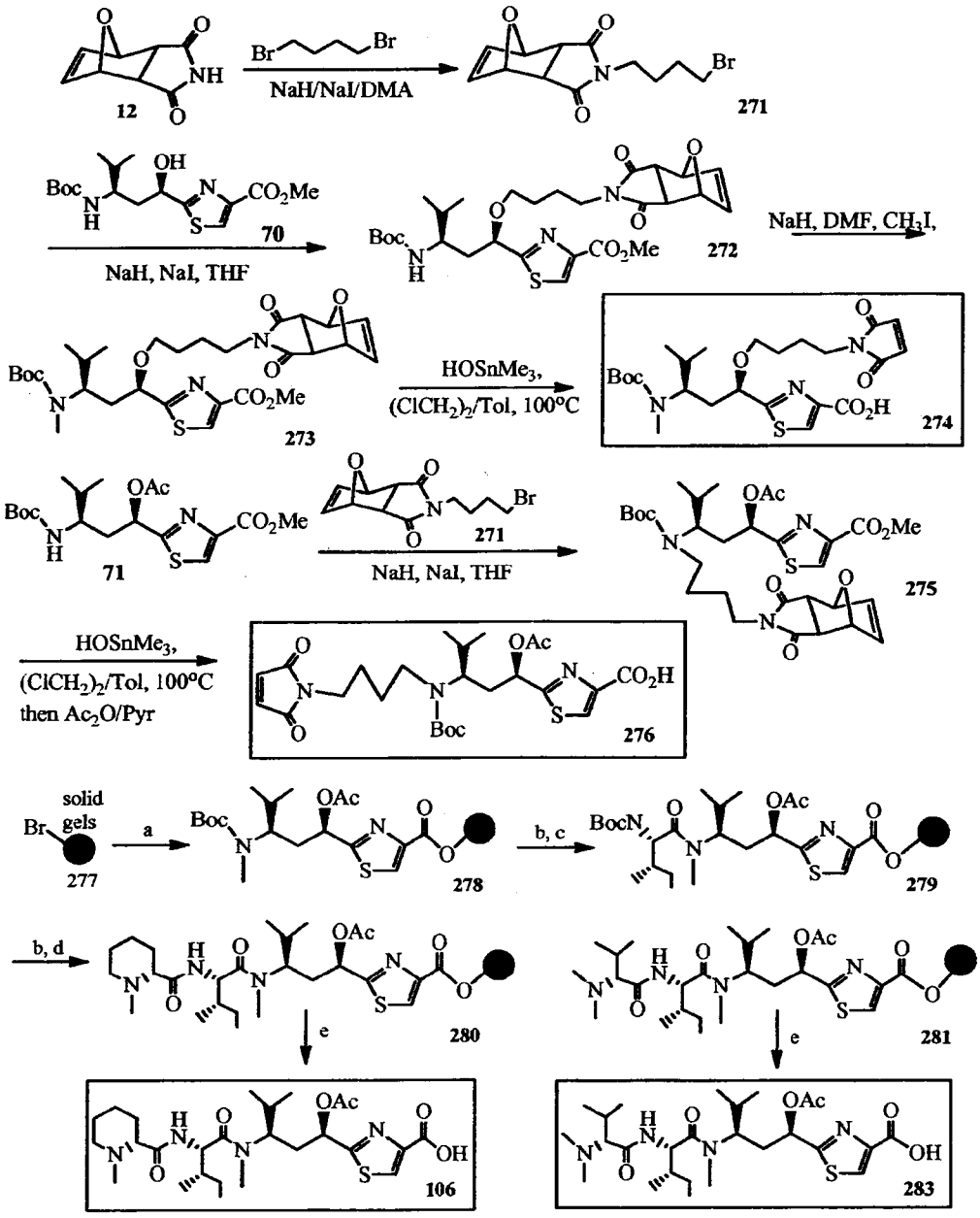

12
NaH/NaI/DMA
271
70
NaH, NaI, THF
272
NaH, DMF, CH3I,
273
HOSnMe3,
(ClCH2)2/Tol, 100°C
274
71
271
NaH, NaI, THF
275
HOSnMe3,
(ClCH2)2/Tol, 100°C
then Ac2O/Pyr
276
solid
gels
277
a
278
b, c
279
b, d
280
e
281
e
106
283

Figure 23.

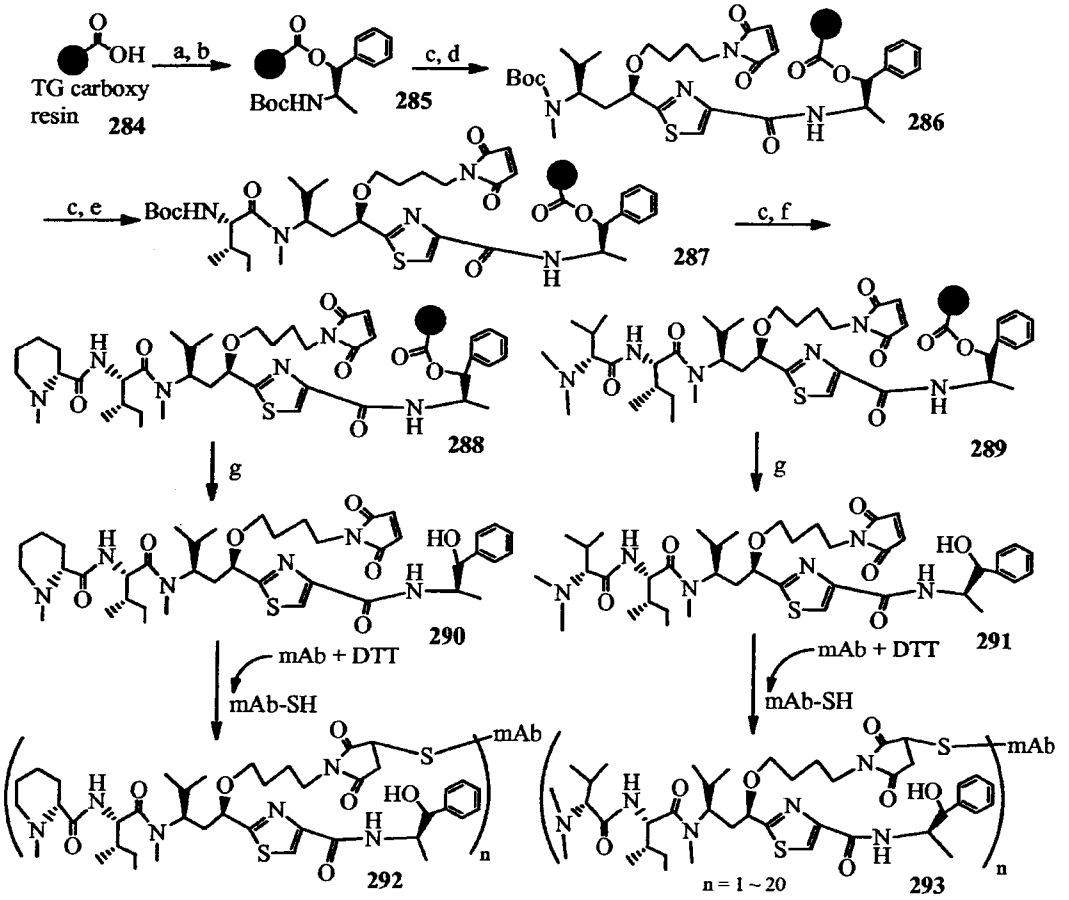
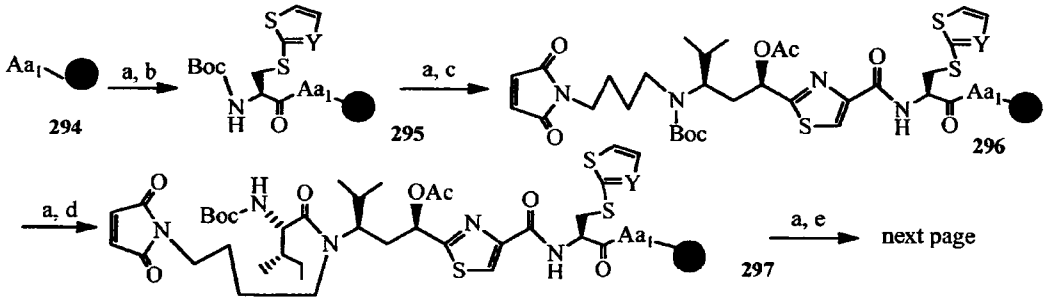

TG carboxy resin
284
a, b
BocHN
285
c, d
Boc
286
c, e
BocHN
287
c, f
288
289
g
g
290
291
mAb + DTT
mAb + DTT
mAb-SH
mAb-SH
mAb
mAb
292
n = 1 ~ 20
293

Figure 24.


294
a, b
295
a, c
296
a, d
297
a, e
next page

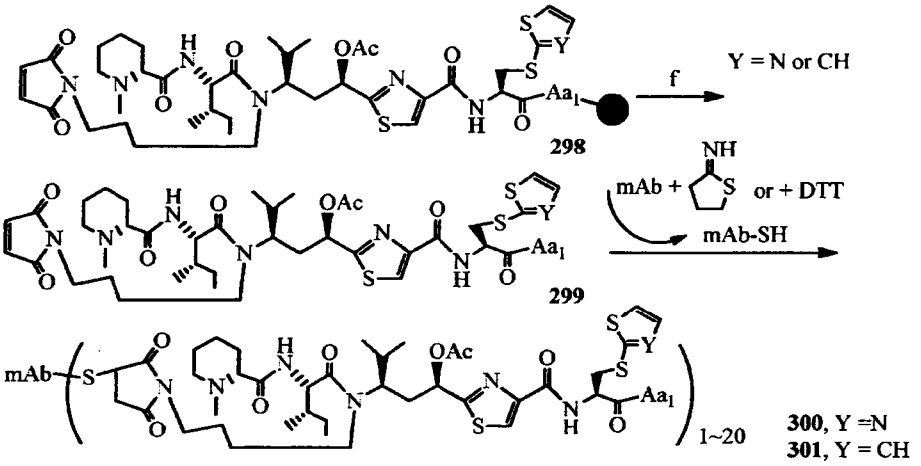
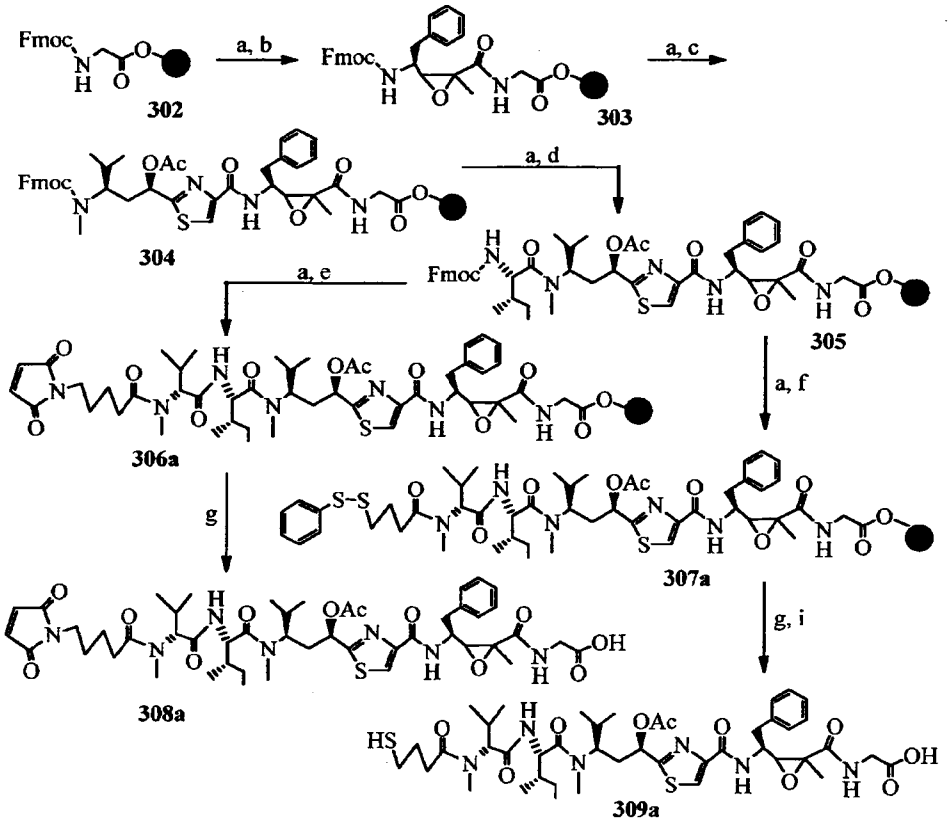

Y = N or CH
f
298
mAb + or + DTT
mAb-SH
299
mAb
1~20
300, Y =N
301, Y = CH

Figure 25


a, b
a, c
302
303
a, d
304
a, e
305
a, f
306a
g
307a
g, i
308a
309a

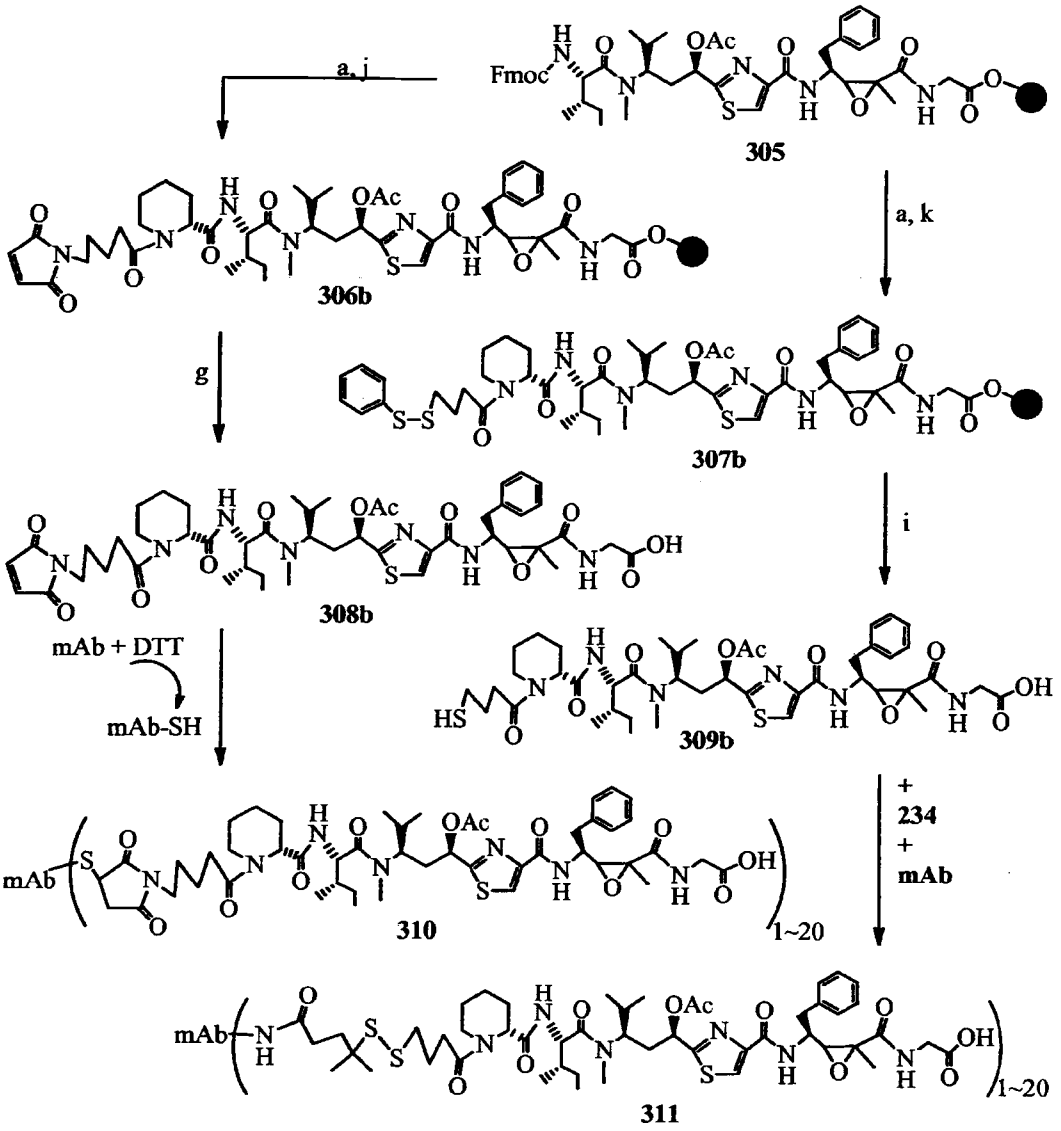

a, j
Fmoc
OAc
305
a, k
306b
g
307b
i
308b
OH
mAb + DTT
mAb-SH
HS
309b
+
234
+
mAb
mAb
310
1~20
311

Figure 26.

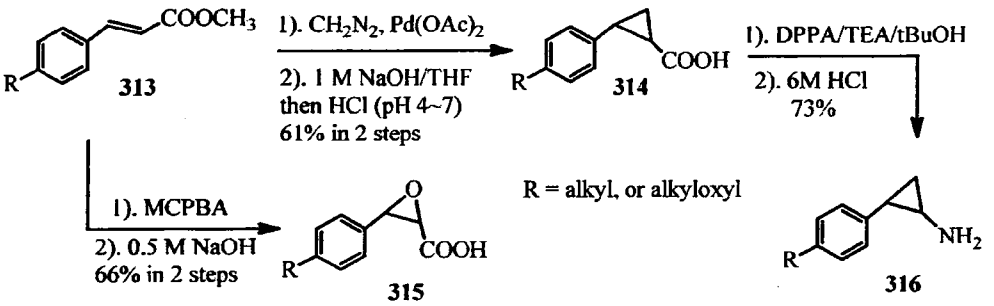


Figure 27.

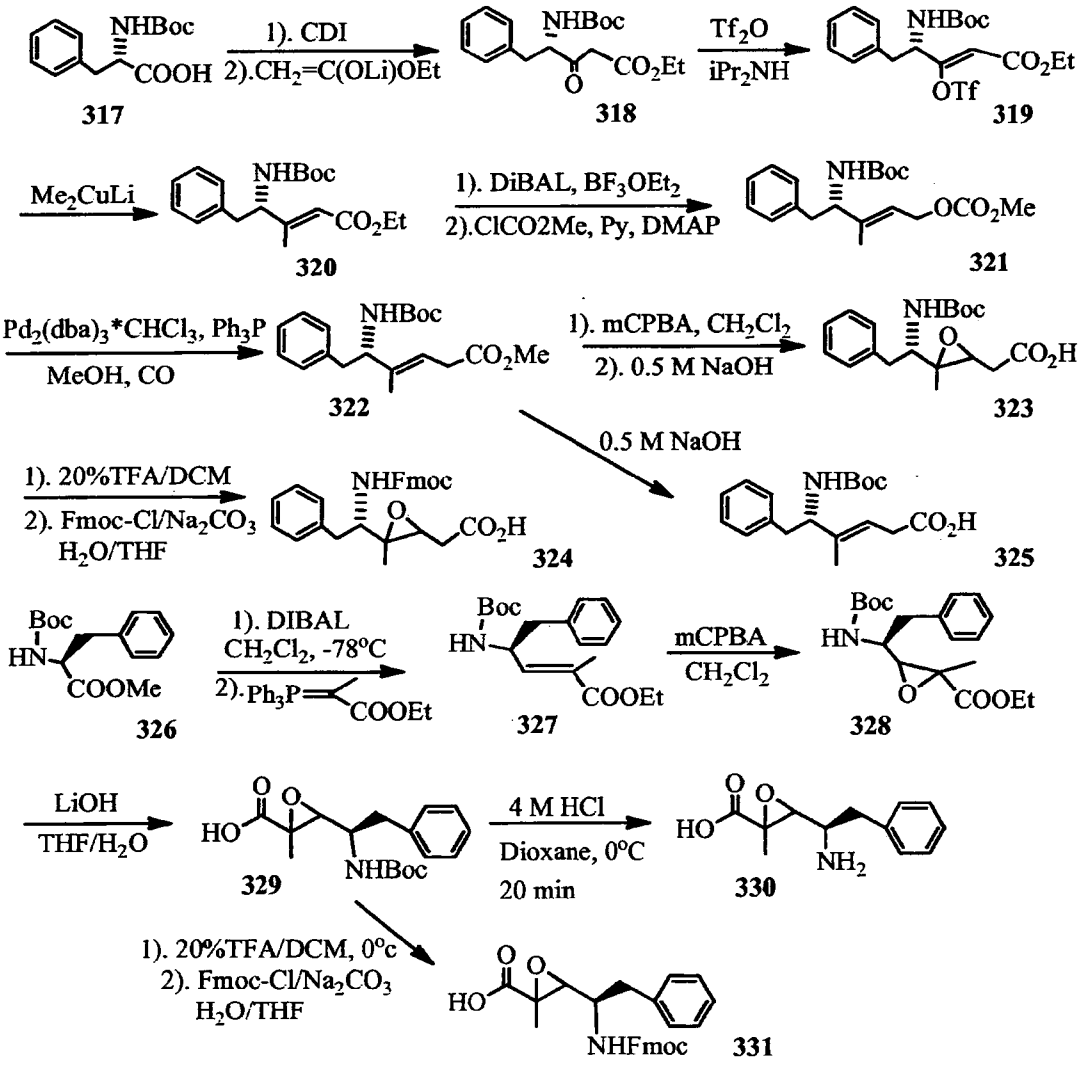


Figure 28.

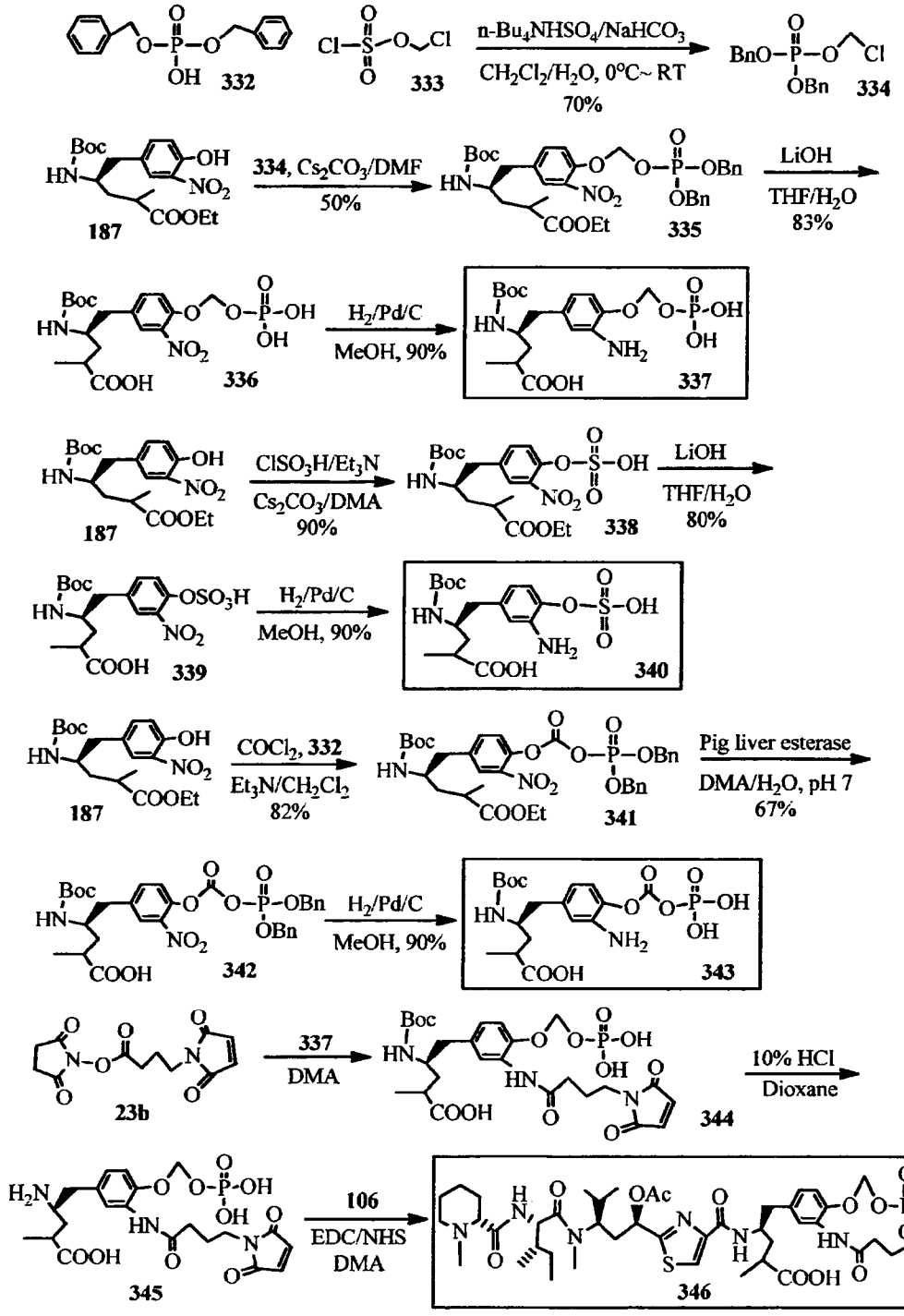

n-Bu4NHSO4/NaHCO3
CH2Cl2/H2O, 0°C~ RT
70%
334, Cs2CO3/DMF
50%
LiOH
THF/H2O
83%
H2/Pd/C
MeOH, 90%
ClSO3H/Et3N
Cs2CO3/DMA
90%
LiOH
THF/H2O
80%
H2/Pd/C
MeOH, 90%
COCl2, 332
Et3N/CH2Cl2
82%
Pig liver esterase
DMA/H2O, pH 7
67%
H2/Pd/C
MeOH, 90%
337
DMA
10% HCl
Dioxane
106
EDC/NHS
DMA

Fig. 29.

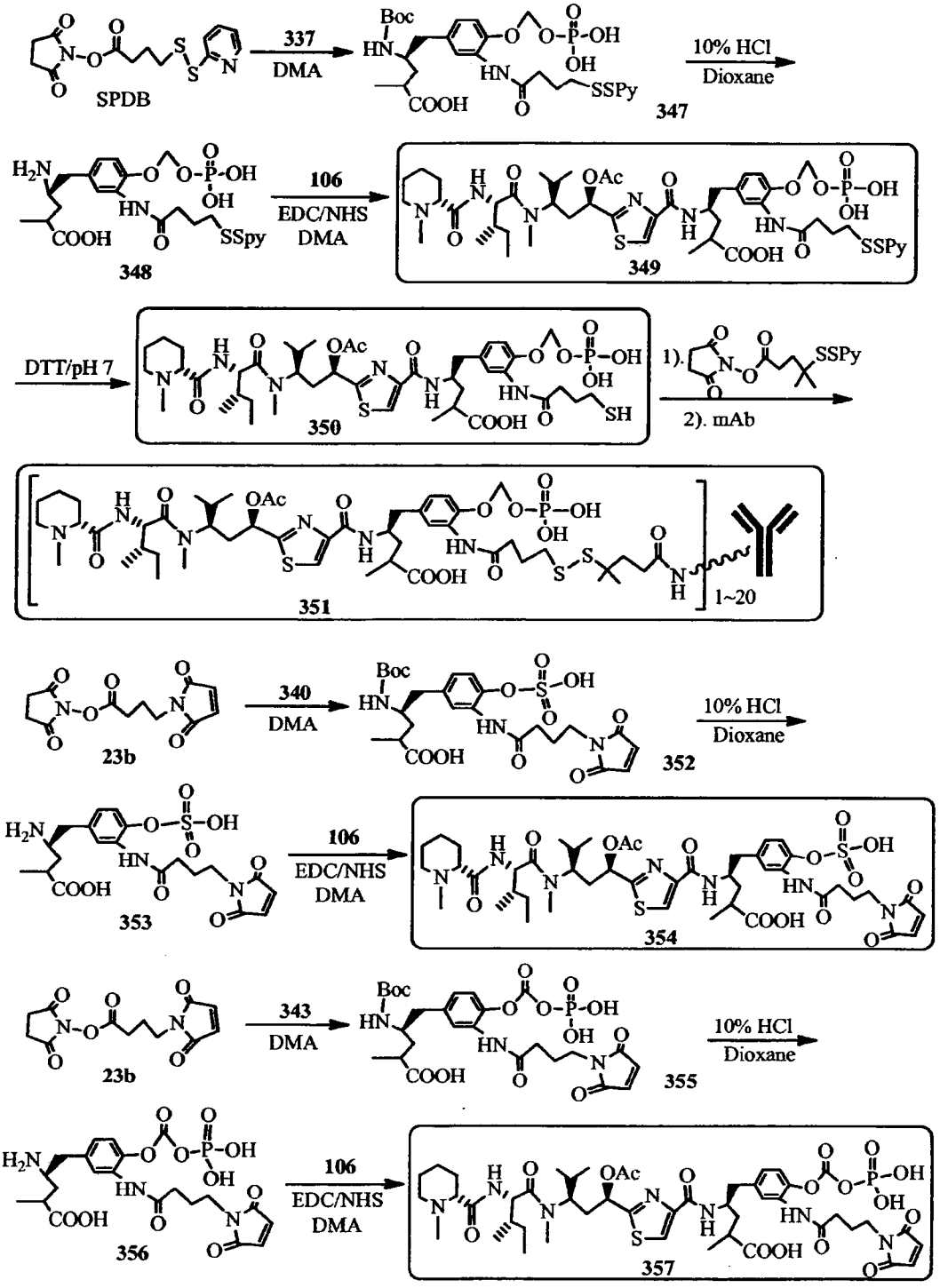

SPDB
337
DMA
347
10% HCl
Dioxane
348
106
EDC/NHS
DMA
349
DTT/pH 7
350
1).
2). mAb
351
1~20
23b
340
352
353
354
343
355
356
357

Fig. 30.

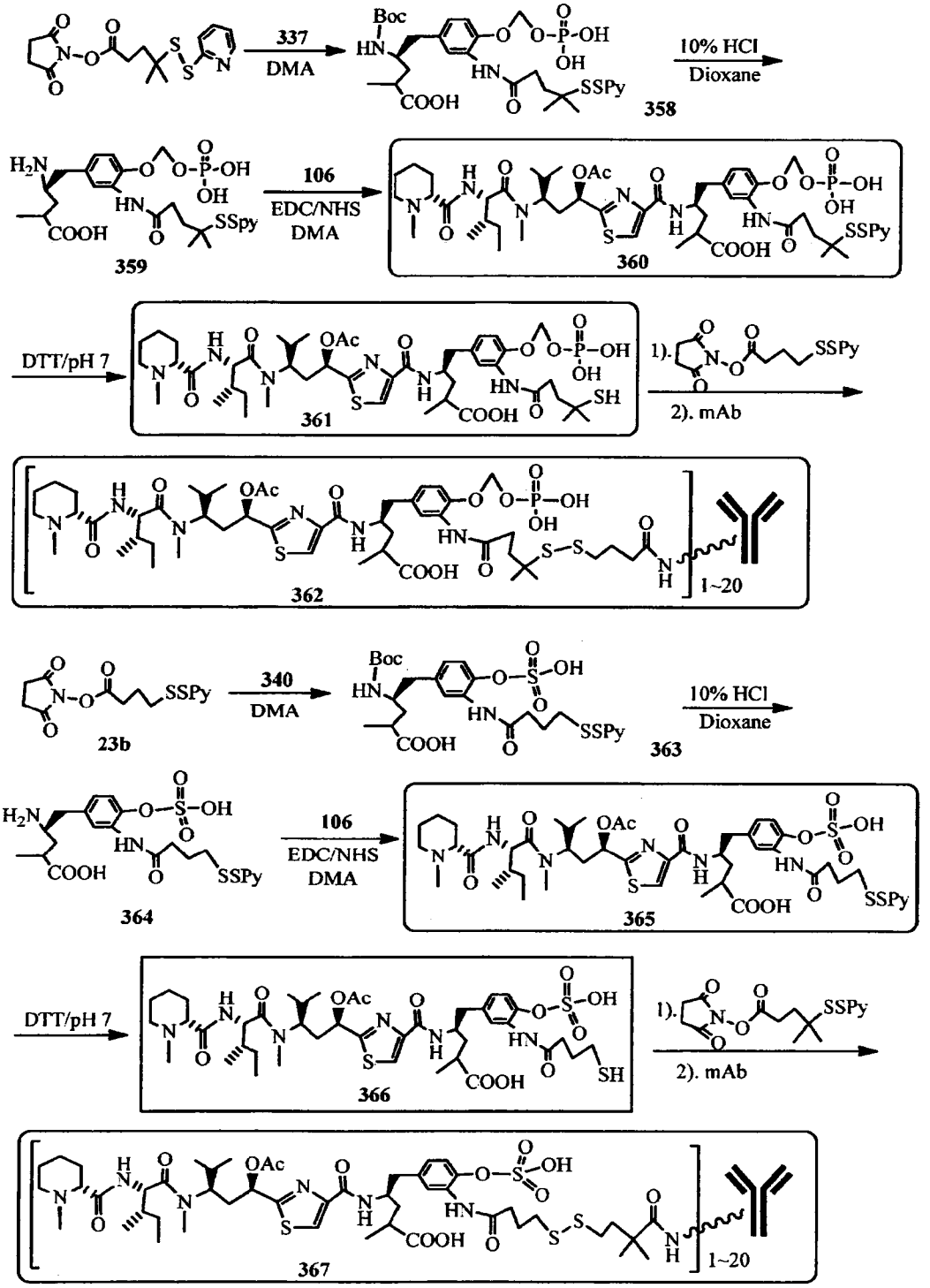

337
DMA
10% HCl
Dioxane
358
106
EDC/NHS
DMA
359
360
DTT/pH 7
361
1).
2). mAb
362
1~20
340
23b
363
364
365
366
367

Figure 31.

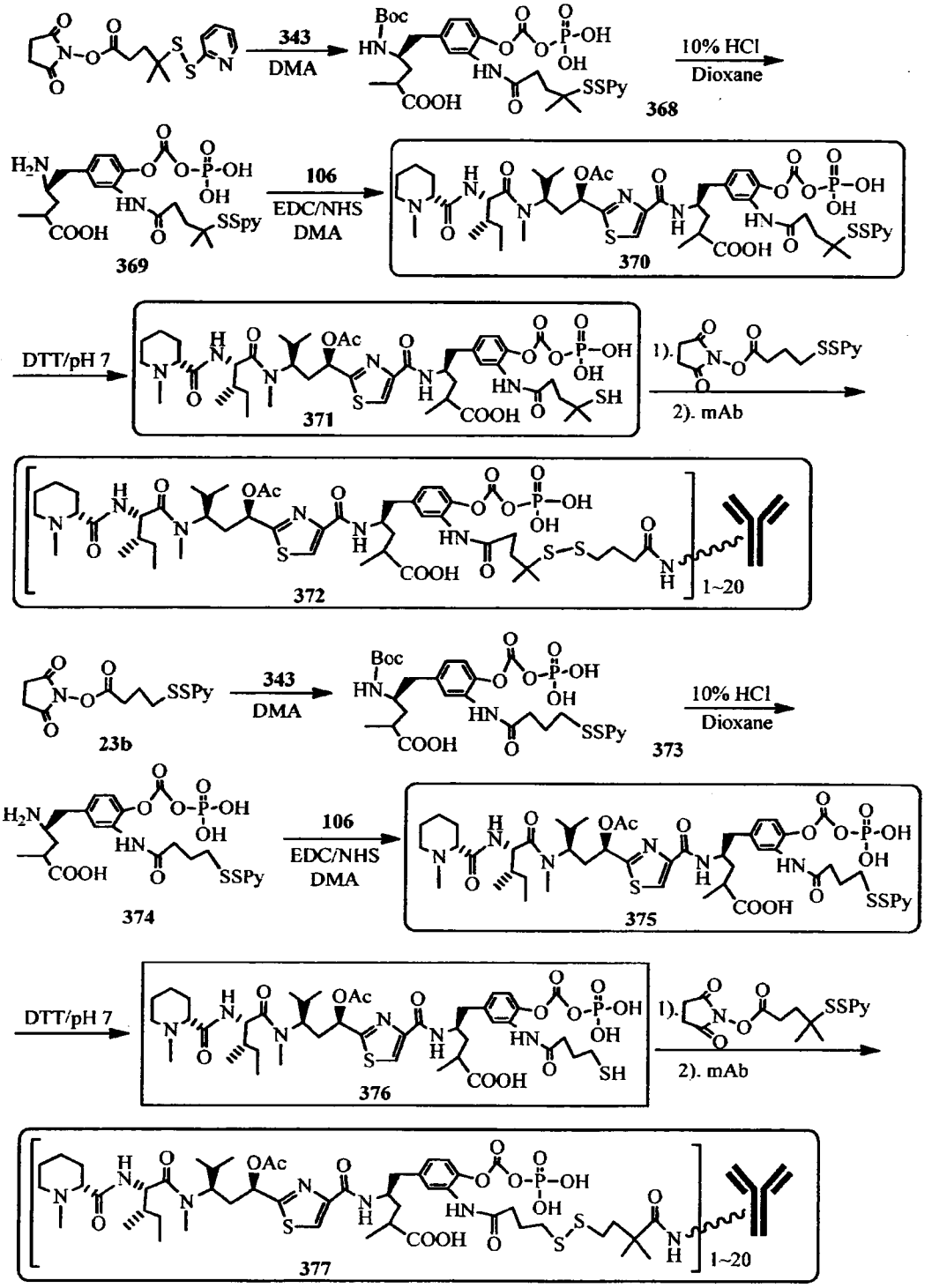

343
DMA
10% HCl
Dioxane
368
106
EDC/NHS
DMA
369
370
DTT/pH 7
371
1).
2). mAb
372
1~20
23b
373
374
375
376
377

Figure 32.

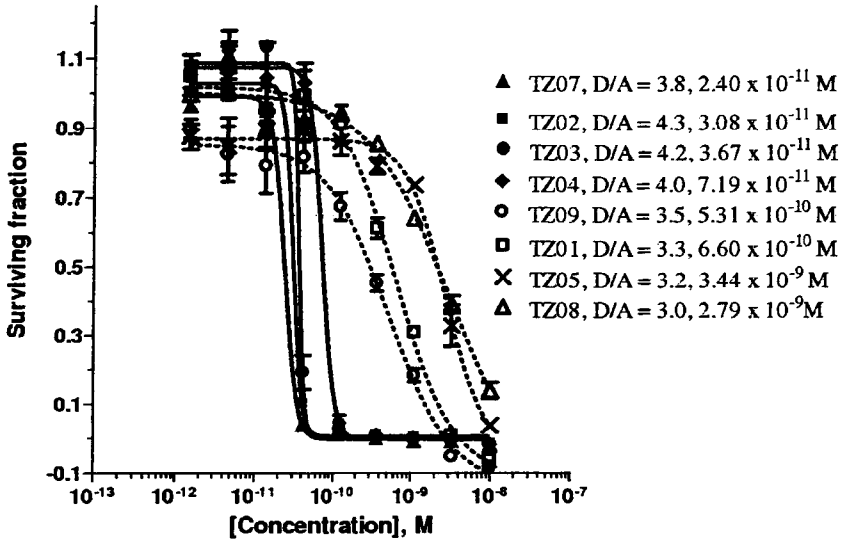


Figure 33.

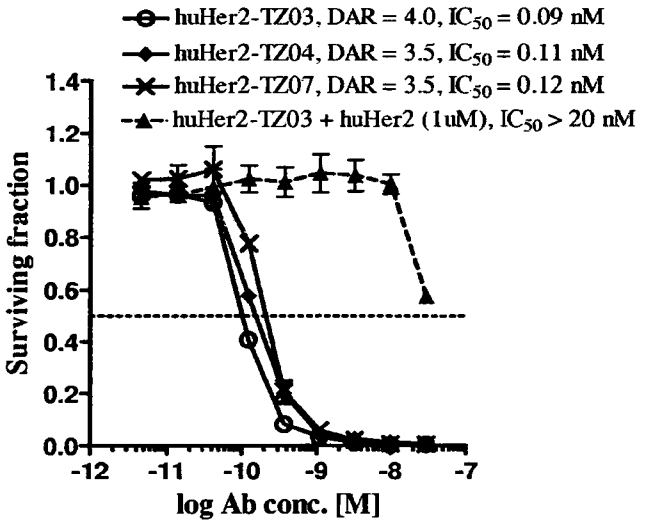


Figure 34.

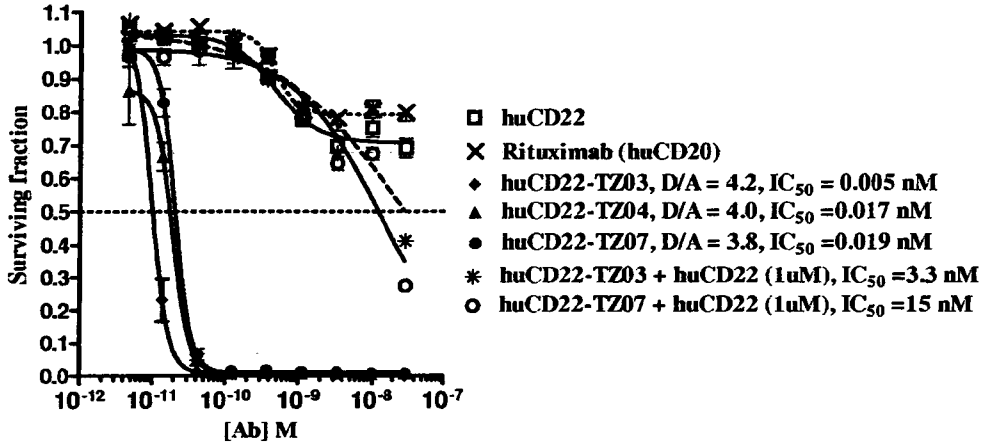
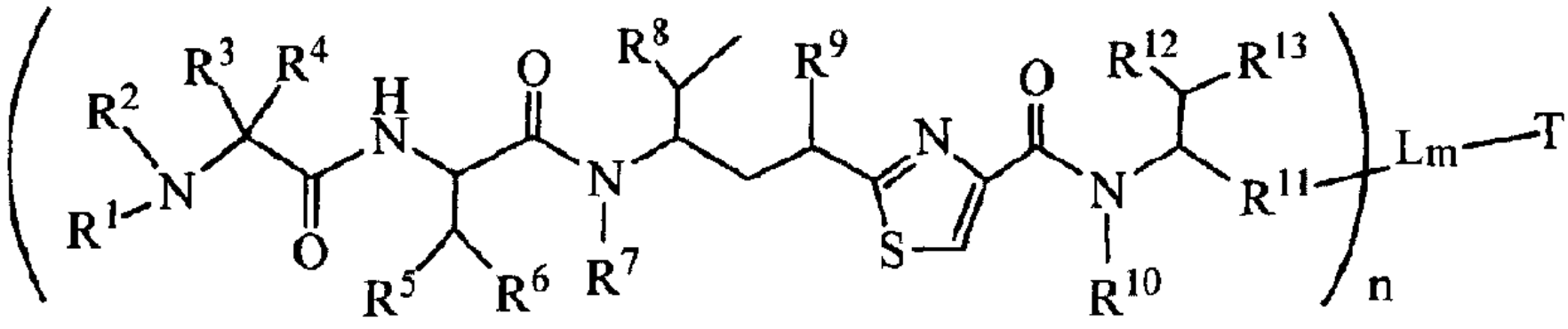

Surviving fraction
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
10-12 10-11 10-10 10-9 10-8 10-7
[Ab] M
huCD22
Rituximab (huCD20)
huCD22-TZ03, D/A = 4.2, IC50 = 0.005 nM
huCD22-TZ04, D/A = 4.0, IC50 =0.017 nM
huCD22-TZ07, D/A = 3.8, IC50 =0.019 nM
huCD22-TZ03 + huCD22 (1uM), IC50 =3.3 nM
huCD22-TZ07 + huCD22 (1uM), IC50 =15 nM

Figure 35.



(V)